Pharmacology Biochemistry & Behavior, Vol. 12, pp. 459–461. Printed in the U.S.A.

Circadian Rhythm of Apomorphine-Induced Stereotypy in Rats

SHIGEYUKI NAKANO, CHIAKI HARA AND NOBUYA OGAWA

Department of Pharmacology, Ehime University, School of Medicine, Shigenobu-Cho Onsen-Gun, Ehime-Ken, 791-02 Japan

Received 31 August 1979

NAKANO, S., C. HARA AND N. OGAWA. *Circadian rhythm of apomorphine-induced stereotypy in rats.* PHARMAC. BIOCHEM. BEHAV. 12(3)459–461, 1980.—The existence of circadian variations of apomorphine-induced stereotypy was determined. Male Wistar rats, standardized to a light-dark cycle (lights on from 7:00–19:00) for three weeks, were injected with apomorphine hydrochloride (1 mg/kg or 3 mg/kg) at one of six times (9:00, 13:00, 17:00, 21:00, 1:00 and 5:00). A significant time-of-day effect was found for apomorphine-induced stereotypy, with highest stereotypic score following injection at 13:00 or 17:00. The circadian rhythm of apomorphine-induced stereotypy was significantly fitted to a single cosine curve with a 24-hr cycle using the least squares method.

Apomorphine Stereotypy Circadian rhythm Chronopharmacology

APOMORPHINE (APM) induces stereotyped behavior in rodents [1]. Evidence suggests that APM produces its behavioral effects by a direct action on central dopaminergic receptors [2, 3, 4]. Since antipsychotic agents such as phenothiazine and butyrophenone derivatives block APM-induced stereotypy, this behavior has been used for screening new compounds with neuroleptic properties.

Recent research indicates that responses to a variety of drugs follow circadian rhythms [11,12]. This study was performed to clarify the existence of circadian variations of APM-induced stereotypy.

METHOD

Male Wistar rats, weighing 150–170 g, were housed four per cage in a light-controlled room (lights on from 7:00–19:00) at a temperature of 24 ± 1°C and a humidity of 60% with food and water ad lib for three weeks. Apomorphine hydrochloride (Merck) was used. Groups of 10 rats each were intraperitoneally given 1 mg/kg APM on 10/29/77 to 10/30/77. Other groups of 8 rats each were intraperitoneally given 3 mg/kg APM on 2/7/78 to 2/8/78. Each group was injected at one of six times: 9:00, 13:00, 17:00, 21:00, 1:00 and 5:00. APM was dissolved in saline just before administration. Each rat was rated for the degree of stereotypy on 0-to-4 scale based on 30-sec observation every 10 min for a period of 1 hr after injection [13]. Rating was as follows: 0=behavior was the same as saline-treated rats; 1=compulsive sniffing; 2=licking the floor or walls of the cage at least once during the observation period; 3=biting the cage wires at least once during the observation period; 4=compulsive continuous biting. The whole observation was done in an adjacent room with exactly the same physical conditions.

Since the experiments for 1 mg/kg and 3 mg/kg dosage were performed on the different date, these two experiments were separately analyzed by analysis of variance. The total scores (sum of the six scores obtained from the 1-hr observation period) of each group were used for the statistical analysis.

To get the best fitted single cosine curve for estimating the rhythm characteristics of APM-induced stereotypy the following model was used [7].

$$y_i = M + A \cos(w \cdot t_i + \phi) + e_i, \quad (i = 1, \ldots\ldots n)$$

where y_i is considered as a single observation at time t_i and n is the number of observations. The model includes the mesor (M), amplitude (A), acrophase (ϕ) and the fixed angular velosity (w) as other components. The e_i are errors associated with the measurement for unknown or unevaluated reasons. In these calculations $w = 360°/\tau$, where τ is a fixed period assumed a priori to characterize the data. In this study $\tau = 24$ hr; hence $w = 15°$/hr. Parameters such as M, A and ϕ at a fixed period of 24 hr were estimated by the least squares method. Then statistical significance of the amplitude was tested under the assumption that errors follow a so-called normal distribution.

RESULTS

A significant time-of-day effect was found for APM-induced stereotypy, $F(5,64) = 2.375$, $p < 0.05$ for 1 mg/kg and $F(5,50) = 2.411$, $p < 0.05$ for 3 mg/kg. Mean total scores at two 9:00 treatments were not significantly different for both 1 mg/kg and 3 mg/kg dosage, although the variability in response between these two treatments was not small for 1 mg/kg dosage which was the lowest completely effective dosage to produce this behavior. The highest stereotypic score was found following injection at 13:00 with 1 mg/kg and at 17:00 with 3 mg/kg (Table 1). The lowest score was following injection at 5:00 with both 1 mg/kg and 3 mg/kg (Table 1).

The circadian rhythm of APM-induced stereotypy was

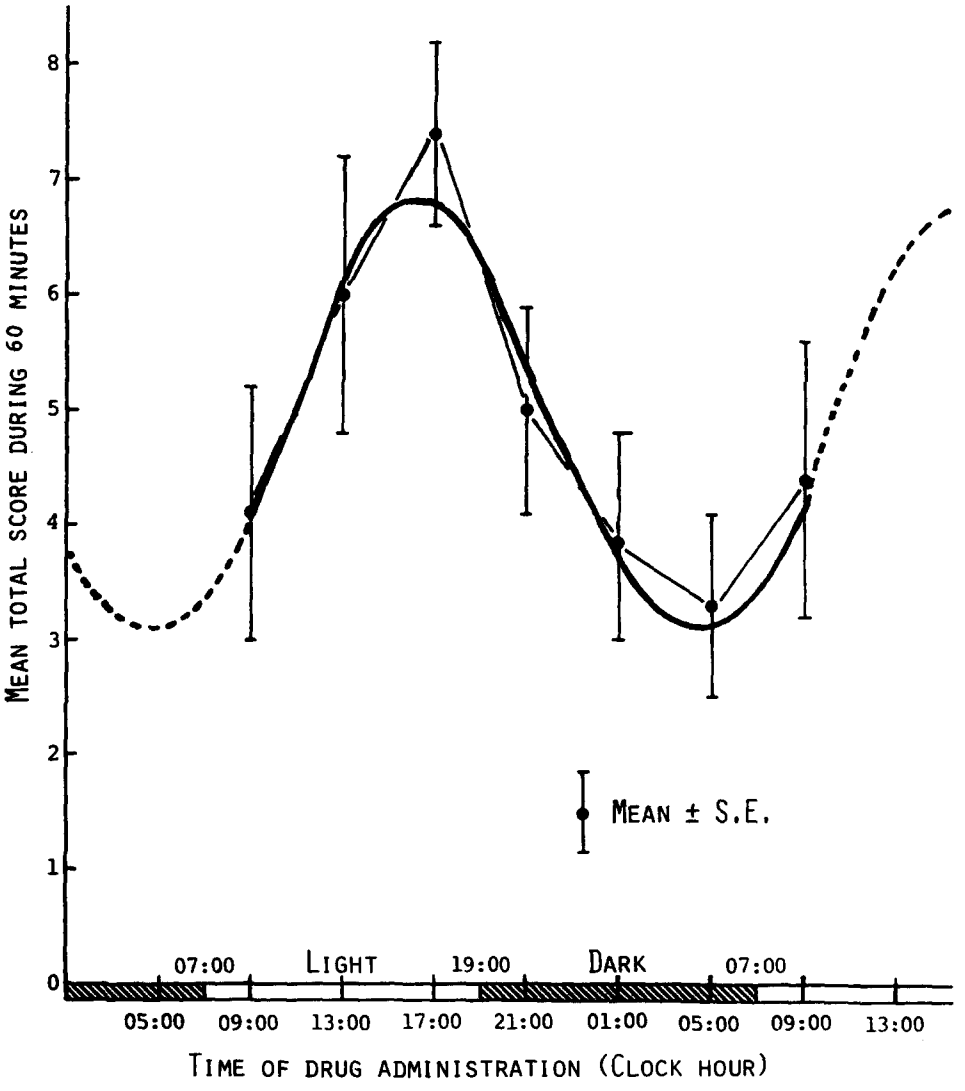

FIG. 1. Circadian rhythm of APM-induced stereotypy (3 mg/kg, IP) and the best fitted single cosine curve calculated by the least squares method. The best fitted single cosine curve is $y_i=5.0+1.9 \cos(15°/hr \cdot t_i - 244°)$.

significantly fitted to a single cosine curve with a 24-hr cycle using the least squares method. The best fitted single cosine curves for 1 mg/kg and 3 mg/kg dosage were $y_i=4.1+1.5 \cos(15°/hr \cdot t_i - 221°)$, $F(2,67)=5.33$, $p<0.01$, and $y_i=5.0+1.9 \cos(15°/hr \cdot t_i - 244°)$, $F(2,53)=3.82$, $p<0.05$, respectively, where y_i is considered as a single observation at time t_i (hours form 00:00) (Table 1, Fig. 1). These two curves were similar in their acrophases from one another.

DISCUSSION

The circadian susceptibility rhythm to APM has been recently reported in rats using artificially reversed light-dark cycle schedule (lights on from 19:30 to 7:30) [9]. Although Nagayama and his coworkers observed APM-induced stereotypy using a different scoring method four times a day, instead of six times in this study, this behavior was pronounced during the light phase and least observable in the dark phase, regardless of the amount of APM administered (0.6 mg/kg to 80 mg/kg). Our data confirm their results. The light-dark cycle seems to entrain the rhythm of this behavior.

A circadian rhythm of drug effects often shows a single cosine wave. The circadian rhythm of APM-induced stereotypy was found to fit significantly to a single cosine curve with a 24-hr cycle using the least squares method. The acrophases for 1 mg/kg and 3 mg/kg dosage were −221° (14:43) and −244° (16:15), respectively. Therefore, the peak is in the latter half of light phase (L-6 hr to L-10 hr) and the trough is in the latter half of dark phase (D-6 to D-10 hr).

The effect of drugs is considered to be influenced not only by the sensitivity of the receptors, but also by the pharmacokinetic aspects of drugs. It has been demonstrated in rats and mice that the activities of the hepatic drug-metabolizing enzymes which are responsible for the inactivation of hexobarbital, imipramine, aminopyrine, exhibit a circadian rhythm [5, 6, 10]. APM is also mainly metabolized

TABLE 1

MEAN TOTAL SCORE (± SE) OF APOMORPHINE-INDUCED STEREOTYPY

Time of injection	Dose of apomorphine: 1 mg/kg, IP (N=10 for each group)	3 mg/kg, IP (N=8 for each group)
09:00	5.1 ± 1.5	4.1 ± 1.1
13:00	6.3 ± 1.2	6.0 ± 1.2
17:00	4.7 ± 0.8	7.4 ± 0.8
21:00	4.2 ± 0.6	5.0 ± 0.9
01:00	3.3 ± 0.4	3.9 ± 0.9
05:00	2.1 ± 0.6	3.3 ± 0.8
09:00	3.1 ± 0.9	4.4 ± 1.2
Statistical significance by analysis of variance	$F(5,64)=2.375$ $0.01<p<0.05$	$F(5,50)=2.411$ $0.01<p<0.05$
Single cosine curve ($y_t=M^* + A\dagger \cos(\omega\ddagger \cdot ti + \phi\S)$) fitted by the least squares method	$M=4.1$ $A=1.5$ $\omega=15°/hr$ $\phi=-221°$ $p<0.01$	$M=5.0$ $A=1.9$ $\omega=15°/hr$ $\phi=-244°$ $0.01<p<0.05$

*M=mesor, †A=amplitude, ‡ω=fixed angular velocity, §ϕ=acrophase (degrees from 00:00).

by the hepatic drug-metabolizing enzymes in rats. Therefore, the circadian rhythm of APM-induced stereotypy might be due to the circadian influence of pharmacokinetics. The circadian susceptibility rhythm to APM in the rat brain has also been suggested to be present [9]. However, the underlying mechanism of its circadian rhythm is not clearly known, since the methods used at the present for measuring APM have a limitation in selectivity and/or sensitivity for pharmacokinetic studies of APM [15].

The circadian rhythm of APM-induced stereotypy demonstrated in the present study does not correlate with daily fluctuation in brain dopamine levels in rats. The pattern of daily fluctuation in brain dopamine has been reported to be "ultradian" rather than circadian [14]. Further studies should be done to clarify this mechanism measuring APM concentrations in the brain and in the plasma using more specific and sensitive analytical technique than the methods used at the present [15].

APM-induced stereotypy is a complex behavior. The various elements of stereotyped behavior may be different entities and have been recently suggested to be elicited from various parts of the brain [8]. If so, such scoring system as used in the present study might be too arbitrary. However, the results shown in this study are still basically important, since a good dose-response curve for APM can be demonstrated using this scoring system.

Thus the existence of circadian rhythm of APM-induced stereotypy might necessitate choice of an appropriate time for studies with APM in rats, although the characteristics of this rhythm should be more precisely defined in the future.

REFERENCES

1. Ernst, A. M. Relationship between the action of dopamine and apomorphine and their o-methylated derivatives upon the CNS. *Psychopharmacologia* **7**: 391–399, 1965.
2. Ernst, A. M. and P. G. Smelik. Site of action of dopamine and apomorphine on compulsive gnawing behavior in rats. *Experimentia* **22**: 837–838, 1966.
3. Ernst, A. M. Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. *Psychopharmacologia* **10**: 316–323, 1967.
4. Fuxe, K. and U. Ungerstedt. Histochemical, biochemical and functional studies on central monoamine neurons after acute and chronic amphetamine administration. In: *Amphetamines and Related Compounds*, edited by E. Costa and S. Garrattini. New York: Raven Press, 1970, pp. 257–288.
5. Holcslaw, T. L., T. S. Miya and W. S. Bousquet. Circadian rhythms in drug action and drug metabolism in the mouse. *J. Pharmac. exp. Ther.* **195**: 320–332, 1975.
6. Jori, A., D. Salle and V. Santini. Daily rhythmic variation and liver drug metabolism in rats. *Biochem. Pharmacol.* **20**: 2965–2969, 1971.
7. Koukkari, W. L., S. H. Duke, F. Halberg and J.-K. Lee. Circadian rhythmic leaflet movements: student exercise in chronobiology. *Chronobiologia* **1**: 281–302, 1974.
8. Melzacka, M., G. Wiszniowska and J. Vetulani. The distribution of apomorphine in rat brain: Possible behavioral correlates. *Pol. J. Pharmac. Pharm.* **30**: 335–345, 1978.
9. Nagayama, H., A. Takagi, E. Nakamura, H. Yoshida and R. Takahashi. Circadian susceptibility rhythm to apomorphine in the brain. *Commun. Psychopharmacology* **2**: 301–310, 1978.
10. Radzialowski, F. M. and W. F. Bousquet. Daily rhtymic variation in hepatic drug metabolism in the rat and mouse. *J. Pharmac. exp. Ther.* **163**: 229–238, 1968.
11. Reinberg, A. and F. Halberg. Circadian chronopharmacology. *Ann. Rev. Pharmac.* **11**: 455–492, 1971.
12. Reinberg, A. Chronopharmacology in man. In: *Chronobiological Aspects of Endocrinology*, edited by J. Aschoff, F. Ceresa and F. Halberg. Stuttgart/New York: Schattauer-Verlag, 1974, pp. 305–337.
13. Rotrosen, J., B. M. Angrist, M. B. Wallach and S. Gershon. Absence of serotonergic influence on apomorphine-induced stereotypy. *Eur. J. Pharmac.* **20**: 133–135, 1972.
14. Scheving, L. E., W. H. Harrison, P. Gordon and J. E. Pauly. Daily fluctuation (circadian and ultradian) in biogenic amines of the rat brain. *Am. J. Physiol.* **214**: 166–173, 1968.
15. Watanabe, H., S. Nakano and N. Ogawa. Determination of apomorphine in plasma by selected ion monitoring. *Biomed. Mass Spectrom.* In press.