Circadian Variations in Anesthetic Requirement and Toxicity in Rats

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The effects of circadian rhythm on cyclopropane and halothane requirements (MAC) and cyclopropane toxicity have been investigated at four-hour intervals in rats synchronized to a standard 24-hour day. Longitudinal and transverse determinations in four groups of animals showed characteristic circadian patterns, with the highest values occurring in the early dark (active) period and lowest values occurring in the early light (inactive) period. Differences in MAC were significant ($P < 0.05$) for each agent, with maximal changes showing 10 to 14 per cent variation from mean values. Circadian variation in cyclopropane toxicity (apneic concentration) was not found. However, calculations of the anesthetic index showed a cyclic pattern similar to that observed for MAC. (Key words: Circadian rhythms; Anesthetic requirement; Anesthetic toxicity; minimum alveolar concentration.)

CENTRAL NERVOUS SYSTEM responses of experimental animals to depressant drugs,[1,2] tranquilizers [3] and local anesthetics [4] vary independent of drug dosage. These responses are rhythmic and are closely related to the cyclic periods of light and darkness in the day. Biological phenomena that have this 24-hour rhythmicity have been designated "circadian rhythms" by Halberg *et al.*[5]

In 1964, Matthews, Marte and Halberg [6] reported that the lethal dose of halothane showed cyclic variation in mice, and stressed the importance of biorhythmicity in the evaluation of drug toxic–therapeutic relationships.[7] Since there have been no reports of circadian effects on anesthetic requirement, we have determined the minimum alveolar concentrations (MAC) of cyclopropane and halothane in rats synchronized to a standard 24-hour day and have analyzed these data for cyclic variation. In addition, the apneic concentration of cyclopropane was determined to evaluate the impact of circadian rhythmicity on toxic–therapeutic relationships.

Methods

One hundred sixteen adult male Sprague-Dawley rats (mean weight ± SD: 400 ± 40 g) were studied in four groups. Animals were housed in groups of two or four in isolation chambers in which environmental influences were rigidly controlled. The housing units (17 cu ft), maintained in an air-conditioned laboratory, were shock-mounted and relatively soundproof. Lighting (two 40-watt Ken. Rad. F40WW lamps) were automatically regulated to produce equivalent periods of light and darkness. For Groups I, II, and IV the light period was from 0800 to 2000 hours. For Group III the light period was from 2000 to 0800 hours. The chambers were entered daily at 0800 for animal feeding and cage maintenance. Purina Lab Chow and water were available *ad libitum*. Environmental synchronization ranged from four to six weeks prior to each study. All experiments were performed between April and August 1969.

Animals in Group I ($N = 70$) were randomly divided into seven equal subgroups ($N = 10$) and studied at different phases of the photoperiod. Measurements of basal metabolic rate (BMR) were made in five animals of each subgroup prior to induction of anesthesia utilizing an open-circuit apparatus.[8] Colonic temperatures of all animals were measured by means of telethermistor probes and recorded.

The minimum alveolar concentration (MAC) [9] of cyclopropane was determined by a previously-described method.[10] Cyclopropane–oxygen mixtures were derived from calibrated flowmeters. Alveolar cyclopropane concentration was calculated from inspired values cor-

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TABLE 1. Cyclopropane Dose–Response Data, Group I (N = 70)

	Light Phase			Dark Phase			
	0800 hours	1200 hours	1600 hours	2000 hours	2400 hours	0400 hours	0800 hours
Per cent of rats moving in response to tail clamp at an inspired concentration of							
22.0 per cent cyclopropane	0	0	20	0	10	20	0
20.5 per cent cyclopropane	10	10	30	30	20	20	0
19.0 per cent cyclopropane	30	40	50	40	30	20	20
17.5 per cent cyclopropane	40	70	90	50	60	50	50
16.0 per cent cyclopropane	70	90	90	90	80	70	60
14.5 per cent cyclopropane	80	90	100	100	100	90	80
MAC (per cent)	16.0	17.0	18.4	17.4	17.0	16.7	15.9
Range representing 1 SD (per cent)	13.6–18.8	15.0–19.5	16.1–20.9	15.1–20.0	14.7–19.6	13.9–20.0	13.3–18.8
BMR (l/m²/hr)	—	7.51 ± 0.67	7.05 ± 0.76†	8.73 ± 1.34	8.01 ± 0.90	8.27 ± 1.45	7.93 ± 0.7
Temperature (C)							
Control	38.6 ± 0.2	38.5 ± 0.4	38.5 ± 0.4	39.1 ± 0.3	38.9 ± 0.1	38.7 ± 0.1	38.3 ± 0.5
Anesthetic	—	37.4 ± 0.5	37.9 ± 0.7	37.2 ± 0.9	37.3 ± 0.7	36.9 ± 0.9	36.2 ± 0.7

rected for dilution by water vapor at body temperature. Gases were delivered at high flows (more than 2.5 l/min) into a small transparent chamber (0.2 l) which covered the rat's head and chest. Anesthesia was induced by subjecting each rat to inhalation of 28 per cent cyclopropane for ten minutes. The concentration was then reduced to 22 per cent for another six minutes. The tail was then clamped and the response noted. The procedure was repeated, reducing the concentration by 1.5 volumes per cent at six-minute intervals, until all animals responded with movement. MAC is defined as the concentration of cyclopropane which prevented movement in response to tail-clamping in 50 per cent of the rats.

Following determination of MAC, and after

TABLE 2. Cyclopropane Dose–Response Data, Group II (N = 15)

	Light Phase			Dark Phase		
	0800 hours	1200 hours	1600 hours	2000 hours	2400 hours	0400 hours
Per cent of rats moving in response to tail clamp at an inspired concentration of						
23.5 per cent cyclopropane	—	—	—	—	—	13
22.0 per cent cyclopropane	7	27	27	20	27	27
20.5 per cent cyclopropane	40	40	53	47	47	33
19.0 per cent cyclopropane	53	60	67	67	73	67
17.5 per cent cyclopropane	87	73	87	93	87	93
16.0 per cent cyclopropane	93	93	100	100	100	100
MAC (per cent)	18.2	18.6	19.2	20.0†	19.2	19.1
Range representing 1 SD (per cent)	16.3–20.2	16.0–21.7	16.7–22.0	17.7–21.7	16.5–21.7	17.0–21.4
Temperature (C)						
Control	38.4 ± 0.6*	38.3 ± 0.4	38.0 ± 0.5	38.7 ± 0.3	38.8 ± 0.4	38.6 ± 0.5
Anesthetic	37.9 ± 0.5*	38.0 ± 0.5	37.5 ± 1.1	38.5 ± 0.3	38.4 ± 0.6	38.4 ± 0.5

* $P < 0.05$.

† Average of four values.

TABLE 3. Cyclopropane Dose–Response Data, Group III (N = 15)

	Dark Phase			Light Phase		
	0800 hours	1200 hours	1600 hours	2000 hours	2400 hours	0400 hours
Per cent of rats moving in response to tail clamp at an inspired concentration of						
22.0 per cent cyclopropane	27	20	13	13	7	13
20.5 per cent cyclopropane	47	40	40	40	33	40
19.0 per cent cyclopropane	73	73	67	60	53	67
17.5 per cent cyclopropane	87	100	87	93	60	93
16.0 per cent cyclopropane	100	—	100	100	80	100
MAC (per cent)	19.2	19.0	18.6	18.6	17.4	18.7
Range representing 1 SD (per cent)	16.9–21.8	16.4–21.4	16.8–20.6	16.6–20.8	15.0–20.4	17.2–20.5
Temperature (C)						
Control	38.2 ± 0.5	38.6 ± 0.4	39.1 ± 0.6	38.2 ± 0.4	38.3 ± 0.5	37.7 ± 0.5
Anesthetic	38.2 ± 0.7	38.4 ± 0.4	39.2 ± 0.5	37.9 ± 0.6	37.7 ± 0.5	37.3 ± 0.9

the rat had recovered from anesthesia, the apneic concentration of cyclopropane was determined. After induction of anesthesia with 28 per cent cyclopropane for ten minutes, the concentration was increased from 34 to 42 per cent in 2-volumes-per-cent increments at six-minute intervals until apnea occurred. Calculation of alveolar cyclopropane was similar to that of MAC. Oxygen concentration was maintained at 50 per cent by the addition of nitrogen to the cyclopropane–oxygen mixtures.

Groups II and III (N = 15) were studied as single groups, with measurements at 0400, 0800, 1200, 1600 and 2400 hours, but in a random sequence with recovery between determinations. Control (colonic) temperature of each rat in these groups was obtained within the first minute following induction of

TABLE 4. Halothane Dose–Response Data, Group IV (N = 16)

	Light Phase			Dark Phase		
	0800 hours	1200 hours	1600 hours	2000 hours	2400 hours	0400 hours
Per cent of rats moving in response to tail clamp at an inspired concentration of						
1.75 per cent halothane	0	0	6	25	6	18
1.50 per cent halothane	44	31	38	56	56	38
1.25 per cent halothane	63	56	63	88	81	88
1.00 per cent halothane	88	94	100	100	94	100
MAC (per cent)	1.33	1.26	1.29	1.45	1.33	1.39
Range representing 1 SD (per cent)	0.98–1.80	1.03–1.53	0.97–1.71	1.21–1.75	1.10–1.61	1.18–1.64
Temperature (C)						
Control	38.4 ± 0.3	38.2 ± 0.5*	38.1 ± 0.5	38.1 ± 0.4	38.5 ± 0.5	38.3 ± 0.3
Anesthetic	38.4 ± 0.8	38.9 ± 0.8*	38.4 ± 0.5	38.3 ± 0.6	38.4 ± 0.8	38.9 ± 0.8

* $P < 0.05$.

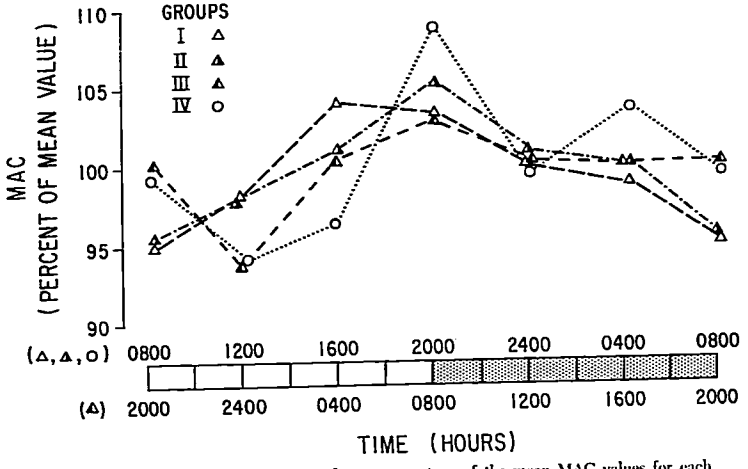

FIG. 1. Data from tables 1–4 expressed as a percentage of the mean MAC values for each group. Group I data have been corrected to account for the effect of temperatures on MAC (see text). No correction was made for the other groups. Significant differences ($P < 0.05$) between the high and low values in Groups I, II and IV were found. Note that the photoperiod for Group III was reversed, but that the phase relationship between the light–dark cycle and MAC was the same as that found in the other groups.

anesthesia. Complete recovery between anesthetic exposures was assured by determining MAC at two- and three-day intervals. Single determinations of the apneic concentration were also made in Groups II and III at the times when MAC values were lowest and highest.

Group IV ($N = 16$) animals were studied in a similar manner to determine halothane requirement over a 24-hour period. However, to permit recovery from anesthesia and and the re-establishment of circadian rhythms, halothane MAC determinations were made at weekly intervals. The technique of halothane administration was similar to that for cyclopropane. Halothane concentration was measured with an ultraviolet monitor which had been calibrated against an infrared halothane analyzer.[11] Anesthesia was induced by subjecting the rat to inhalation of 3.0 per cent halothane for ten minutes, after which the concentration was reduced to 2.25 per cent for another ten minutes. The concentration was then reduced in 0.25-per cent decrements at ten-minute intervals until all rats responded with movement to tail-clamp stimulation.

Data from all experiments were analyzed by plotting the percentage of animals responding, that is, exhibiting movement or apnea, on the ordinate (probit transformation) and the anesthetic concentration (log scale) on the abscissa. This method provides a linear representation of the normally sigmoid-shaped dose–response curve, thereby allowing estimation of MAC or apneic concentration (ED_{50}), the range of one standard deviation ($ED_{16\text{-}84}$), and the slope sensitivity of the response curve.[12] Zero and 100 per cent responses were not used in the calculations of MAC. However, in order to have at least three points in the toxicity calculations, the zero and the 100 per cent responses were plotted at one and 99 per cent, respectively (table 5). Significant differences between paired and unpaired groups were determined utilizing student's t test.

Results

Cyclopropane and Halothane Requirements

Dose–response MAC data for all groups are shown in tables 1 to 4. Characteristic circa-

dian patterns were observed in all groups. The highest values occurred at, or just prior to, initiation of the dark (active) phase and the lowest values occurred during the first half of the light (inactive) phase. Differences between the high and low values were significant ($P < 0.05$) for Groups I, II and IV. Figure 1 shows the MAC data for each group expressed as percentage of mean for all groups. Values in Group I have been corrected to account for the effect of temperature using an independently-determined correction factor of 3.3 per cent reduction in MAC per degree C decrease (unpublished data). Temperature corrections were not required for the other groups. The average variation in cyclopropane MAC was 10 per cent. The difference in the halothane group tested was of a similar magnitude, amounting to 14 per cent. Control temperatures in all groups showed typical circadian patterns for rats,[12-15] with the highest values occurring in the dark (active) phase (fig. 2). Temperatures of anesthetized rats were less predictable, probably due to variations in the duration of anesthesia and, therefore, the amount of cooling. Variations in Group I were similar to variations in temperature (table 1) and also correlated with the observed patterns of physical activity.

Cyclopropane Toxicity

Apneic concentrations of cyclopropane (Group I) showed no significant variation throughout the 24-hour period (table 5). Values at all time periods ranged from 99 to 101 per cent of the mean value. Single values of the apneic concentrations measured in Groups II and III at 0800 and 1600 hours, to correspond with the low and high MAC values in Group I, also were not different (35.5 *vs.* 35.8 per cent cyclopropane). Calculation of the anesthetic index, that is, the apneic concentration divided by MAC at each time period, showed circadian variation, ranging from 92 to 108 per cent of the mean value. The lowest values occurred in the late light (inactive) period and the highest values corresponded to the late dark (active) periods.

Discussion

The calculation of alveolar cyclopropane and halothane concentrations based on inspired anesthetic concentrations was considered valid for the determination of MAC for

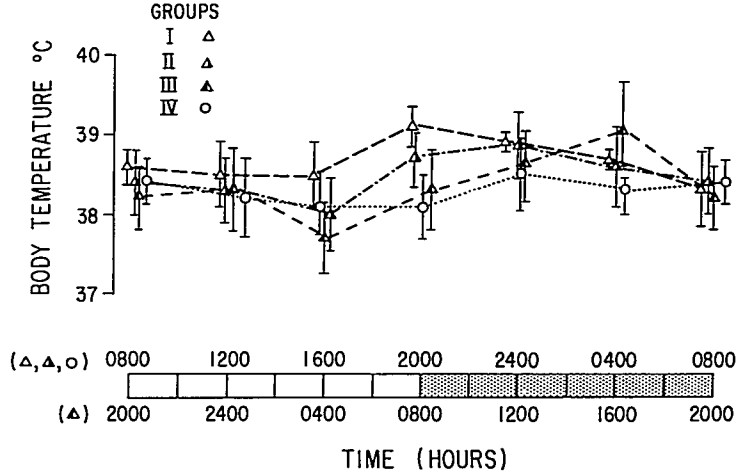

Fig. 2. Mean (±SD) body temperatures of all groups. Note that the highest values occurred during the dark (active) phase. Although the light–dark cycle was reversed in Group III, a normal phase relationship between light–dark cycle and body temperature was found.

the following reasons. At the time of painful stimulus, the otherwise-possible failure of the alveolar (arterial) concentration (partial pressure) to attain equilibrium with the inspired concentration was avoided by approaching alveolar concentration from a higher level and, in the case of cyclopropane, using a relatively high concentration (28 per cent), which minimized the effect of uptake on reducing alveolar concentration.[16] Furthermore, the effect of cyclopropane on reducing alveolar concentration is small after ten minutes. This interval was exceeded at least two or three times prior to determination of MAC.

Halothane, which is more soluble than cyclopropane, might be expected to maintain a significant alveolar-to-inspired difference for a relatively longer interval. However, the work of Salanitre and Rackow [17] shows that the approach of the alveolar (expired) halothane concentration to the inspired level is more rapid in the infant than in the adult. In studies of infants, when halothane was inspired at constant concentration, 80 per cent equilibration was reached in 30 to 40 minutes. This rapid approach of the alveolar concentration toward the inspired concentration is believed to result from the proportionately greater ventilation and cardiac output (per unit mass) possessed by the smaller and more metabolically active organism. Anesthetic uptake in the rat probably occurs at a rate even more rapid than that in the infant, since in rats cardiac output values are 15–20-fold greater on a weight basis than in adult man.[18] Finally, the rectilinear dose–response relationships, with lack of skewness, obtained in our study, suggest that equilibrium between inspired and alveolar anesthetic partial pressures did exist at the time of tail-clamp stimulation. The necessity of progressing from a lower to a higher concentration of cyclopropane for the determination of apneic concentrations obviously precluded the use of the method described for the MAC determinations. Failure of the alveolar concentration to reach equilibrium with the inspired cyclopropane concentration conceivably might result in an overestimation of alveolar cyclopropane concentration. However, any error in the estimation of apneic concentration would be minimized by the relatively narrow range of cyclopropane concentrations at which apneic response occurred. Furthermore, any error would be common to all groups, which would tend to preserve the relative differences between groups.

Our results show that MAC is significantly influenced by circadian rhythmicity. The failure to observe a statistically significant variation in cyclopropane toxicity (apnea) appears to be at variance with the variations in halothane susceptibility (death) reported by Matthews *et al.*[6,7] This discrepancy may be related to the different criteria used for the definition of toxicity. Another explanation may be related to differences in the uptake and distribution of the two agents when administered at constant inspired concentration. During halothane anesthesia, the alveolar concentration may be markedly altered by changes in ventilation and circulation.[19,20] An insoluble anesthetic, such as cyclopropane, produces a level of anesthesia that is relatively stable in the presence of such changes, particularly with the technique used in the present study. These factors may be important in considerations of drug kinetics, particularly since circadian variations in arterial blood pressure and peripheral resistance are well documented.[21,22] Variations in drug effects, therefore, may be related to different levels of anesthesia rather than altered sensitivity to the anesthetic drug *per se*. We tested this hypothesis by determining the percentages of animals made apneic when cyclopropane (40 per cent) and halothane (5 per cent) were administered at constant inspired concentrations. Measurements made at times when MAC was lowest and highest showed 40 to 50 per cent variation in the numbers of animals that became apneic with each agent. These results are similar to those reported by Matthews.

In considering possible mechanisms for the variations in anesthetic requirement found in this study, it is useful to consider the phase relationships between MAC and other physiologic cycles. Both the phase (as related to the photoperiod) and the magnitude of our temperature and metabolic activity values agree with those of others.[13–15] Similar physiologic cycles have been observed in man.[23] However, in man temperature and oxygen uptake, as well as other respiratory,[24] thermoregulatory [25] and cardiovascular [21,22] cycles, generally reach peak amplitudes late in the

TABLE 5. Cyclopropane Dose–Response Data, Group I (N = 70)

	Light Phase			Dark Phase			
	0800 hours	1200 hours	1600 hours	2000 hours	2400 hours	0400 hours	0800 hours
Per cent of rats apneic at an inspired concentration of							
36.0 per cent cyclopropane	10	10	10	0	10	0	0
38.0 per cent cyclopropane	60	60	50	40	20	10	30
40.0 per cent cyclopropane	90	100	80	100	80	80	80
42.0 per cent cyclopropane	100	—	100	—	90	100	100
Apneic concentration (per cent)	35.5	35.1	35.8	35.8	36.5	35.7	36.5
Range representing 1 SD (per cent)	33.9–37.3	33.7–36.4	34.0–37.6	34.8–36.7	34.4–38.8	35.5–38.1	25.1–37.9
Temperature (C) Anesthetic	—	37.2 ± 0.4	37.8 ± 0.3	37.2 ± 0.6	37.3 ± 0.6	37.0 ± 0.7	36.8 ± 0.6
Apneic concentration/MAC	1.08	0.98	0.92	0.97	1.01	1.05	1.08

active (light) period. In the nocturnal rat these peaks commonly appear at the beginning of the active (dark) period. Therefore, in comparing rat and human circadian variations the physiologic cycle of temperature, rather than the photoperiod, which is the presumed synchronizer, is usually used as a reference frame for phase comparison. The maximal values for MAC in the present study show good correlation with the time of greatest physical and metabolic activity.

Cyclic changes in MAC also correlate with central nervous system activity. Heninger *et al.*[26] showed circadian variation in cerebral evoked responses as well as in electroencephalographic activity. Similar variations in central nervous system activity have been shown for seizure thresholds in rodents.[27–29] Another variable which appears to be in phase with anesthetic sensitivity is that of catecholamine excretion in man [30, 31] and brain tissue levels in rats.[32] Norepinephrine cycles also may be important as related to the observations of Miller *et al.*,[33] who showed a 30 per cent reduction in anesthetic requirement following depletion or inhibition of cerebral nervous system norepinephrine levels. The norepinephrine cycle is also dependent, at least in part, on the sleep–wakefulness cycle.[34] A further relationship between circadian variations in humoral influences and changes in MAC that we observed is that of serum corticosterone levels in mice.[1] Although a similar phase synchronization does not of itself prove a causal relationship, Selye's findings of an anesthetic inhibiting effect of spironolactone [35] and the similar phase relationships of ethanol toxicity [1] and duration of pentobarbital anesthesia [2, 36] make these observations provocative.

The 10–14 per cent circadian variation in anesthetic requirement may not appear to be great enough to warrant serious consideration. Recalling, however, that the well-known 2 per cent circadian rhythm in deep body temperature appears to be a reflection of a rhythm in peripheral arterial blood flow 20 times as great in amplitude,[22, 25] it is prudent to consider these cyclic phenomena in the evaluation of therapeutic–toxic relationships as well as in the conduct of physiologic experimentation. We believe that temporal influences should be considered a "fourth dimension" to the usual pharmacologic triad of dose, stimulus and response.

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