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论文

NO与Ca^{2+}对蚕豆保卫细胞气孔运动的互作调控

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摘要：

以蚕豆(*Vicia faba* L.)为材料研究NO和Ca^{2+}对蚕豆气孔运动及质膜K^+通道的影响。结果表明，10 mmol L^{-1} Ca^{2+}和100 μmol L^{-1} NO供体SNP均有效抑制气孔开放，NO清除剂c-PTIO不能缓解Ca^{2+}抑制气孔开放，相反胞外加入0.1 mmol L^{-1} Ca^{2+}可以明显加强NO对气孔开放的抑制程度，该现象可被La^{3+}(Ca^{2+}通道抑制剂)缓解。以膜片钳技术记录全细胞K^+电流发现，胞外10 μmol L^{-1}或100 μmol L^{-1} SNP均可选择性抑制蚕豆保卫细胞质膜内向K^+通道，追加0.1 mmol L^{-1} Ca^{2+}可显著激活质膜外向K^+通道，且可被La^{3+}所缓解，然而0.1 mmol L^{-1} Ca^{2+}单独作用并不影响质膜外向K^+通道活性。10 mmol L^{-1} Ca^{2+}单独处理可激活质膜外向K^+通道，但不能被c-PTIO缓解。分别用Ca^{2+}和NO专一的荧光探针Fluo-3-AM和DAF-2DA标记蚕豆保卫细胞原生质体，检测胞内Ca^{2+}和NO的水平变化发现，100 μmol L^{-1} SNP明显诱导胞内Ca^{2+}积累，但10 mmol L^{-1} Ca^{2+}并不能诱导NO在细胞内积累。记录保卫细胞质膜Ca^{2+}通道电流发现，NO可明显激活质膜Ca^{2+}通道。表明NO有效抑制气孔开放，可能主要通过激活质膜Ca^{2+}通道，提高胞内Ca^{2+}，激活质膜外向K^+通道促进K^+外流，同时，可选择性抑制内向K^+通道阻止K^+内流，两种途径共同作用抑制气孔开放。然而，胞外10 mmol L^{-1} Ca^{2+}对气孔和质膜K^+通道活性的调节并不依赖于NO。

关键词： 钙离子 一氧化氮 保卫细胞 质膜K^+通道 信号转导

Crosstalk of NO with Ca^{2+} in Stomatal Movement in *Vicia faba* Guard Cells

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Abstract:

Previous studies suggested that both NO and Ca^{2+} can serve as a signalling intermediate in ABA, H_2O_2-induced stomatal movement. However, Its mechanism(s) of action is not well defined in guard cells and, generally, in higher plants. In this study, extracellular 10 mmol L^{-1} Ca^{2+} significantly inhibited stomatal opening, which was not alleviated by carboxy PTIO (c-PTIO, a NO scavenger). Sodium nitroprusside (SNP, a NO donor) showed effects of inhibition on stomatal opening at concentration of 10 or 100 μmol L^{-1}. However, 0.1 mmol $L^{-1}$$Ca^{2+}$facilitated NO-inhibited stomatal opening, which was alleviated by $LaCl_3$ (a Ca^{2+}channel inhibitor) at concentration of 1 mmol L^{-1}. To gain further insights into Ca^{2+} function in NO-regulated stomatal movement, we patch-clamped *Vicia faba* guard cell protoplasts in a whole-cell configuration. In the absence of extracellular Ca^{2+}NO inhibited inward rectifying K^+ current at concentration of 10 or 100 μmol L^{-1}, but have little effects on outward rectifying K^+ current. NO significantly activated outward rectifying K^+ current, when $CaCl_2$ was added to the bath solution, at concentration of 0.1 mmol L^{-1}, which was alleviated by $LaCl_3$. In contrast, 0.1 mmol L^{-1} $CaCl_2$ alone had little effects on inward or outward rectifying K^+ current. Extracellular Ca^{2+}significantly inhibited inward rectifying K^+ current and activated outward rectifying K^+ current at concentration of 10 mmol L^{-1}, which was not alleviated by c-PTIO. A single-cell analysis of cytosolic Ca^{2+} and NO using Ca^{2+}specific fluorescence probe Fluo-3-AM and DAF-2DA revealed that 100 μmol L^{-1} SNP evidently induced accumulation of Ca^{2+} in the guard cells, which was partially alleviated by $LaCl_3$, but 0.1 or 10 mmol L^{-1} $CaCl_2$ had few effects on the accumulation of NO in the guard cells. These results indicated that NO promotes influx of Ca^{2+} into cytoplasm through Ca^{2+} channels to activate outward rectifying K^+ channels and promotes K^+ eflux, alternatively, NO inhibits inward rectifying K^+ channels and blocks K^+ influx, thus inhibiting stomatal opening and preventing the excessive loss of water in plants. In addition, extracellular Ca^{2+} at concentration of 10 mmol L^{-1} modulatesstomatal movement and plasma membrane K^+ channels of *Vicia* guard cells in a NO-independent signaling pathway.

Keywords: Calcium Nitric oxide Guard cell Plasma membrane K^+ channels Signal transduction

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