The Effects of Constant Light and Light Pulses on the Circadian Rhythm in the Eye of Aplysia

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Received December 6, 1973

Summary. 1. A circadian rhythm in the frequency and amplitude of compound action potential (CAP) from the isolated eye of Aplysia persists for a week or more in vitro in constant darkness. This rhythm has a period of about 26 hours (Figs. 1, 2) in a specific culture medium and may express several periodic amplitude components (Fig. 3).

2. Constant light (LL) of low intensity shortens the period (Fig. 4) and reduces the range of oscillations. Higher intensity LL results in a further reduction in range, a greater variability of CAP frequency from hour to hour, alterations in the period, and possibly rhythm splits (Fig. 5).

3. Pulses of light given at specific points in the circadian cycle shift the phase of the rhythm (Fig. 7). The resulting phase response curve (Fig. 8) is similar to response curves for the activity of diurnal animals and potassium pulses on the eye rhythm.

Introduction

The brain has been shown to be important in the entrainment of some circadian rhythms to light-dark cycles (Menaker, 1968; Adler, 1970) and in some instances the brain is clearly shown to be the receptor for the zeitgeber stimulus and the site of the circadian clock that controls rhythmic functions (Truman and Riddiford, 1970; Truman and Sokolove, 1972). Another brain structure, the eye of Aplysia, has an inherent circadian rhythmicity in spontaneous dark activity (Jacklet, 1969a). The eye rhythm can be phase shifted by light and entrained by light-dark cycles in vitro (Jacklet, 1971; Eskin, 1971). Thus, under normal circumstances this eye serves the animal as a very sensitive detector of light, as a receptor for the zeitgeber stimulus and as a source of circadian timing information. The further characterization of the responses of the eye rhythm to light is of interest for several reasons: (1) the responses are important in determining if this rhythm in vitro is comparable to other rhythms studied in whole animals, (2) the responses might indicate something about the mechanism of the rhythm, (3) a phase response
curve for light pulses is needed to compare to the phase response curve for potassium pulses (Eskin, 1972) to investigate the idea that depolarization of the membrane is a natural mechanism for phase shifting the rhythm. Accordingly, the responses of the eye rhythm in vitro to constant light and constant darkness interrupted by light pulses were studied.

Methods

*Aplysia californica* were obtained from Pacific Bio-Marine (Venice, Ca.) and kept in LD 12:12 in Instant Ocean tanks (15°C) prior to experimentation. A preparation of the eye and attached optic nerve was dissected free of the animal and placed in 100 ml of culture medium regulated at 15°C for long term continuous recording of the optic nerve activity. The optic nerve was drawn into a tubing electrode (PE 10 or 20) in the recording chamber and the electrical activity was led off via a silver chloride/silver wire, amplified with a Tektronix 122 and recorded on a Grass polygraph. The recording chamber was housed in a light-tight box so that the eyes could be maintained at constant temperature in constant darkness or constant light from an incandescent illuminator. Phase response curves were generated by interrupting constant darkness with 1 hour pulses of light at appropriate phases of the rhythms. The culture medium consisted of 80% artificial sea water (ASW), 10% *Aplysia* blood and 10% nutrient mixture. The ASW composition in millimoles/liter was: NaCl, 425; KCl, 10; CaCl₂, 10; MgCl₂, 22; MgSO₄, 26; NaHCO₃, 2.5. The *Aplysia* blood has a similar ionic composition to sea water (Hayes and Pelluet, 1947) and was filtered (0.22 μm millipore) before it was added to the medium. Twenty-five ml of nutrient mixture contained: 2.5 ml of MEM vitamin solution (100×, Gibco), 2.5 g dextrose (Baker), 5 ml MEM amino acids (50×, Gibco), 2.5 ml non-essential amino acids (100×, Gibco), 2.5 ml L-glutamine (200 mM, Gibco), 2.5 ml penicillin-streptomycin solution (10000 units, Gibco), 6.7 ml 7.5% NaHCO₃, 2.7 ml 0.5 N NaOH and ASW. The final culture medium was filtered (0.22 μm, millipore) and the pH was adjusted to 7.8. This culture medium is modified from the medium of Strumwasser and Bahr (1966).

Results

*Circadian Rhythm in DD*

The eye is spontaneously active in constant darkness (DD). This activity is recorded in the optic nerve as a triphasic compound action potential (CAP) that is naturally synchronized by mechanism (electroconic coupling) inherent to the eye (Jacklet, 1973). The CAP frequency varies with a circadian rhythm that is precise. The period of the rhythm varies (Jacklet, 1971) according to the medium composition, it is 22–24 hours in ASW alone and 26–27 hours in the culture medium used in these experiments. The rhythm persists quite clearly in culture for a week or longer as shown in Fig. 1. The temporal characteristics of the periodic frequency changes are: a rapid increase at onset, a sustained rate for about 10 hours and then a gradual decrease. During the week several changes occur in the rhythm: the period usually lengthens by 5% or so, the duration of the active phase lengthens with a corresponding decrease