Circadian Rhythms: Variation in Sensitivity of Isolated Rat Atria to Acetylcholine

Abstract: *Experiments were performed at two time points in the 24-hour cycle to determine whether isolated right atria of rats would vary in sensitivity, with a circadian rhythm, to two concentrations of acetylcholine. The beating rate of the atria decreased more in response to the drug if the atria were isolated at 1100 than if isolated at 2300 hours.*

Recently, several workers have reported circadian variations in mammalian susceptibility to toxic doses of such agents as ethanol, SU-4885, and x-irradiation (*1*). In addition, Tharp and Folk (*2*) have shown that the beating rates in isolated rat hearts vary with a circadian rhythm. The present study was initiated to determine if the spontaneously beating isolated right atrium of the rat would show differences in sensitivity to drugs, depending on the time of day the atrium was isolated. Acetylcholine was chosen as the test drug. Two times were chosen, one in the middle of the light part and the other in the middle of the dark part of a 24-hour cycle.

Charles River male albino rats weighing between 250 and 600 g were used. The rats had continuous access to Purina rat chow and water. They were kept in a soundproof, lightproof room which was maintained at a temperature of 24°C. A 12-hour light–12-hour dark schedule was maintained, the light beginning at 0600, and the dark at 1800 hours. This schedule was continued for at least 1 month before the experiments were begun. Three sets of experiments were completed; one in October, one in November, and one in May. Experiments were performed on eight rats at a time, from 1100 to 1300, and from 2300 to 0100. The animals were anesthetized with ether, the thorax was opened, and the heart was removed intact. The ventricular tissue was trimmed away and the left atrium removed. Silk thread was tied in a small loop to the interatrial tissue, and a longer piece was tied to the auricular appendage of the right atrium. The loop served to fasten the atrium in a glass chamber. The longer thread was tied to a strain gauge. An ink-writing recorder was used to display records of force and rate. The atria were then immersed in Krebs-Henseleit bicarbonate solution (*p*H 7.4) at 37° ± 0.2°C, and were allowed to equilibrate for 30 minutes. Acetylcholine, available in sealed ampules of 100 mg, was dissolved in Krebs-Henseleit solution and diluted immediately for use. Acetylcholine was added in 1-ml volumes to 120 ml of Krebs-Henseleit solution in the chambers to produce final concentrations of 1 and 10 μg/ml. The force and rate were recorded continuously.

The data were subjected to an analysis of variance which indicated that the 1100 and 2300 values were significantly different at $p < 0.01$. To determine which of the values were significantly different, t tests were performed. Table 1 shows the results.

The control rates were not significantly different ($p > 0.2$), a result which was somewhat surprising, since the heart rates in the living animal are different in the light and dark parts of the 24-hour period (*3*). The percentage decrease in rate at both concentrations of acetylcholine was greater if the atria were isolated at 1100 than if isolated at 2300. The 50 percent effective concentration, determined graphically, for the atria isolated at 2300 is 1.6 times greater than for those isolated at 1100 (5.7 μg/ml and 3.6 μg/ml, respectively). A similar trend was noted for the force measurements, but since the rate grossly affects this measurement, the results for the force are not reliable, show large variations, and do not show statistical significance.

It would appear that the isolated atrium of the rat possesses a controlling mechanism for the change in sensitivity to acetylcholine. This sensitivity appears to vary, being greater if the rats have been in light for 5 hours than if they have been in the dark for 5 hours. Thus, if an extremely accurate measurement of such a preparation is desired, the time of day at which the experiment is performed is of importance.

It is also possible that the rate changes seen in isolated hearts are due to differences in the sensitivity of the heart or of the pacemaker to endogenous rate-controlling agents. The mechanisms involved are as yet unknown. The acetylcholinesterase activity of the atria isolated at similar times is currently being studied. Experiments using adrenergic drugs as test agents are planned.

Table 1. Mean rates (beats/min) of atria isolated at 1100 and at 2300 hours. The light cycle began at 0600 and the dark cycle began at 1800 (N = 214 for each group).

Acetylcholine concentration	Mean rate ± S.E.		Rate difference	t	p
	Atria isolated at 2300	Atria isolated at 1100			
Control	273.6 ± 2.1	270.3 ± 2.0	3.3	1.25	> 0.2
1.0 μg/ml	202.0 ± 4.4	187.1 ± 5.1	14.9	2.20	< 0.05
10.0 μg/ml	113.7 ± 6.2	94.0 ± 6.1	19.7	2.26	< 0.05

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References and Notes

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Color Vision in the Adult Female Two-Spotted Spider Mite

Abstract. *Responses of the summer form of the adult female two-spotted spider mite,* Tetranychus urticae *Koch (Acarina: Tetranychoidea) placed in near-ultraviolet and green light are photopositive. The independent variation of these responses requires the presence of separate receptor systems.*

The summer form of the adult female two-spotted spider mite (*Tetranychus urticae* K.) responded positively to white light; variations of this response, particularly with changes in humidity, were used as the basis for definition of sedentary and dispersal phases within a population (*1*). Mites were presented with bands of the spectrum from 350 to 700 mμ at 25-mμ increments, the bands being equal in energy and spectral distribution. A curve (*2*) illustrating the mites' behavioral response as