

## A Slowing Down of Circadian Rhythms by Lithium Ions

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(Z. Naturforsch. **28 c**, 733–736 [1973]; received July 16, 1973)

Circadian rhythm, lithium, *Kalanchoe*, *Meriones*, depression

Under the permanent influence of lithium ions the circadian rhythm of movement of *Kalanchoe* petals is effectively lengthened but a pulse administered up to 12 hours has no influence.

Lithium ions could also be demonstrated to similarly slow down the circadian activity rhythm of a small mammal (*Meriones crassus*).

It is hypothesized that the therapeutic effect of lithium salts in endogenous depressions in human beings may result also from its acting on the human circadian system.

Lithium ions are known to affect organisms from the bacterial to the human level of organization. They influence enzymes, cytoplasm, mitochondria, glycolysis and respiration, bioelectric potentials, ciliary and amoeboid movement, and are causative of a number of morphogenetic effects in protozoa, animals and plants<sup>1</sup>.

An additional and hitherto unknown role of the lithium ions in slowing down a circadian rhythm in the petal movements of *Kalanchoe* has been reported recently<sup>2</sup>. A concentration of 1 mM LiCl was demonstrated to lengthen the period by more than one hour. I have extended these experiments systematically to different concentrations of the LiCl solution. The effect of LiCl, administered in the form of pulses has also been studied. Finally this report includes also results of experiments in which Li ions were offered in drinking water to the desert rat *Meriones crassus* to detect possible changes in the period length of the locomotory activity due to Li.

### Methods

Flowers of *Kalanchoe blossfeldiana* (strain Göttingen) were used. Methods of growing plants and inducing them to flower have been described<sup>3</sup>. For the technique of handling flowers and automatically recording the petal movement see Engelmann *et al.*<sup>4,5</sup>. Plants were kept in LD cycles of 12:12 hours<sup>6</sup> and flowers plucked at the end of a light period and mounted. Recording was done under constant conditions of temperature and physiological darkness<sup>7</sup>.

LiCl (Merck) was added to the 0.2 M sucrose solution in the recording cuvettes when offered

throughout the experiment. In pulse experiments the acrylic glass plates holding the flowers were transferred to a petri dish containing the desired concentration of LiCl in 0.2 M sucrose solution. At the end of the exposure excess LiCl was washed off thoroughly before the plates were returned to the recording unit.

The locomotory activity of *Meriones crassus*<sup>8</sup> was measured with the aid of a running wheel cage of 40 cm diameter. Each revolution of the wheel cage closed a microswitch which activated the pen of an event recorder. The recording paper moved at a uniform speed of 48 cm per day. The animals were housed in individual cages during the study and had access to food and nesting material placed in a glass tank connected to the wheel cage through a corridor. 2 mg of LiCl<sup>9</sup> was administered in 10 ml drinking water daily. The recording units were placed in a temperature controlled room with weak continuous red light<sup>10</sup>. The strip containing the activity recording for each day was pasted directly below the strip of the previous day on charts.

### Results

#### *Increase of the period length under the influence of LiCl in Kalanchoe*

In Fig. 1 an example of the petal movement of a single flower in 3 mM LiCl solution is shown in addition to that of a control. The mean period length is 23.6 hours compared to 22.3 hours in the control. This daily gain of 1.3 hours leads to a 6.5 hours shift of the maximum of petal opening in 5 days in respect to the control. In Table I further data are presented for the different cycles and means compiled. The data for the flowers showing maximum lengthening of the period are at the top of the Table.

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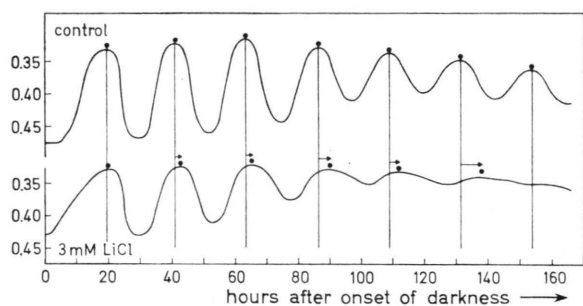


Fig. 1. Circadian petal movement of *Kalanchoe* flowers during 7 days of constant conditions of temperature and physiological darkness (weak green light) under continuous influence of 3 mM LiCl (below) as compared to a control (above). Abscissa: Time in hours after transfer from LD 12 : 12 into DD. Ordinate: Voltage of the Wheatstone bridge as a measure for the degree of opening of the flowers. ● Maximum of flower opening. Vertical lines indicate position of control maxima. Transverse arrows show the shift of the maxima due to the increase in period length (time interval between 2 maxima) by Li.

Table I. Period lengths of 1st to 5th cycle of *Kalanchoe* petal rhythm in 3 mM LiCl (upper part) and control (lower part). Five single flowers were recorded in each case. Mean period length of single flowers is given in the right column. Mean period length of the different cycles are in the lowest row of each part. The difference of periods of different cycles between treated and untreated flowers (indicating the slowing down of the rhythm of treated flowers) are in the lowermost row.

#### 3 mM LiCl, period lengths [hours]

| number of period                  | 1           | 2    | 3    | 4    | 5    | $\bar{\tau}$ |
|-----------------------------------|-------------|------|------|------|------|--------------|
| flower number 1                   | 23          | 25.5 | 24.5 | 25   | 36.5 | 26.9         |
| 2                                 | 26.5        | 27   | 24.5 | 24   | 25   | 25.4         |
| 3                                 | 23          | 26   | 24.5 | 25.5 | 19   | 23.6         |
| 4                                 | 22.5        | 23   | 23.5 | 22.5 | 25   | 23.3         |
| 5                                 | 22.5        | 24   | 22.5 | 24   | 23   | 23.2         |
| average<br>period<br>length/cycle | $\tau_{Li}$ | 23.5 | 25.1 | 23.9 | 24.2 | 24.5         |

#### Controls, period lengths [hours]

| number of period            | 1        | 2    | 3    | 4    | 5    | $\bar{\tau}$ |
|-----------------------------|----------|------|------|------|------|--------------|
| flower number               | 1        | 21.5 | 23.5 | 24.5 | 21.5 | 22.8         |
|                             | 2        | 24.5 | 24.5 | 23   | 19   | 22.6         |
|                             | 3        | 21   | 23   | 22.5 | 23.5 | 22           |
|                             | 4        | 22.5 | 23.5 | 23.5 | 21.5 | 20.5         |
|                             | 5        | 22   | 23   | 23   | 22   | 21           |
| average period length/cycle | $\tau_C$ | 22.3 | 23.5 | 23.3 | 21.5 | 21.4         |
| difference                  |          |      |      |      |      |              |
| $\tau_{Li} - \tau_C$        |          | 1.2  | 1.6  | 0.6  | 2.7  | 4.3          |

Results of experiments using different concentrations of LiCl are shown in Fig. 2. The period length of the controls is indicated by a horizontal line (22.8 hours). At a concentration of 0.1 mM the

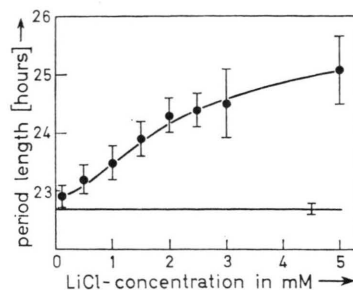


Fig. 2. Dependence of the period length of the *Kalanchoe* petal movement on the concentration of LiCl (abscissa). Period length of the control indicated by a horizontal line. Vertical lines show the standard errors. 5 flowers per group, 15 control flowers.

period length is not affected and no significant difference to the control is found. At the extreme of 5 mM LiCl fewer opening and closing cycles are completed and the flowers are irreversibly damaged and show severe signs of wilting in the course of the experiment.

The increase in period length is clearly owing to the Li ions and not to the Cl ions. This becomes obvious by the fact that other salts of Li ( $\text{Li}_2\text{SO}_4$ ,  $\text{Li}_2\text{CO}_3$ ) lengthen the period as well as LiCl, whereas other chloride salts ( $\text{KCl}$ ,  $\text{MnCl}_2$ ,  $\text{CaCl}_2$ ,  $\text{CoCl}_2$ ) are ineffective even in much higher concentrations.

LiCl given in the form of a pulse rather than permanently does not affect the petal movement: Period length and phase are unaltered as in the control, if concentrations of 5 mM are applied up to 12 hours. Higher concentrations (7.5 mM) are already lethal to most of the flowers, even when given for only 4 hours.

#### Action of Li on other organisms

For reasons presented in the discussion we have also tried to influence the free running period length of the circadian rhythms in mammals through the administration of a daily dose of LiCl in water. Fig. 3 illustrates the activity pattern of a male desert mouse *Meriones crassus* over a period of 48 days under constant temperature of 20 °C and in red light. The two lines connecting the daily onsets of activity *before* and *after* Li administration show a slight change in the period length during the free

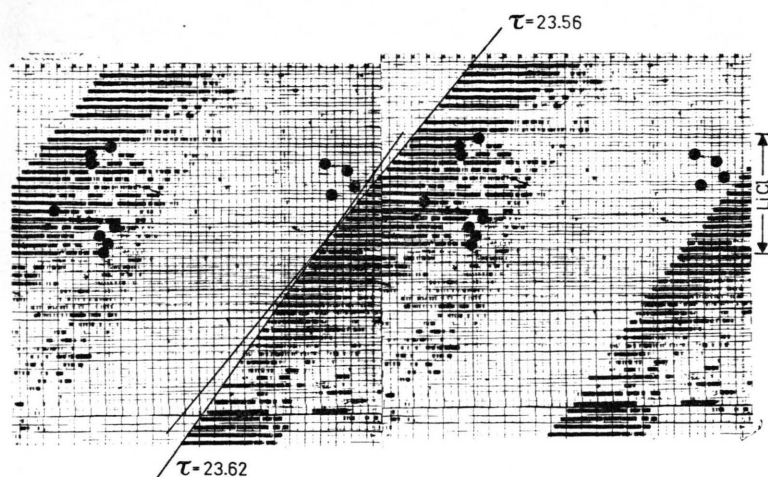


Fig. 3. Actogram of the locomotory activity of a male desert rat (*Meriones crassus*) under constant conditions of temperature (20 °C) and light (weak red). After 8 days of free run a LiCl solution was offered for 15 days. The time of daily administration is indicated by black dots. The recording was continued further for further 23 days without LiCl. The two lines connecting the daily onsets of activity before and after Li administration show slightly different slopes corresponding to a period length  $\tau$  of 23.56 and 23.62 hours respectively.

run. Whereas the period length is 23.56 hours before the treatment, it increases to 23.62 hours as a consequence of the Li treatment.

### Discussion

The present findings clearly indicate, that LiCl added to the sucrose solution, in which flowers of *Kalanchoe* are maintained, effectively increases the period length of the circadian petal movement. From the data in Table I it can, however, be seen, that the variability in the resulting period lengths is quite large, ranging from about 25 hours to 23 hours<sup>11</sup>.

This variability might be due to differences in the uptake of Li in the different flowers, which in turn might depend on transpiration rate, undetected damage to the flowers during the process of plucking them from the plants and other factors.

The period length of the first cycle of Li treated flowers is usually shorter than later ones. This might be due to the time Li needs to enter the system.

A concentration of 0.1 mM LiCl does not seem to have any effect on the period length, as seen from Fig. 2. Even slight effects should have been detectable, since they add up to considerable differences after several cycles. This is not the case. With increasing concentrations of LiCl, however, the period length increases. A concentration of 5 mM LiCl is already lethal, and the flowers close completely, wilt and die after displaying a few oscillations with increased period length and decreasing amplitude.

If pulses of LiCl would have yielded a response curve consisting of phase delays only — as has been found with pulses of heavy water in the *Kalanchoe* petal rhythm<sup>12</sup> — the lengthening of the period by permanently offered Li could have been the result of a continuous action of many pulses. But this is, in view of the results of Li pulse experiments, unlikely.

Another possibility is that Li lengthens the period by introducing some "resistance" between the light sensitive oscillator and driven oscillations, so that the speed of the latter is slowed (kind of slippery belt<sup>13</sup>). This possibility is currently being subjected to experimental verification.

Kandeler<sup>14</sup> reported Li to inhibit flower induction in the long day plant *Lemna gibba* and to promote flower induction in the short day plant *Lemna perpusilla*, both under long day conditions. He attributes this to an action of Li on phytochrome. The present findings of Li acting on the circadian rhythm of *Kalanchoe* might, however, point to another way of interpreting these results: If Li increases the period length of the circadian rhythm, the photoperiodic time measurement, which is done according to Bünning's hypothesis by a circadian rhythm, would change, thus leading to a different critical daylength in *Lemna*.

Li is used in human patients suffering under depressions<sup>15</sup>. Since this mental disorder has been interestingly linked with derangements of circadian rhythms<sup>16</sup>, the bearing of the findings of Li effects on the *Kalanchoe* rhythm might turn out to be of greater importance. A number of working hypo-

theses are currently being discussed and experiments being planned in this regard at the Nervenklinik of Tübingen university and this institute. As a first step in this direction we have started to monitor the circadian activity rhythm of small mammals treated with Li. Although the change in period length in the case shown in Fig. 3 is rather small, it seems to us encouraging to continue this work. Apart from the general implications of Li affecting the circadian rhythm it may prove useful

in the understanding and therapy of depressions in humans.

I thank Dr. M. K. Chandrashekar, Dr. A. Johnsson, Dr. H. G. Karlsson, and Dr. A. T. Winfree for critically reading the manuscript and suggesting improvements. This work has been supported by the Schwerpunktprogramm "Biologie der Zeitmessung", Deutsche Forschungsgemeinschaft (En 42/14).

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<sup>6</sup> Abbreviations used: L, light; D, darkness; LD 12 : 12 hours, cycles of alternating 12 hours light and 12 hours darkness.

<sup>7</sup>  $22.5 \pm 0.5$  °C, weak green light of 20 lux.

<sup>8</sup> Kindly provided by Dr. E. Kulzer.

<sup>9</sup> In the last 4 days of treatment 4 mg of LiCl daily.

<sup>10</sup>  $20.0 \pm 0.5$  °C, weak red light 660 nm, intensity at the level of the animals  $15 \text{ erg cm}^{-2} \text{ sec}^{-1}$ .

<sup>11</sup> Neglecting the unusually long last period of flower number 1 (LiCl).

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