Circadian Rhythmicity in Excised Samanea Pulvini

I. SUCROSE-WHITE LIGHT INTERACTIONS

ESTHER SIMON,2 RUTH L. SATTER, AND ARTHUR W. GALSTON
Department of Biology, Yale University, New Haven, Connecticut 06520

Received for publication March 8, 1976 and in revised form June 4, 1976

ABSTRACT

The rhythmic movement of excised Samanea saman pulvini incubated in H2O or 50 mm sucrose was monitored during extended periods of white light (cool white fluorescent, 2,000 ft-c) darkness, or alternating white light (16 hr) and darkness (8 hr). In continuous white light, the rhythm damps at an intermediate angle after only one cycle, whether pulvini are incubated in sucrose or H2O. The rhythm also damps after the first cycle when darkened pulvini are incubated in H2O, but it persists for several cycles if sucrose is available. Sucrose depresses the mesor (average angle) during extended dark periods in Samanea, as in Albizzia julibrissin, but it increases the mesor if supplied during white-light-dark cycles. With the latter irradiation schedule, oscillations persist for several cycles whether pulvini are supplied with H2O or sucrose, but closure is incomplete when pulvini are incubated in sucrose.

When Samanea saman plants grow in their native tropical habitat, leaflets are horizontal (open) in daylight and vertical (folded together, or closed) during the night. Leaflets also open and close with a circadian periodicity in constant darkness, providing evidence for an endogenous circadian oscillator (13). The leaflet movement rhythm in Samanea, as in the related plant Albizzia julibrissin, is controlled by differential turgor changes in pulvinal motor cells, in turn regulated by migration of K+ and Cl- ions (16, 18-20) possibly related to changes in transmembrane potential (15). Samanea is a particularly useful plant for studies of light-rhythmic interactions, since each pulvini is an autonomous system whose rhythm and photoregulated turgor changes persist after the pulvinus has been excised and laminar tissue removed (18, 19). The photoreceptors and the oscillating system, including the clock and the ions that regulate motor cell turgor and pulvinar movements, must be located in the pulvinus or attached rachilla section.

In Samanea, as in Albizzia (17) and other plants (4, 6), the amplitude of the oscillations damps rapidly during DD unless sucrose or other suitable carbohydrate is available. This paper reports the effect of sucrose on rhythmicity in excised Samanea pulvini. Experiments were conducted during LD and LL as well as DD, to provide information on rhythmic behavior under both natural and free running conditions.

1 This work was supported by a grant from the Spanish Ministerio de Educaci6n y Ciencia to E. S. and by National Science Foundation Grant BMS74-24269 to R. L. S. and A. W. G.
2 Present address: Departamento de Fisiologia Vegetal, Facultad de Ciencias, Universidad de Barcelona, Barcelona, Spain.
3 Abbreviations: DD: continuous darkness; LD: 16-hr white light-8-hr dark cycles; LL: continuous white light.

MATERIALS AND METHODS

Samanea saman (Jacq.) Merrill4 plants grown from seed in the greenhouse, were transferred to controlled chambers at least 1 week before experimental use. The chambers provide a 16-hr photoperiod (about 2,000 ft-c cool white fluorescent light with about 10% incandescent supplement) at 24 C ± 2 C, and relative humidity of 60% ± 10%. These chambers and light sources were used for experiments in LL and LD. The beginning of the dark period (DD = 0) coincided with the regular "lights off" signal; thus, continuous darkness represents an extension of the usual 8-hr dark period. Experiment was run for 120 to 150 hr. All measurements and experimental manipulations during the dark periods were done under a dim green safelight.

Secondary pulvini from the third to eighth youngest fully expanded leaves were used in all experiments. The rachis, cut transversely 1.5 cm below pulvini to separate the paired pinnae from the plant, was then split along its longitudinal axis to separate the two paired pulvini, which became experimental and control partners. Laminar tissue was removed, but a small (2.5 cm) section of rachilla was left attached to the pulvinus for immersion in water or 50 mM sucrose (see reference 18 for further details). Solutions were changed daily to minimize bacterial contamination. Six to eight pairs of pulvinus were used for each treatment. Angles between the rachis and rachilla were estimated at 2-hr intervals by comparison with angles on a chart.

Preliminary experiments, conducted to determine the effect of cutting, revealed that leaflets tend to close for a short time following excision. This causes a slight disturbance in the maximum of the first cycle but it does not affect subsequent behavior. To avoid variability, pinnae were always excised at DD = 2.5 hr.

RESULTS

Figure 1 shows the movement of pulvini supplied with water or sucrose during prolonged DD. The rhythm of pulvini supplied with H2O damps in the open position after two cycles. By contrast, oscillations persist for at least 1 week if sucrose is available, although both the amplitude of the oscillation and the mesor or median angle (11), decrease with time. To determine whether loss of K+ from the pulvinus to the bathing solution might be responsible for such damping, K+ efflux was monitored during 3 days in DD (Table I). T. Three to 5% of pulvini K+ was lost to the solution during the 1st day; this is probably due in part to the effect of excision, since there was only 1 to 2% loss on subsequent days. We were surprised to find more leakage with sucrose than with H2O, although K+ losses in all cases were too small to account for the large decrease in amplitude noted in Figure 1. Damping can be prevented by conversion of Pr to Pfr at an appropriate part of each rhythmic cycle (21).

4 Synonym: Pithecolobium saman (Jacq.) Bentham.
In LL, the rhythm damps at an intermediate angle (50°) after the first cycle, whether pulvini are in H₂O or sucrose (Fig. 2). Rhythms in other organisms are known to damp rapidly in high intensity LL. When pulvini are transferred to DD after 68 hr of LL, the H₂O controls open slightly, but without circadian oscillations, whereas sucrose-treated pulvini are relatively immobile for about 36 hr, then start to oscillate once again. The amplitude of these circadian oscillations is about one-half that of dark controls at 120 to 144 hr (Fig. 1).

In 16-hr L–8-hr D cycles (Fig. 3), pulvini in H₂O oscillate for at least 96 hr with only a small decrease in amplitude; pulvini in sucrose also oscillate, but with a smaller amplitude and larger mesor. Thus, exogenous sucrose interferes with closure during LD, although it promotes closure during DD. The latter generalization holds when pulvini are transferred to DD after 96 hr of LD; those in sucrose continue to oscillate with decreasing mesor, whereas those in H₂O cease oscillating but open slightly.

It makes no difference whether 50 mM sucrose is supplied during the 16-hr light or 8-hr dark portions of LD cycles, even though the duration of exposure to sucrose is twice as high under the former conditions.

Mathematical Analysis. The free running rhythm of pulvini in sucrose during DD was analyzed by computer to determine the harmonic composition of the oscillations. The pattern was corrected to compensate for progressive decrease in the amplitude of the oscillation (damping) prior to power spectrum (Fourier) analysis. Four replicate experiments, each containing eight pulvini, were analyzed; data from two typical experiments appear in Figure 4. The power spectrum has a typical biharmonic composition, with a maximum between 25 and 21 hr and a secondary component at about 4.5 hr. There is also a suggestion of a third peak at about 6 hr.

**DISCUSSION**

The remarkable persistence of oscillations during long dark periods when sucrose is available indicates that excised *Samanea* pulvini should be excellent systems for investigating circadian rhythmicity. However, it is important to recognize that behavior of excised pulvini differs somewhat from that of the intact system.Incomplete closure during the first cycle DD (Fig. 1) is one

<table>
<thead>
<tr>
<th>Incubation Period</th>
<th>H₂O Efflux %</th>
<th>Sucrose Efflux %</th>
</tr>
</thead>
<tbody>
<tr>
<td>hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–24</td>
<td>3.0 ± 1.6</td>
<td>4.3 ± 2.9</td>
</tr>
<tr>
<td>24–48</td>
<td>1.0 ± 0.5</td>
<td>2.0 ± 1.1</td>
</tr>
<tr>
<td>48–72</td>
<td>1.0 ± 0.5</td>
<td>2.1 ± 1.2</td>
</tr>
</tbody>
</table>

*Fig. 1. Oscillations in the angle of *Samanea* pulvini supplied with H₂O or sucrose during DD. Pulvini attached to a small section of rachilla were excised at DD = 2.5 hr.*

*Fig. 2. Oscillations in the angle of excised Samanea pulvini supplied with H₂O or sucrose during 76-hr LL followed by DD.*

Fig. 3. Oscillations in the angle of excised Samanea pulvini supplied with H₂O or sucrose during four 16-hr light–8-hr dark cycles followed by DD, or during seven LD cycles.

Fig. 4. Power spectrum showing the harmonic composition of the pulvinal movement rhythm of excised Samanea pulvini supplied with sucrose during DD. The two graphs represent replicate experiments.

such example, for this effect was not noted in intact plants (7). Such differences might be due to injury effects, to disruption of the usual flow of metabolites between the pulvinus and other parts of the plant, or to the inverted system used in our experiments (the rachilla is in solution and the rachis above it, although this portion of the rachis is basal to the rachilla in the intact plant) (18).

Comparative Analysis of the Effects of Sucrose during DD, LD, and LL. The effect of sucrose in maintaining rhythmicity during DD resembles that already reported for petal and leaf movements (6, 9, 17). Sucrose probably substitutes for a product formed in the light during photosynthesis, and necessary for expression of the rhythm. Sucrose-dependent metabolic events such as synthesis of ATP and organic anions, have been discussed in relation to Albizia experiments (17).

Sucrose inhibits closure in LL or LD, even though it is promotive in DD. However, one must consider that different regulatory systems control leaflet movement in light and in darkness. The phytochrome system regulates leaflet movement during long dark periods (21) but it is not clear whether Pfr also influences leaflet movement in white light. However, at least two pigment systems are known to be involved during the white light period: the photosynthetic system, and a blue absorbing pigment (7) that probably resembles the flavoprotein recently characterized in other systems (3, 12, 14).

Even though sucrose is required for Pfr action during DD, (8) we do not know how it affects action of the blue absorbing pigment. Red and blue light have opposite effects on leaflet movement in Coleus (9); red regulates phase advances, while blue regulates phase delays. Identification of the blue absorbing pigment in Samanea and investigation of its role in circadian rhythmicity might be helpful in analyzing sucrose action in white light.

Other investigators (2, 5) have suggested that oscillations in energy metabolism are an important component of the circadian clock; if so, the combined effect of photosynthetically produced carbohydrates and 50 mm exogenous sucrose might exceed the regulatory capacity of the pulvinal system, since the pulvinus contains a normal complement of chloroplasts. The resumption of oscillations 36 hr after pulvini are transferred from LL to DD (Fig. 2) is consistent with this view, for one would except a gradual attrition of carbohydrate reserves during darkness. An oversupply of sucrose could be deleterious to excised pulvini, since excision disrupts the source-sink relationships that govern sucrose transport in the intact plant (22). The "sucrose oversupply" hypothesis can be tested experimentally by comparing the effects of gradations in light intensity and sucrose concentration.

Externally supplied sucrose, which must cross plasmalemma membranes to enter pulvalinal cells, might have quite different effects than endogenous carbohydrates synthesized in chloroplasts. Arisz (1) reported that both white light and sucrose promote C₁⁻ absorption in Vallisneria leaves but through different mechanisms. A sucrose-dependent cotransport system, similar to that regulating H⁺ uptake in Chlorrella (10), could be involved, and might explain the small increase in ion efflux noted in Table 1.

LITERATURE CITED


