Effect of D_2O on the Circadian Rhythm of Petal Movement of *Kalanchoe*

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Heavy Water, Circadian Rhythm, Petal Movement, *Kalanchoe*

D_2O lengthens the free running period of the circadian petal movement of isolated *Kalanchoe* flowers by 1.6% per 10% heavy water. This corresponds to findings reported in the literature for other organisms. 100% D_2O administered in the form of 4 hour pulses at various phases of the circadian cycle lead to phase responses zero to maximally 1.5 hour delays. No advancing phase shifts occur. Possible ways in which lengthening of the period occurs are discussed.

The slowing down of circadian rhythms by heavy water has been demonstrated in unicellulars, plants and animals and seems to follow the same quantitative relationships (about 2% lengthening of the period per 10% D_2O)[1] (but see also [2]). This feature has been claimed to suggest fundamental similarities of the underlying rhythmic mechanisms. In connection with findings of slowing *rhythms of higher frequencies* with D_2O it has been hypothesized that ionic balances across cellular membranes are influenced and are thus parts of the clock mechanism [1]. Recent findings of ions being involved in circadian movements of plants [3, 4] support this view.

We have studied the influence of D_2O on the *Kalanchoe* petal movement rhythm offered continuously as well as for shorter periods of time. We will discuss different general modes of action of D_2O on circadian rhythms and present an alternative interpretation to that of a direct change of the oscillating system by D_2O leading to a lengthening of the period.

Material and Methods

Isolated flowers of *Kalanchoe blossfeldiana* exhibit a circadian rhythm of opening and closing the petals under conditions of constant temperature and in physiological darkness of weak green light for about one week if mounted in a 0.2 M solution of sucrose. Methods of rearing the plants [6] and details of recording the rhythm have been described elsewhere [7, 8].

Desired concentrations of D_2O [9] were prepared and sucrose added to give the standard 0.2 M solution. For experiments in which D_2O was offered in the form of 4 hour pulses the flowers were transferred with the acrylglass plates in which they were mounted from the recording cuvettes to dishes containing the heavy water/sucrose solution. The times of maximum flower opening were determined from the plotted curves and the distances between the maxima used for the calculations of period lengths. Phase shifts due to D_2O pulses were determined in respect to undisturbed controls. The temperature was maintained at 22.5 ± 0.5 °C.

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Results and Discussion

I. Dependence of the period length on the concentration of D_2O offered continuously

The period length progressively lengthens in a linear manner with increasing concentrations of D_2O (Fig. 1). The regression line has the form

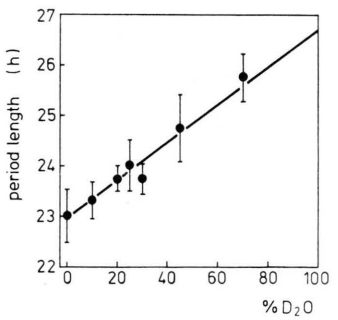

Fig. 1. Dependence of the period length (ordinate) of the *Kalanchoe* petal rhythm on the concentration of D_2O (abscissa). Vertical bars: standard errors.

$y = 22.97 + 0.038\,x$. Thus a 10% increase in the D_2O concentration leads to a 1.6% increase in the period length. At 70% D_2O the period length has reached 25.8 hours. At 100% the period length could not be determined since only one opening

with a low amplitude was observable. At this concentration the flowers showed severe signs of wilting.

II. Effect of 4 hour pulses of D_2O

4 hour pulses of 100% D_2O do not damage the flowers. They lead to phase shifts of the petal rhythm the amount of which depends on the affected phase of the circadian cycle. Fig. 2 shows in the

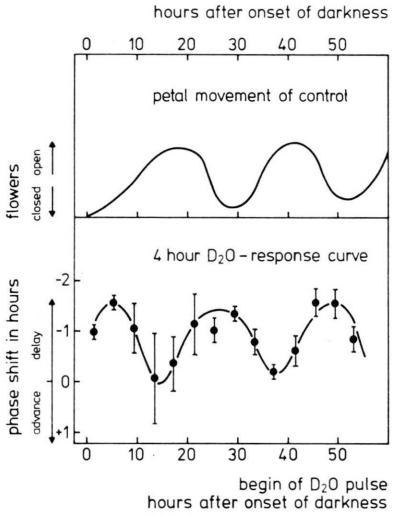

Fig. 2. Upper part: Petal movement of a control flower after having been entrained by a 12:12 hour light-dark-cycle and released into physiological darkness (=weak green light) at time 0. Lower part: Phase response curve of petal rhythm to a 4 hour pulse of 100% D_2O. Abscissa: Onset of D_2O pulse in hours after release into darkness. Ordinate: phase shift due to the D_2O pulse, in hours.

upper part the petal movement of a control flower after release into darkness at time 0. From the lower part of Fig. 2 it can be seen, that D_2O pulses delay the rhythm maximally (about 1.5 hours) at times at which the petals are closed. No phase shift takes place if D_2O pulses are administered during the time of fastest opening of the flowers (15 and 38 hours after onset of darkness). *No advancing phase shifts are observed.* The form of the phase response curve is repeated in later cycles.

For discussions concerning the mechanism of the D_2O action on circadian rhythms see [1, 2]. We would like to discuss the action of D_2O on circadian rhythms in a more formal way to avoid speculations on the mechanism by which D_2O acts. Some of the possible ways of D_2O in retarding circadian rhythms are schematically shown in Fig. 3 (other alternatives, *e. g.* simultaneous actions at several of

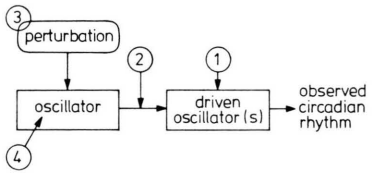

Fig. 3. Schematic representation of the modes of action of D_2O in slowing the circadian rhythm. See text.

the discussed points, are of course conceivable).

1. D_2O could act on a driven oscillation, but the driving oscillation is unaffected by it.

2. D_2O could act on the coupling between the driving and the driven oscillator as has been discussed and compared to a slippery belt action [10].

3. D_2O acts only as a perturbation, and a continuous application of D_2O acts like a continuous repetition of perturbations. The overall effect is thus the sum of all the effects of single perturbations. The amount of phase shift per cycle can be deduced from the response curve towards 4 hour D_2O pulses if the phase shifting action of foregoing D_2O pulses is taken into account. Since 4 hour pulses bring forward delays only, the overall effect would be a general delay which shows up as a lengthening of the period.

4. D_2O acts on an essential part of the oscillator, thus changing the frequency of the oscillation.

Under the assumption that the driving oscillator (④ in Fig. 3) ist the light sensitive oscillator, the modes of action of 1. and 2. can be dismissed: We have found that under D_2O the phase response curves towards light pulses are changed in such a way as one would expect if the light sensitive oscillator has been slowed by the action of heavy water [11]. Proposition 3 (D_2O acts as a perturbation) could be tested in the following way: A 4 hours D_2O pulse is given during the phase of maximum responsiveness of the cycle (see Fig. 2) and repeated in several cycles (the phase shifts induced by the foregoing pulses being taken into account). This should add up to a considerable phase shift after several cycles, whereas the same treatment with daily recurring cycles at minima of responsiveness towards D_2O pulses should hardly shift the rhythm at all.

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[1] J. T. Enright, Z. vergl. Physiol. **72**, 1–16 [1971].
[2] C. S. Pittendrigh, P. C. Caldarola, and E. S. Cosbey, Proc. nat. Acad. Sci. USA **70**, 2037–2041 [1973].
[3] R. L. Satter and A. W. Galston, Bioscience, in press.
[4] W. Engelmann, Z. Naturforsch. **28 c**, 733 [1973].
[5] W. Engelmann and V. Vielhaben, Z. Pflanzenphysiol. **55**, 54–58 [1965].
[6] W. Steinheil, Z. Pflanzenphysiol. **62**, 204–215 [1969].
[7] W. Engelmann, H. G. Karlsson, and A. Johnsson, Intern. J. Chronobiol. **1**, 147–156 [1973].
[8] W. Engelmann, I. Eger, A. Johnsson, and H. G. Karlsson, in preparation.
[9] Roth, Karlsruhe, 99.7 atom%.
[10] H. B. Dawse and J. D. Palmer, Biol. Bull. **143**, 513–524 [1972].
[11] A. Maurer and W. Engelmann, in preparation.