

BRIEF COMMUNICATION

Involvement of nitric oxide in the mechanism for stomatal opening in *Vicia faba* leaves

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Abstract

Nitrite, as well as the nitric oxide (NO) donor *S*-nitroso-*N*-acetylpenisilamine (SNAP), was found to increase the aperture of stoma on *Vicia faba* leaf peels. The results demonstrated here suggest that the nitrite-dependent NO production pathway would be involved in the signal transduction for stomatal movements.

Additional key words: active oxygen, hydrogen peroxide, nitrite, *Viagra*.

Hydrogen peroxide (H₂O₂) is a reactive oxygen species (ROS) that can diffuse across lipid bilayers, thereby functioning as an intra- and intercellular signaling molecule in plants and animals (Finkel 1998, Corpas *et al.* 2001). It has been established in animal systems that NO, a reactive nitrogen species, is also involved in signal transduction (Wendehenne *et al.* 2001). Because NO exhibits anti-microbial activity in addition to its signaling function, the molecule has attracted much interest from plant scientists, particularly in the field of pathogen response (Beligni and Lamattina 1999, Bolwell 1999, Wendehenne *et al.* 2001). Recent papers have suggested that H₂O₂ is involved in abscisic acid-induced stomatal closing (Pei *et al.* 2000). Here we demonstrate that in addition to ROS, NO also acts as a signal transduction molecule involved in the mechanism of stomatal movements.

Fully expanded leaves (3rd, 4th and 5th) of *Vicia faba* L. were used for measurements of stomatal aperture. Epidermal peels were carefully detached from the abaxial surface of the leaves in a medium which contained 30 mM KCl, 10 mM MES-KOH (pH 6.15) and 1 mM EGTA. The peels were treated with the medium which contained 5 mM *S*-nitroso-*N*-acetylpenisilamine (SNAP, Dojindo, Kumamoto, Japan), 1 mM nitrite or ethanol extract of 5 mM *Viagra*[®] (sildenafil citrate, Pfizer, N.Y., USA) under vacuum for 1 min and then incubated for

20 min under a room light. After the incubation, 10 μM H₂O₂ was added to the peels on a slide glass. Stomatal apertures were determined from measurements of 10 - 30 digitised images of stomata using a microscope *Microphoto-FXA* (Nikon, Tokyo, Japan) connected to a digital image camera (*Coolpix 990*, Nikon).

After the treatment of peels with an NO donor SNAP, stomatal apertures significantly increased in contrast with the control (Fig. 1). Stomatal aperture was also increased by nitrite (NO₂⁻) that can be converted to NO by the activity of endogenous nitrate reductase (NR) (Yamasaki *et al.* 1999, Yamasaki and Sakihama 2000, Yamasaki 2000). *Viagra*, which inhibits cyclic GMP (cGMP) degradation in the NO signaling pathway of humans by inhibiting the activity of phosphodiesterase 5 (Terrett *et al.* 1996), significantly suppressed the H₂O₂-induced stomatal closing (Fig. 2). These results suggest that an NO-dependent *Viagra*-sensitive signaling pathway similar to humans is involved in the stomatal movements.

The stomatal aperture responds to a range of stimuli including light, humidity, CO₂, growth regulators and air pollutants (Zeiger 1990, Pei *et al.* 2000, Lake *et al.* 2001). The molecular mechanisms for sensing this variety of stimuli have not yet been revealed. Many papers have conclusively shown that complex signal transduction pathways in guard cells are involved in the regulation of

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Abbreviations: ROS - reactive oxygen species; SNAP - *S*-nitroso-*N*-acetylpenisilamine.

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stomatal movements (Zeiger 1990, Schroeder *et al.* 2001). Mata and Lamattina (2001) have recently reported that NO induces stomatal closure under drought conditions, results apparently opposite to those presented here. It is known that Ca^{2+} is involved in both opening and closing stomatal mechanisms by varying the concentration (McAinsh *et al.* 2000). We consider it plausible that NO could exhibit dualism on the stomatal movements like Ca^{2+} . Further investigation is required to

clarify the concentration of NO required to induce each movement. Although many papers have suggested the presence of mammalian-type NO synthase in plants, a consensus on the intracellular source of NO has not yet been reached. To date, NR is the only known plant enzyme that produces NO (Yamasaki *et al.* 1999, Yamasaki *et al.* 2001). Nitrite-dependent stomatal opening (Fig. 1) implies that NR would be responsible for NO production in signal transduction system of guard cells.

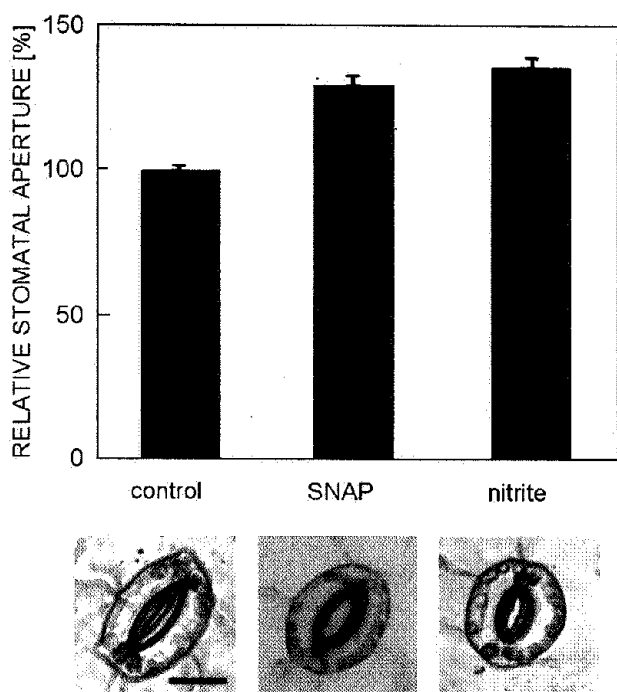


Fig. 1. Induction of stomatal opening by NO. Values are means \pm SE; $n = 10 - 30$. Scale bar: 20 μm .

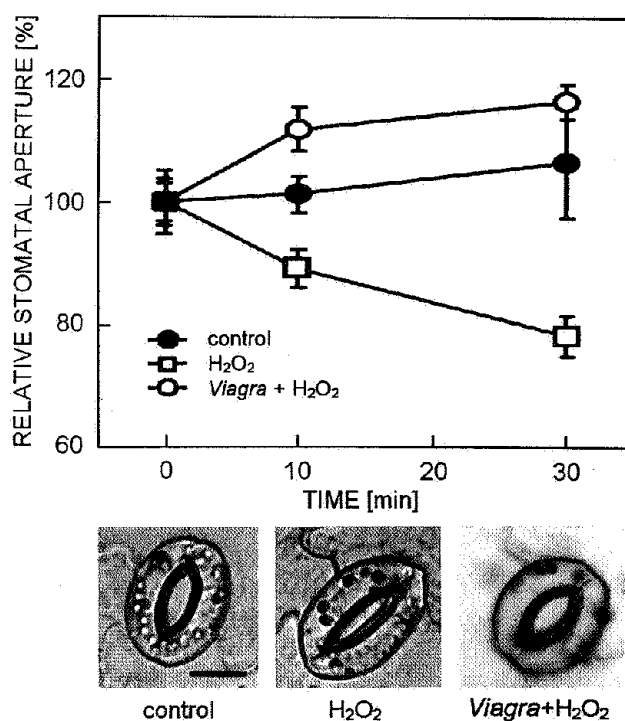


Fig. 2. Inhibition of H_2O_2 -induced stomatal closing by Viagra. Values are means \pm SE; $n = 10 - 30$. Scale bar: 20 μm .

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