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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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

a function of the wavelength presented showed that most photopositive responses occurred at 375 mμ, a large number of photopositive responses being stimulated by a broad range of wavelengths centered around 525 mμ. We have investigated the dynamic range of the behavioral response (that is, the range of intensities within which an increase causes a corresponding increase in the behavioral response) in the near-ultraviolet (UV) and green light.

The mites were reared on lima beans in continuous illumination (cool, white fluorescent) at 28°C and 60 percent relative humidity (*4*). They were tested at 25° ± 1°C and 18 ± 5 percent relative humidity (except as noted) under a 20-watt red fluorescent light, to which they were indifferent (*2*). The mites were kept in the test room 1 to 2 hours before the behavioral test. A flat-black test arena (30 by 14.5 cm) with a central zero area (2.5 cm) was used. A diverging light source (18°) was presented at an angle of incidence of 15°, which intersected the test area in a parabola. The behavioral response was scored as a response index (RI).

$$RI = \frac{(\text{Positive mites}) - (\text{Negative mites})}{(\text{Positive} + \text{negative} + \text{zero mites})} \times 100$$

After 1 minute, the mites in the area toward the light were scored as positive, the mites in the central area were scored as zero, and the mites away from the light were scored as negative. This measure, giving essentially the same information as the more complex tracking of individual mites (*2*), was used since vector information was not required in this study. In some tests wavelengths of 380 mμ and 530 mμ were added with a half-mirror and were presented at 15°. By presenting, along a common axis, opposing lights at 15° to the test area we measured the effect of these wavelengths when subtracted. Light intensity was measured at the center of the zero area with a radiometer (Yellow Springs Instruments). We nulled the dark current to zero using a Wratten 88A infrared pass filter in series with the filter used.

In the first tests, the mites were collected from newly infested plants and were used without any preselection. The mites responded randomly to the following spectral regions at intensities up to 1 mw/cm²: violet, Wratten 36 plus 2A; blue, Wratten 45; red, Klett 680 mμ; near-infrared, Wratten 88A; and green, Wratten 40 (3 mw/cm²). Only the blue region of the near-UV (Wratten 39) stimulated an increased response as its intensity was increased. Using low passfilters, we found the stimulating region of the spectral region passed by Wratten 39 (down 2 optical density units at 310 mμ and 480 mμ) to be between 360 and 400 mμ. This finding agrees with the peak response found at 375 mμ (*2*) and indicates the absence of response in the near-UV below 360 mμ. Responses of mites subjected to green light, however, were either photopositive ("green +") or photonegative ("green −"). Mites were separated into these two classes by being placed in the center of an eliptical area (15 by 70 cm, outlined with Tanglefoot) illuminated from the end at 15° with green light (Wratten 40, intensity 0.3 mw/cm²). After 1 hour the mites were piled up at each end ("green −" and "green +"). We tested them for photoresponse, using these narrow-band filters: one with a maximum wavelength of 380 mμ, 50 percent transmission, 40 mμ bandwidth; and one with a maximum wavelength of 530 mμ, 50 percent transmission, 40 mμ bandwidth.

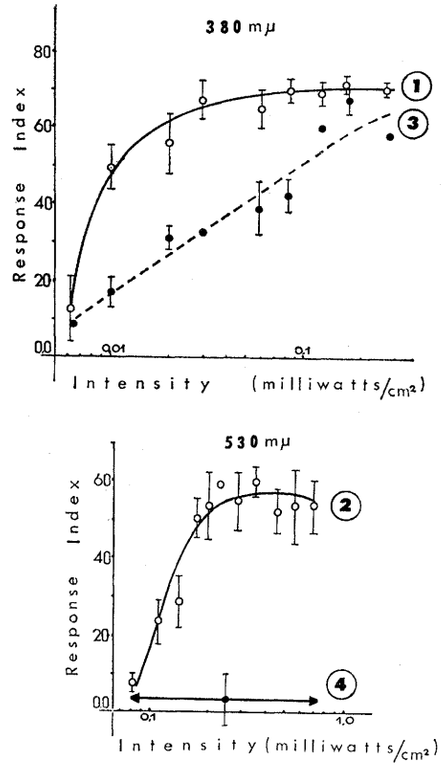

Fig. 1. Changes in behavioral response (RI) with increasing intensity at 380 mμ and 530 mμ. Curves 1 and 2, obtained with mites giving photopositive responses to green light; curves 3 and 4, obtained with those giving photopositive responses to green light. Each curve is based on the photo response of 3000 animals. The small bars represent two standard deviations.

The "green +" mites subjected to light of both 380 mμ and 530 mμ had a steep threshold of response with a dynamic range limited to a half log cycle of increasing intensity (Fig. 1). The group of "green −" mites at 380 mμ reached the same response index as the "green +" fraction, but its response curve was linear and its dynamic range covered 1.5 log cycles. The "green −" mites showed a random response at 530 mμ with an average response index close to zero. When light of wavelengths of 530 mμ and 380 mμ was added at intensities below their saturation levels, only the "green +" mites showed a summation of response, whereas the "green −" mites showed their normal response to the 380 mμ component. When the effects of 530 mμ and 380 mμ were subtracted with opposing lights at their saturation values (380 mμ, 0.1 mw/cm²; 530 mμ, 0.3 mw/cm²) the "green +" mites gave a response index of zero while the group of "green −" mites showed its normal response to 380 mμ. As previously mentioned (*1*), the response to white light of unselected mites varied with ambient humidity. When mites were held at a relative humidity of 55 percent for 1 hour and then tested at the same humidity, the response of the "green +" mites decreased to zero at 530 mμ while their response at 380 mμ was essentially unchanged.

Our results demonstrate that the responses of this animal to green and to UV light are independent and require separate receptor systems. Spectral sensitivity in the green region, around 500 mμ, is common in arthropods. Insects also see in the near-UV, but the maximum sensitivity of insects is to light with wavelengths of 340 to 350 mμ (*5*). Anatomical and behavioral studies indicate that the front pair of eyes, which contain photoreceptors for UV and green light, are scanning point-detectors and that the rear pair of eyes, which contain a receptor for UV light, are omnidirectional.

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Cyanoacetylene in Prebiotic Synthesis

Abstract. *Cyanoacetylene is a major nitrogen-containing product of the action of an electric discharge on a mixture of methane and nitrogen. It reacts with simple inorganic substances in aqueous solution to give products including aspartic acid, asparagine, and cytosine.*

In the synthesis of purines from hydrogen cyanide (*1*) we have shown that 4-aminoimidazole-5-carbonitrile (I) is an intermediate (*2*). This led us to consider β-aminoacrylonitrile (II), and hence cyanoacetylene, as possible prebiotic precursors of the pyrimidines (Fig. 1). The prebiotic synthesis of pyrimidines from β-aminoacrylonitrile has previously been suggested by Oro (*3*). We now report on the synthesis of cyanoacetylene in an electric discharge and on its conversion to biologically important products.

We have studied the effects of an electric discharge on a number of gases and gas mixtures including hydrogen cyanide, cyanogen and acetylene, hydrogen cyanide and acetylene, and methane and nitrogen. In these cases, cyanoacetylene was detected in appreciable amounts; in fact, it was always the most abundant volatile nitrogen-containing product other than hydrogen cyanide. The sparking of a mixture of methane and ammonia resulted in an efficient conversion to hydrogen cyanide. Cyanoacetylene was never present in more than trace amounts; however, it would have been destroyed rapidly in the presence of ammonia. We now describe our experimental work only for the methane-nitrogen system, which we believe to be interesting in the context of prebiotic synthesis.

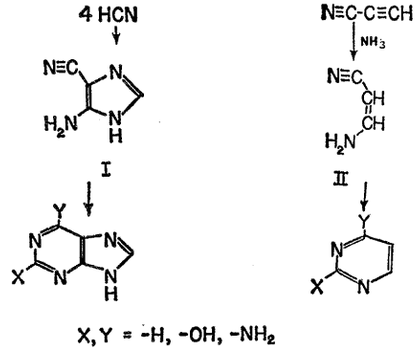

Fig. 1. Proposed pathways for purine and pyrimidine synthesis.

In a typical experiment, a round-bottom flask was charged to atmospheric pressure with 20 cm^3 of methane and 88 cm^3 of nitrogen (each > 99.9 percent pure). A spark was generated across a tungsten electrode gap of 5 mm with an Electro-technic BD-10 high-frequency generator (tesla coil) operating at maximum output. Periodic analyses were made by gas chromatography at 30°C on a 3.6-m column of 1,2,3,-tris(2-cyanoethoxy) propane on Gas-Chrom Q, with a flame-ionization detector.

Gaseous products and their retention times (minutes) were: methane (1.5), acetylene (1.6), various hydrocarbons (2 to 4), diacetylene (6.2), cyanoacetylene (10.4), hydrogen cyanide (12.4), and benzene (15.1). The cyanoacetylene concentration was maximum after about 25 minutes, when its integrated response was 8.4 percent that of the hydrogen cyanide. The cyanoacetylene slowly decreased thereafter, whereas the hydrogen cyanide concentration eventually doubled to an integrated response which was 42 percent that of the original methane.

The identities of the various products were established by comparison of retention times with those of authentic samples, and by collection of the compounds in the individual peaks and comparison of their infrared and ultraviolet spectra with those of authentic samples. The ultraviolet spectrum of cyanoacetylene is highly characteristic and leaves no doubt concerning the identity of this material.

We have not attempted to study the mechanism of cyanoacetylene synthesis. However, it has been shown that this compound is produced by the action of active nitrogen on acetylene (*4*).

Aspartic acid was one of the amino acids obtained by Miller in his classic experiments on the synthesis of amino acid precursors from mixtures of methane, ammonia, and water in an electric discharge (*5*). It was suggested that the aspartic acid was formed by the action of ammonia and hydrogen cyanide on acrolein. However, it seemed possible to us that after part of the ammonia had decomposed to nitrogen, some or all of this aspartic acid might have been formed in the following sequence of reactions.

$$CH_4 + N_2 \xrightarrow{\text{spark}} H\text{-}C{\equiv}CC{\equiv}N$$

$$HC{\equiv}CC{\equiv}N + NH_3 \rightarrow NH_2CH{=}CHCN$$

$$NH_2CH{=}CHCN + CN^- \xrightarrow{H^+} CNCHCH_2CN \quad (NH_2)$$

$$NCCHCH_2 \ (NH_2) \xrightarrow{\text{hydrolysis}} \text{asparagine}$$

$$\xrightarrow{\text{hydrolysis}} \text{aspartic acid}$$

We have now confirmed that a mixture of 0.1*M* cyanoacetylene, 5.0*M* ammonia, and 1*M* HCN heated at 100°C for 24 hours yields aspartic acid (10 to 15 percent) and asparagine (5 to 10 percent).

A number of pyrimidine syntheses starting with propiolic acid or β-aminocrotonic acid derivatives have been described (*6*). It was not surprising, therefore, that under severe conditions we obtained cytosine by fusing cyanoacetylene with urea in a sealed tube; uracil was obtained similarly from propiolamide. Our attempts to obtain cytosine under more plausible prebiotic conditions have met with some success.

When an aqueous solution containing 1.0*M* potassium cyanate and 0.1*M* cyanoacetylene was heated to 100°C for 1 day, a 5 percent yield of cytosine was obtained. When the same mixture was allowed to stand at room temperature for 7 days, cytosine was obtained in 1 percent yield. The cytosine was identified chromatographically by direct comparison in three solvent systems. Furthermore the ultraviolet spectrum of the eluted cytosine was identi-