Effects of Lithium on Stomatal Regulation

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Recent findings indicate that lithium belongs to the group of rather exclusive agents which affect the period of circadian clocks: The period of the circadian petal movements of *Kalanchoë* is increased by about 2 hours in 5 mM LiCl [1, 2]. In the present paper we will report an action of lithium on a much more rapid oscillation, which under certain circumstances occurs in stomatal regulation. These oscillations are found to be remarkably slowed down by lithium, in analogy to the circadian oscillations of *Kalanchoë*.

Material and Methods

Cultivation of seedlings, *Avena sativa* (c. v. Blenda), followed a program described elsewhere [3]. In the experiments the transpiration rate of a single eight days old plant kept in a cuvette was measured. Stabilized carrier gas (air; 20% RH, 26 °C) passed the leaf (1.5 cm/s) and the relative humidity of the carrier gas leaving the cuvette was calibrated to the transpiration rate of the plant (mg water per hour). An oscillatory stomatal regulation appears under suitable circumstances which causes transpiratory oscillations as shown in Fig. 1 A [3–6]. The recordings were made in constant light intensity (white light, LUMA 250 W P8; 2000 lx) and temperature (26 °C), and the period of the oscillations was about 25 min.

Lithium was given to both oscillatory *intact* plants and oscillatory *excised* plants (without root system). Sustained stomatal oscillations can be maintained in excised plants if the xylem resistance to water flow is increased by an external physical pressure on the leaf base [4]. Lithium in the medium around the plant stem will then enter the xylem vessels at the site of the excision and the transpiration stream will bring the lithium ions to the stomatal regions. Lithium solutions were used as a medium either for a short time ("Li^+-pulse") or continuously ("Li^+-step").

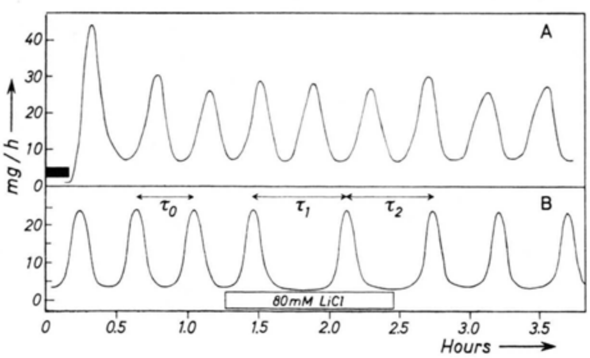

Fig. 1. A. Transpiratory oscillations of an oat plant with excised root system and deformed xylem vessels. Oscillations are initiated by a dark pulse (black area). B. Transpiratory oscillations of a plant treated as in A, but given 80 mM LiCl during the period indicated. The normal period length of the oscillations, τ_0, is increased after the application of lithium. τ_1 and τ_2 indicate the period length of the first and second cycle after the lithium treatment.

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Results

Fig. 1 A shows the normal oscillatory transpiration of an excised plant exposed to xylem deformation. The transpiratory water stream originates from a vessel containing distilled water. Figure 1 B shows results from an experiment in which the distilled water was substituted by 80 mM LiCl-solution for ≈ 1 hour (Li^+-pulse). This caused an immediate and very pronounced period lenghtening; τ_1 as defined in the Figure is roughly 40 min, τ_2 36 min and τ_0 25 min.

Data on the effects of *Li^+-steps* are compiled in the Table. τ_1 and τ_2 denote the period length of the first and second cycle after the Li^+-step. All Li^+-steps are administered starting when the transpiratory oscillation is at a minimum.

Table. Period lengths of transpiratory oscillations of plants under different treatments. For definition of τ_0, τ_1, τ_2 see Figure 1 B. Number of plants investigated: 5 when 40 mM LiCl, KCl or NaCl was used, 2–3 in the other cases.

	Excised plant		Intact plant	
Treatment	τ_1/τ_0	τ_2/τ_0	τ_1/τ_0	τ_2/τ_0
80 mM LiCl	1.6	1.5	—	—
40 mM LiCl	1.4	1.6	1.0	1.0
20 mM LiCl	1.2	1.2	—	—
10 mM LiCl	1.0	1.0	—	—
40 mM $LiNO_3$	1.4	1.7	—	—
80 mM KCl	1.1	1.1	—	—
40 mM KCl	1.0	1.0	1.0	1.0
40 mM NaCl	1.0	1.0	1.0	1.0
Control	1.0	1.0	1.0	1.0

Li⁺-steps given to excised plants

LiCl gives a concentration dependent increase of the period length. The weaker the solution is, the longer time is required to cause a certain lengthening of the period time. This lengthening can be interpreted as caused by an accumulation of lithium in the stomatal regions during the Li^+-steps.

The action on the amplitude of the oscillations is negligible during the cycles tabulated (*i.e.* the change in the period length is not due to a coupling between frequency and amplitude in the non-linear stomatal control system, *cf.* discussion in ref. 7). However, after a certain time – dependent on the strength of the lithium solution – the oscillations are mostly damped out.

The lengthening of the period is due to the lithium ions and not to the chloride ions. $LiNO_3$ gives the same effects as LiCl. KCl and NaCl do not cause period changes even at high concentrations.

Li^+-steps given to intact plants

Lithium does not affect the period length or the amplitude of the transpiratory oscillations if given in the root medium to an intact plant, see Table. (The same is valid for KCl and NaCl.)

Li^+-pulses given to excised plants

Pulses of lithium ions were given to oscillatory, excised, plants. The experiments showed that the lithium effects at lower concentrations are reversible: The period returned to its value previous to the pulse. In an experiment with a 25 min pulse of 40 mM LiCl subsequent period lengths were typically as follows: 26, 25, 27, 38, 31, 27, 28, 28 min. (The Li^+-pulse was applied during the 3rd cycle.)

Discussion

The method of xylem deformation, used to maintain oscillations of the stomata of excised plants, was a prerequisite for the present experiments. Lithium ions can not (or only very slowly) penetrate the root barrier system of intact plants and, therefore, it is difficult to study lithium effects on stomatal dynamics of intact plants.

Lithium ions change the curve shape of the stomatal oscillations in a characteristic way: The phase between two transpiratory peaks is prolonged while the transpiratory peaks themselves do not change their shape (see Fig. 1 B). This means that the stomata need a longer time to start the opening when lithium is present but once opening has begun, the opening and the subsequent closing movements are fairly independent of the lithium concentration (at least when concentrations are not too high).

Lithium is known to act on bioelectric potentials in plants and animals, on enzyme reactions, on respiration etc [8]. It causes irreversible changes when added for longer periods to muscles but it does not decrease the excitability in shorter experiments [8]. Often lithium and sodium ions are reported to show similar effects: They are both inhibitors of certain potassium activated enzyme reactions (*e.g.* [9, 10]), they give rise to an appreciable membrane potential across isolated frog skins [11–13] etc.

In analogy with the evidence mentioned above it would be natural to expect the lithium ions to interfere with membrane processes in the guard cells. Simulations, based on a model for stomatal regulation proposed by Cowan [6], were made to see if a change in the membrane permeability of the guard cells could account for the phenomena reported here. A decrease of the guard cell permeability in the model gave a period lengthening and a change in the curve shape as in the lithium experiments, but it was not possible to maintain a constant amplitude simultanuously. Probably Cowan's model needs further non-linearities in the stomatal parameters to account for the lithium effects.

During the stomatal regulation potassium is transported across the guard cell membranes, *e.g.* [14], and it has been proposed that water passively follows this ion [15]. Therefore, it is not unreasonable to suggest that a potassium activated reaction is responsible for the rate limiting processes of the present oscillatory system. Lithium might then act as an inhibitor and slow down the oscillations.

Finally we would like to mention that the oscillatory stomatal movements discussed here, are modulated in a circadian fashion in some species [16]. Since lithium strongly influences the short-term stomatal movements it is possible that it also interferes with the circadian stomatal regulation.

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