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Circadian rhythm in the function of central 5-HT_{1A} receptors is endogenous in nature

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Abstract. In a recent study, we found a circadian rhythm in the response of central serotonin (5-$HT)_{1A}$ receptors to 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT). In the present study, the 8-OH-DPAT-induced 5-HT behavioural syndrome was examined in rats kept under continuous dark (DD) conditions for 6 days. The results revealed a circadian rhythm in the behavioural response to 8-OH-DPAT under DD conditions. The pattern of this rhythm was similar to that observed under the light-dark conditions, except for the phase delay that corresponds to the period of free-running rhythm. These results strongly suggest that the rhythm in the function of central 5-HT_{1A} receptors is driven by the endogenous oscillator.

Key words. Circadian rhythm; serotonin (5-$HT)_{1A}$ receptors; 5-HT syndrome; 8-OH-DPAT; endogenous rhythm.

In the central serotonergic system, there are many rhythms characterized by periods of approximately 24 h, such as changes in the levels of serotonin (5-HT) [1, 2], 5-hydroxyindole acetic acid [2, 3], 5-hydroxytryptophan [2], tryptophan [2], and in the number of receptor binding sites [4–6]. It has usually not yet been determined, however, whether these rhythms are endogenous or exogenous. The exception is the rhythm in 5-HT levels, which has been investigated in three studies [7–9]. Disregarding regional differences in the brain, the authors came to three different conclusions: an endogenous rhythm [9], an exogenous rhythm [8], and an endogenous rhythm that is influenced by light [7].

It has been suggested that the functions of the central 5-HT-ergic system, based on central 5-HT_{1A} [10] and 5-HT_{2A} receptors [11–13], exhibit circadian rhythms. To our knowledge, however, no studies have examined whether the rhythms of the 5-HT functions are endogenous or exogenous. In a previous study [10], we demonstrated that there is a circadian rhythm in the responsiveness to the selective 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT). This finding suggests the existence of a circadian rhythm in the function of central postsynaptic 5-HT_{1A} receptors. The purpose of the present study was to determine, using continuous dark (DD) conditions, whether the rhythm in the responsiveness to 8-OH-DPAT is endogenous or exogenous.

Materials and methods

Animals. Male Wistar rats were housed in groups of two and subjected to a 12 h light-dark (LD) cycle (light from 18.00 to 06.00 h) at 24.0 ± 0.5 °C with 50 ± 5% relative humidity. After 3 weeks of adaptation to the standard LD cycle, all rats were transferred to DD conditions at the end of the dark period, then exposed to DD conditions for a period of 6 days. Food and water were available ad libitum. The animals were acclimatized to the handling required for the experiments. Each rat was used only once.

Experimental protocols. The experiments were performed in a soundproof darkroom at the same temperature as the housing room. The animals were 7 weeks old and weighed 250 to 330 g. To habituate the rats to the experimental environment, each rat was placed in a separate, clear plastic experimental case (44 × 26 × 19 cm) 40 min before drug administration. The behavioural responses to the drug were observed under a dim red lamp.

Six groups of 8 rats were given subcutaneous (sc) injections of 0.25 mg/kg 8-OH-DPAT at one of the following times of day: 00.00, 04.00, 08.00, 12.00, 16.00, and 20.00 h. This dose was the same as that in our previous study [10] and was selected from a dose-response study of 8-OH-DPAT-induced 5-HT behavioural syndrome. After each injection, the same behavioural indices as in the aforementioned study [forepaw treading (FPT), head weaving (HW), and flat body posture (FBP)] were recorded for 45 s every 5 min over an observation period of 90 min. FPT and HW were measured by counting the number of times the behaviour was performed during the observation period, while FBP was scored on a 4-point scale: 0 = absent, 1 = equivocal, 2 = present, and 3 = intense. Behavioural responses were assessed by two parameters: total score and duration. Total score was defined as the sum of the FPT and HW counts, or the sum of the FBP scores for the observation period

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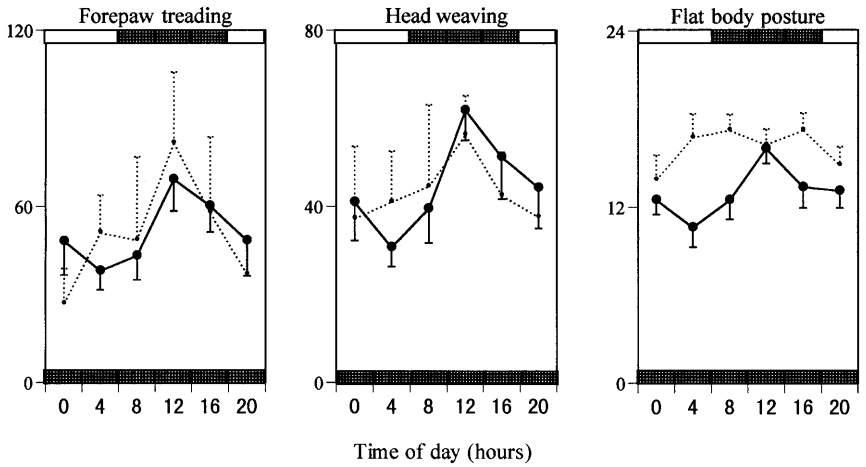

Figure. Circadian rhythms in the total score of behavioural responses to 8-OH-DPAT (0.25 mg/kg, sc) in rats under continuous dark conditions for 6 days. Values are mean ± SD of 8 animals. The dotted line is drawn from the data of a study under light-dark conditions that was published previously (ref. 10). The black bar corresponds to the dark phase. $P < 0.001$ (ANOVA and least squares cosine fitting method).

following drug administration. Duration was defined as the length of time the behavioural response lasted, from appearance to disappearance. The results of the assessment of behavioural responses were expressed as mean ± SD.

Drug. 8-OH-DPAT, obtained from Research Biochemicals, Inc. (Natick, MA, USA), was dissolved in 0.9% saline immediately before use. The concentration of drug solution was 0.5 mg/ml.

Results

Significant circadian rhythms in the response to 8-OH-DPAT were observed in the total scores of FPT, HW and FBP, and in the durations of FPT, HW and FBP ($P < 0.001$, ANOVA). The circadian rhythms of the total scores are shown in the figure. The patterns of the circadian rhythms of durations were similar to those of the total scores. All parameters had peak responses at 12.00 h, i.e. scores of 69.3 ± 11.5 (FPT), 61.9 ± 7.4 (HW), and 16.0 ± 1.1 (FBP) for the total scores, and 36.3 ± 3.5 min (FPT), 36.3 ± 2.3 min (HW), and 36.3 ± 3.5 min (FBP) for the duration.

The acrophases (peak time) of each rhythm, calculated by the least squares cosine fitting method, occurred at 14.35 (FPT), 14.29 (HW), and 14.11 (FBP) h for the total score, and at 13.43 (FPT), 13.20 (HW), and 13,37 (FBP) h for the duration ($P < 0.001$).

Discussion

The present study revealed that the circadian rhythm in the responsiveness to 8-OH-DPAT is maintained under DD conditions. Under LD conditions, the acrophases of the rhythms of the 8-OH-DPAT-induced responses occurred at 11.44 (FPT), 11.05 (HW) and 10.28 (FBP) h for the total score, and 10.38 (FPT), 10.46 (HW) and 10.00 (FBP) h for the duration [10]. In comparison with this rhythm, the acrophases observed under DD conditions (average duration of 5.5 days) had an average 3 h and 12 min delay in the total score and an average 3 h and 5 min delay in the duration. This suggests that the phase of each parameter had an average 34 min delay per day under the DD conditions, and that the rhythm free-runs with an actual period of 24 h and 34 min. On the other hand, we also observed circadian rhythms in the FPT and HW responses following the intracerebroventricular administration of 8-OH-DPAT (Nagayama and Lu, unpublished data); the response patterns were similar to those observed following sc administration of 8-OH-DPAT. This indicates that the circadian rhythms observed in these behavioural responses to the sc administration of 8-OH-DPAT are independent of pharmacokinetic factors. Together with the evidence that the 8-OH-DPAT-induced 5-HT behavioural syndrome is the result of direct stimulation of postsynaptic 5-HT_{1A} receptors [14], the results presented here strongly suggest that the function of central postsynaptic 5-HT_{1A} receptors has an endogenous circadian rhythm.

Previous studies have reported the absence of a circadian rhythm in 5-HT_1 receptor function under LD conditions [11, 15]. The discrepancy in the results between these previous studies and our studies may be derived from the several methodological differences, such as using the non-selective 5-HT_{1A} receptor agonist in one of the previous studies [11], and observing hypothermia in mice, most likely the consequence of stimulation of presynaptic 5-HT_{1A} receptors [16, 17], in the other [15]. Our previous publication [10] addresses this in detail.

Although circadian rhythms in the brain 5-HT-ergic system have been studied extensively, few studies have

examined whether these rhythms are endogenous or exogenous [15]. Based on the findings that daily fluctuations of 5-HT levels are found in the hypothalamus and olfactory bulbs of the hamster under LD conditions, but not under continuous light and DD conditions, Ferraro and Steger [8] concluded that daily fluctuations in brain 5-HT levels are driven by the photic cycle and are not circadian in nature. In contrast, other studies found that the rhythms of 5-HT levels in rat raphe nuclei are regulated by an endogenous oscillator [7, 9] and by light [7]; this was based on the evidence that the rhythms are maintained under DD conditions, that the 5-HT peak detected on day 3 in DD conditions is about 12 h out of phase with the peak found under LD conditions, and that there is a significant increase in 5-HT levels soon after animals are re-exposed to continuous light [7]. In the present study, the rhythm in the 8-OH-DPAT-induced behavioural response persisted in DD conditions; the pattern of the rhythm observed under DD conditions was similar to that observed under LD conditions, except for the phase change that corresponded to the period of the free-running rhythm. This suggests that light, beyond its function as a Zeitgeber, did not have an observable effect on the rhythm of 5-HT_{1A} receptor function. From the above, we suggest that the rhythm in the response to 8-OH-DPAT, with respect to the influence of light, is unlike the rhythm in the 5-HT levels. For this reason, it is unlikely that the rhythm in 5-HT_{1A} receptor function is generated directly from the rhythm of 5-HT levels. As such, we suggest that the rhythm in 5-HT_{1A} postsynaptic receptor function is driven by an endogenous circadian oscillator, without being mediated by the rhythmic change of 5-HT levels. Nevertheless, the possibility that the rhythm in 5-HT_{1A} postsynaptic receptor function is additionally influenced by circadian cycle-dependent changes in other central transmitter systems [12] cannot be ignored.

Many studies have reported on rhythms in the drug effects [19]; few, however, have conducted the experiments under DD conditions. Furthermore, it is possible that for observing a group mean of behaviours, such as in the present study, placing animals under DD conditions for too long could bring about an apparent disappearance of circadian rhythm, as each animal's rhythm is expected to free-run with its own period. Nagayama et al. [20] applied the appropriate duration of DD conditions and concluded that the rhythms of the response to a drug (haloperidol) were driven by an endogenous oscillator. The present study has indicated that a circadian rhythm in the behavioural effects elicited by 8-OH-DPAT persists in DD conditions, adding further support to the hypothesis that the rhythms in the drug effects are endogenous. These results may be important for any clinical application of the rhythms in the drug effect.

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