

A role for ethylene in low-oxygen signaling in rice roots

Short Communication

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Received October 4, 2005

Accepted December 4, 2005

Published online April 20, 2006; © Springer-Verlag 2006

Summary. Inhibitors of action and synthesis of ethylene (Ag^+ , norbornadien, Co^{2+}) were able to reduce the level of γ -aminobutyric acid (Gaba) in rice roots during the development of an anaerobic environment. The inhibitory effect was reversed by the addition of the G protein activator 5'-guanylylimidodiphosphate. Gaba accumulation was modulated by the presence of CO_2 (inhibitor of ethylene action and synthesis) and stimulated by 2-chloroethylphosphonic acid (ethefon). These findings are consistent with a role of ethylene during a low-oxygen stress.

Keywords: γ -Aminobutyric acid – Ethylene – Hypoxia – *Oryza sativa* – Root – Signal transduction

Introduction

The response of plants to stress conditions implies that, after the stress is sensed, the signal is transduced inside the cell and second messengers are produced. The first signal identified for the anaerobic stress was Ca^{2+} (Subbaiah et al., 1994). The Ca^{2+} signal mediates changes in gene expression, protein metabolism and ion channel activities (Subbaiah et al., 1994; Manjunath et al., 1999; Aurisano et al., 1995a; Reggiani and Laoreti, 2000). By using the known stress target Ca^{2+} /calmodulin (CaM)-dependent glutamic acid decarboxylase, it was possible to elucidate many components of the anaerobic signal transduction pathway. This enzyme produces γ -aminobutyric acid (Gaba) which can be easily quantified (Aurisano et al., 1995b). In this way, G proteins, phospholipase C (PLC) and inositol 1,4,5-triphosphate (IP_3) were identified as components of the anaerobic signal transduction pathway (Aurisano et al., 1995a, 1996; Reggiani and Laoreti, 2000).

In maize roots, the aerenchyma formation during hypoxia induced by ethylene involves G proteins, PLC and IP_3

(He et al., 1996; Drew et al., 2000). In hypoxia, rice ethylene production is greatly enhanced for the induction of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (Yang and Hoffman, 1984; Cohen and Kende, 1987). Ethylene synthesis is instead halted under anoxia since the conversion of ACC to ethylene by ACC oxidase requires molecular oxygen. The likeness between the signal transduction pathways for aerenchyma formation and anaerobic Gaba accumulation suggests to verify if ethylene play a role during the development of an anaerobic environment. For this reason, I made use in the present study of ethefon (ethylene releasing), cobalt (ethylene synthesis inhibitor), silver, and norbornadien (ethylene action inhibitors).

Materials and methods

Ethefon, AgCl , CoCl_2 , dansyl chloride and 5-guanylyl-imidodiphosphate (GMP-PNP) were purchased from Sigma-Aldrich (Milano, Italy). Bicyclo[2.2.1]-hepta-2,5-dien (norbornadien) was from Fluka Chemie AG (Buchs, Switzerland).

Seeds of rice (*Oryza sativa* L. cv. Arborio) were sterilized and germinated in petri dishes as previously described (Reggiani, 1997). Six seedlings were aerobically incubated for 1 h in 20 ml of 1 mmol/l MES-Tris (pH 6.0) supplemented with AgCl , norbornadien, CoCl_2 , ethefon and GMP-PNP as described in Fig. 1 and Table 1. The aerobic pre-loading with various substances is a good method to study the anaerobic stress response when they have no effect in air (Subbaiah et al., 1994; Aurisano et al., 1995a). After 1 h, the seedlings were put in a jar for anaerobiosis (Merck, Darmstadt, Germany) in which oxygen was consumed by activation of BBL GasPack Plus (Becton Dickinson, Cockeysville, MD, U.S.A.). According to the manufacturer's protocol, 60 min after activation the oxygen concentration ranged from 0.2 to 0.7% and CO_2 concentration ranged from 4.6 to 6.5%. 1 h 40 min after activation, the oxygen concentration was less than 0.2%. In one set of experiments, 25 ml of 30% (w/v) KOH were added to the jar to trap CO_2 .

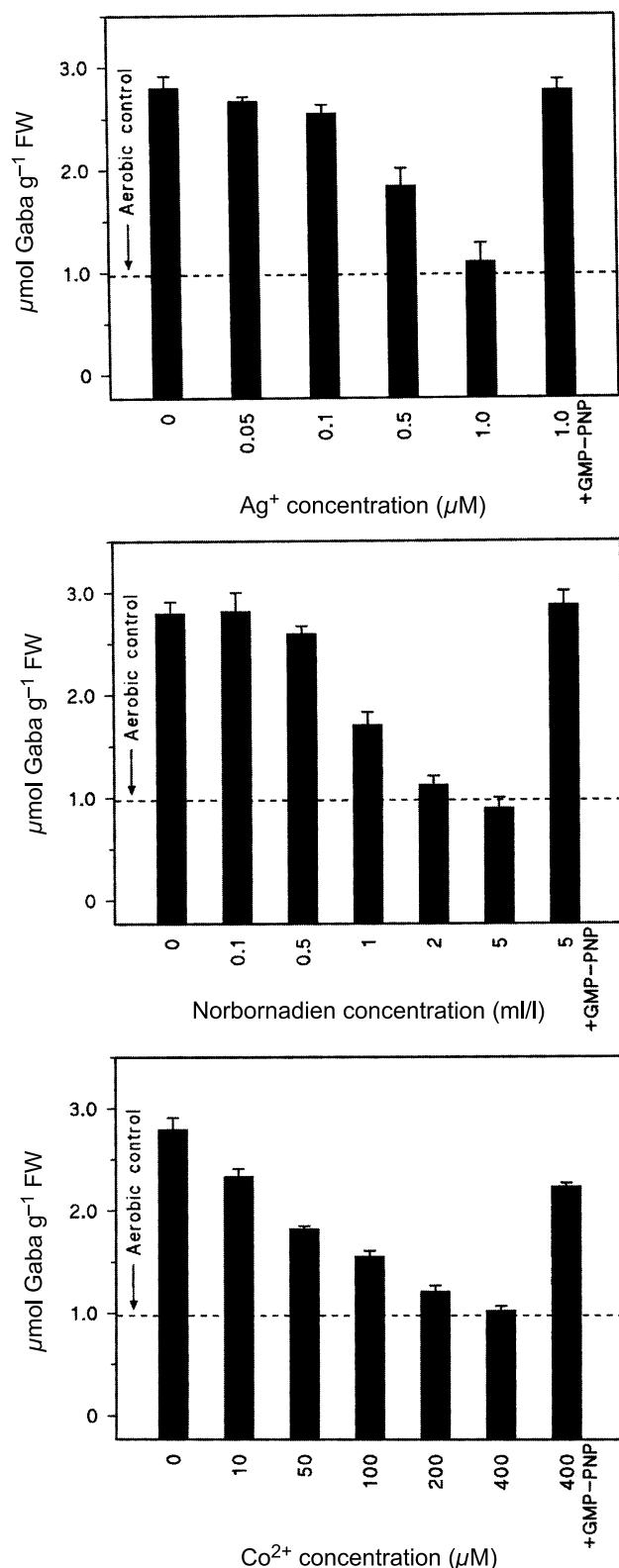


Fig. 1. Effects of Ag⁺, norbornadien and Co²⁺ at various concentrations on the GABA level in rice roots subjected to 3 h of oxygen-deficit stress. Arrows indicate the level of GABA before imposition of stress conditions (aerobic control). Each bar is the mean of at least 3 independent experiments \pm SE

Table 1. Accumulation of GABA in rice roots during 3 h of anaerobic treatment

Treatment	GABA (mmol g ⁻¹ FW)
Aerobic control	0.95 \pm 0.05
3 h anoxia	2.80 \pm 0.11
+30% KOH	3.59 \pm 0.14
+1 mM Ethefon	3.28 \pm 0.09
+1 mM Ethefon, 1 μM Ag ⁺	1.19 \pm 0.14
+1 mM Ethefon, 5 ml/l NSD	0.98 \pm 0.18
+1 mM Ethefon, 400 μM Co ²⁺	1.44 \pm 0.14

The level of GABA did not show a significant change after 1 h in aerated condition in the presence of ethefon (1.08 ± 0.14 μmol g⁻¹ FW). Values are the mean of at least three replicates \pm SE

After the treatment, the roots were excised and ground in a mortar with 0.6 mol/l HClO₄ (100 mg FW per ml) and the homogenate was centrifuged at $13,000 \times g$ for 15 min. GABA was derivatized with dansyl chloride and assayed by HPTLC as previously described (Aurisano et al., 1993).

Results and discussion

In the present study, I examined the effect of inhibitors of ethylene action (Ag⁺, norbornadien) and synthesis (Co²⁺) on the accumulation of GABA by 3 h stress experiments. The level of GABA was $0.95 \mu\text{mol g}^{-1}$ FW in air and became $2.80 \mu\text{mol g}^{-1}$ FW after 3 h of treatment (Fig. 1). Ag⁺ inhibited anaerobic GABA accumulation at concentrations $>0.1 \mu\text{M}$ and with complete inhibition at $1 \mu\text{M}$. Norbornadien began to inhibit GABA accumulation at a concentration of 1 ml/l and it abolished GABA accumulation at 5 ml/l. Inhibition of anaerobic GABA accumulation was also observed in the presence of the ethylene synthesis inhibitor Co²⁺. In this case, complete inhibition was obtained at a concentration of $400 \mu\text{M}$ Co²⁺ (Fig. 1). These data would indicate that ethylene synthesis and signal are required to induce GABA accumulation. For all the inhibitors, the addition of $100 \mu\text{M}$ GMP-PNP (G proteins activator) restored GABA accumulation (Fig. 1), suggesting that the inhibition was upstream of G proteins stimulation.

Since the BBL GasPack Plus produces in jars an enriched-CO₂ atmosphere and CO₂ is an inhibitor of ethylene production and perception (Gorny and Kader, 1996; de Wild et al., 2003), an experiment was made in the presence of 30% KOH (CO₂ trap). Table 1 shows that, in the presence of the CO₂ trap, GABA accumulation increased of $0.79 \mu\text{mol g}^{-1}$ FW. Furthermore, the addition of 1 mM ethefon to the incubation medium can increase GABA accumulation of $0.48 \mu\text{mol g}^{-1}$ FW. The addition of Ag⁺ or NBD to ethefon-treated seedlings inhibited completely GABA accumulation while Co²⁺ showed a residual

accumulation ($0.49 \mu\text{mol g}^{-1}$ FW) similar to the increase in Gaba concentration due to the ethefon treatment (Table 1). These data suggest that rice roots are responding to ethylene (endogenously produced or added) and that this response may be modulated by the release of CO_2 (produced *in vivo* by fermentative processes). Since Co^{2+} is an inhibitor of ethylene synthesis, this had no effect on the quote of Gaba accumulation induced by ethefon.

The data taken as a whole indicate that ethylene is the signal, upstream G proteins, inducing Gaba accumulation during the transition to anaerobic conditions. There is an apparent discrepancy between the fact that ethylene synthesis is stopped in lack of oxygen and the effect of the ethylene synthesis inhibitor Co^{2+} (Fig. 1). However, it has to be considered that complete anoxia is not reached immediately and during the hypoxic transition (at least 100 min in my experimental conditions) ethylene synthesis would be greater than in air (Yang and Hoffman, 1984; Cohen and Kende, 1987). The fact that ethylene induces Gaba accumulation through a G protein-mediated pathway would indicate that the hormone extends its action from hypoxia to the first hours of anoxia through the signal transduction pathway previously described (Reggiani and Laoreti, 2002).

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