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The Effect of Ouabain on the Stomatal Opening and Closing of *Vicia fava.*

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Abstract

The effect of ouabain on the stomatal opening and closing of epidermal strips of *Vicia fava* was observed both in the light and dark.

Experimentation revealed that, by adding ouabain, both in the light and dark there was a tendency for the closed stomata to open, while there was a tendency for the open stomata not to close as compared with the control.

Introduction

Fujino (1959, 1967) first proposed that the stomatal movement was caused by an active transport of potassium ion into and out of the guard cells, and that ATP in the guard cells was involved in the uptake potassium ion, while ATPase in the guard cells was involved in the excretion of potassium ion from the guard cells. Thus, it should be noted that he considered both stomatal opening and closing as active processes.

The active uptake of potassium ion on the stomatal opening has been largely confirmed by other workers (Fischer, 1968; Fischer and Hsiao, 1968; Sawhney and Zelitch, 1969; Willmer and Mansfield 1969 a, b, 1970; Humble and Hsiao, 1970).

Thomas (1970 a, b,) considered that an ATPase linked potassium transport system was involved in the stomatal movement of tobacco and *Vicia fava.*

Cardiac glycoside, i. e. ouabain (G-strophathin) has been used extensively as a specific inhibitor of an ATPase-dependent, Na^+ and K^+ transport system in animal cells.

Chattopadhyay and Brown (1966) and Raven (1967) in their respective study of barley root and *Hydrodictyon africanum* showed that an ATPase-dependent, Na^+

Abbreviation : **PCMB** : p-chloromercuribenzoate

and K^+ transport system was present and the system was inhibited by ouabain. In plant cells, however, an ATPase has been generally considered to be insensitive to ouabain (Dodds and Ellis, 1966; Dodd, Pitman, and West, 1966; Hodges, 1966; Atkinson and Polya, 1967).

In this paper, the effect of ouabain on the stomatal opening and closing of *Vicia fava* will be described.

Materials and Methods

Vicia fava cultivated in a green house during the spring of 1975 was used in this experiment as materials.

Open samples: At about twelve on the day of experiment, entire leaves were cut off and trasferred to the experimental room, and was preexamined by a microscope to ensure that the stomata were wide open. Leaves with open stomata (appoximately 14μm) were used as open samples. When the stomata did not open to about 14μm in width, the leaves were floated on the water for about 2 hours under the light of about 8000 lux at 30℃. Virtually most stomata opened to about 14μm in width.

Closed samples: On the evening of the previous day, entire leaves were cut off and transferred to the experimental room, and kept floating on the water in an incubator at 30℃ for 1 night. By this pretreatment, when the stomata did not completely close, epidermal strips were immersed in distilled water for 30 min. Virtually most stomata closed completely, and the leaves with closed stomata were used as closed samples.

Abaxial epidermis with the fully open stomata or completely closed stomata were taken respectively from the same leaf about 5×5 mm in size. Five strips were immersed in 5 ml of the bathing medium, and kept in the light of 8000 lux and dark for 4 hours, mantaining a temperature of 30℃. At least 20 stomata per each strip were measured at the middle of the strips by a microscope every 1 hour. Each value is an average of at least 100 individual stomatal apertures.

Control medium for the stomatal opening was 60 mM phosphate buffer of pH 6.0 containing 75 mM KCl, while control medium for the stomatal closing was 60 mM phosphate buffer of pH 6.0 without KCl.

The final concentration of ouabain added to the both control medium was 0.1 mM, 0.01 mM and 0.001 mM.

Resuts

a) The effect of ouabain on the stomatal opening.

When completely closed epidermal strips were incubated in the control medium, the stomatal opening was also occurred in the dark. In the light, the stomatal apertures were 2.7μm after 1 hour and 7.0μm after 4 hours, and was 2.0μm after

1 hour and 3.2μm after 4 hours in the dark. Thus, the rate of stomatal opening in the dark was slower than in the light (Fig. 1A, 1B).

In the light, the final apertures after 4 hours at 0.1 mM, 0.01 mM, and 0.001 mM ouabain were 8.3μm, 10.0μm, and 9.1μm respectively. Thus, the rate of opening was greatest at 0.01 mM ouabain.

In the dark, when 0.1 mM, 0.01 mM, and 0.001 mM ouabain were supplied to the strips, the stomata opened to 4.0μm, 5.2μm and 4.5μm after 4 hours respectively. Thus, the rate of stomatal opening in the dark was slower than in the light, and the apertures were widest at 0.01 mM (Fig. 1A, 1B).

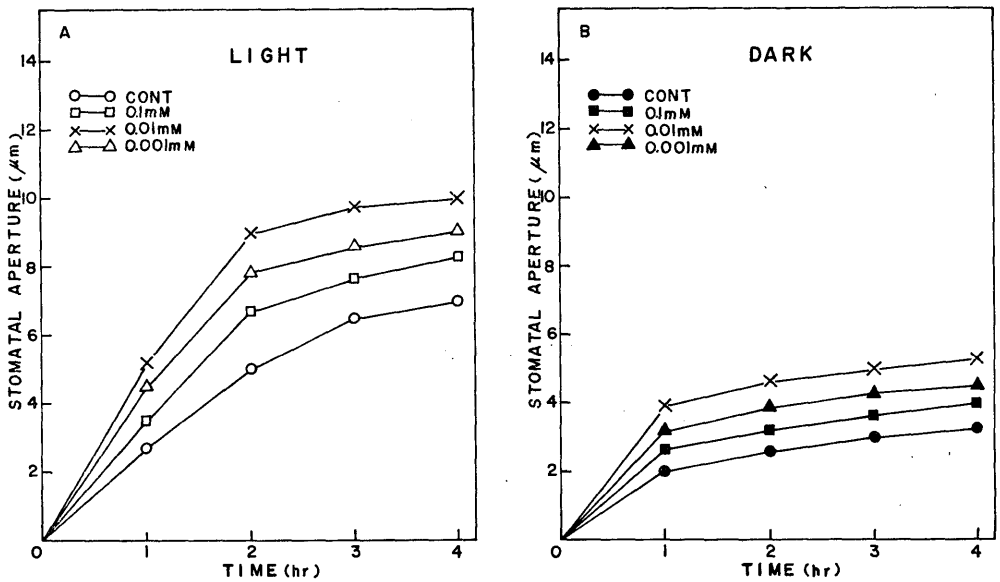

Fig. 1. The effect of ouabain on the stomatal opening of *Vicia fava*. A: in the light, B: in the dark.

b) The effect of ouabain on the stomatal closing.

When fully openined strips were incubated in the control medium, the stomatal closing was considerably rapid both in the light and dark (Fig. 2A, 2B). The apertures decreased from 14μm to 10μm after 1 hour and 7 μm after 4 hours in the light, and to 8 μm after 1 hour and 4.8μm after 4 hours in the dark. Thus the rate of stomatal closing in the dark was greater than in the light.

In the presence of ouabain, the stomatal closing in the light and dark significantly inhibited remaining widely open as compared with the control. At a concentration of 0.1 mM, 0.01 mM, and 0.001 mM ouabain, after 4 hours the stomatal apertures decreased from 14μm to 8.9μm, 12.0μm and 10.6μm in the light, and to 6.8μm, 10.0μm and 8.3μm in the dark respectively. The inhibition of stomatal closing was greatest at 0.01 mM both in the light and dark (Fig. 2A, 2B)

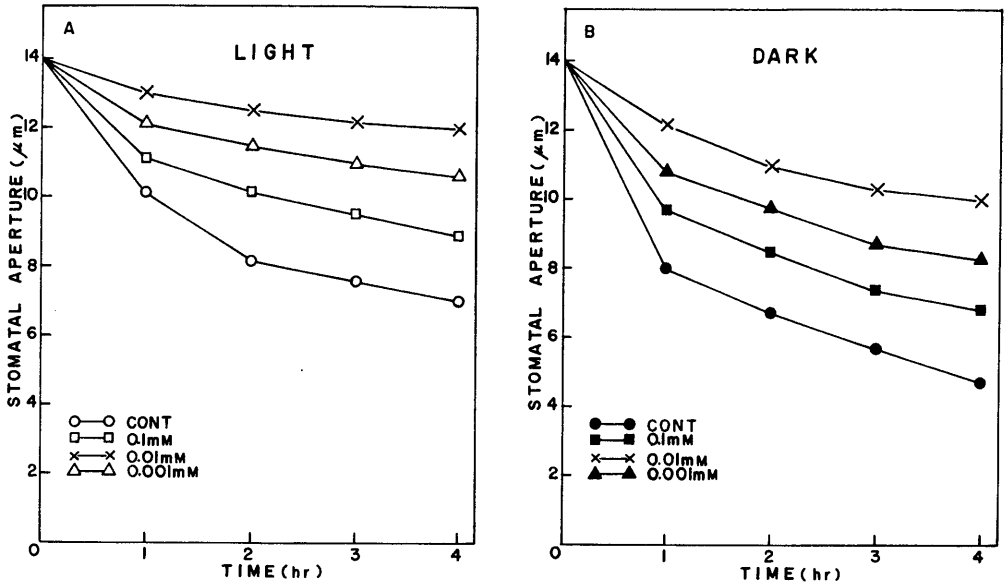

Fig. 2. The effect of ouabain on the stomatal closing of *Vicia fava*. A: in the light, B: in the dark.

Discussion

Thomas (1970 a) showed that open stomata of epidermal strips of tobacco and *Vicia fava* reduced rapidly in apertures by the addition of ouabain. Turner (1973) also showed that ouabain induced the stomatal closoure in the light but had no effect in the dark in *Phaseolus vulgaris*.

On the other hand, Pallaghy and Fischer (1973) showed that ouabain had no effect on the stomatal opening in the light plus CO_2-free air in *Vicia fava*.

The present result is in contrast to those results. By the addition of ouabain, the stomatal opening was significantly accelerated, resulted in wider apertures than in the control. While, the stomatal closing was significantly inhibited remaining widely open as compared with the control.

The inactivation of ATPase and the acceleration of the stomatal opening by the addition of PCMB and monoiodoacetic acid found by Fujino (1967, 1969) are similar to the present result.

Ouabain may have same effect on the stomatal movement as PCMB and mono iodoacetic acid. ATPase may be inactivated by ouabain, and the inactivation may bring about the stomatal opening.

Fujino (1967) proposed that the stomatal movement depended on the balance of ATP and ATPase, and that ATP and ATPase were mainly involved in the stomatal opening and closing respectively.

The stomatal closing is considered to be an active process (Fujino, 1959, 1967), and it may be geared by the energy released by the hydrolysis of ATP. The inhibi-

tion of the stomatal closing by the addition of the ouabain may be caused by the reduction of the energy for the active excretion of potassium ion.

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