EFFECT OF FARNESOL ON VASCULAR SMOOTH MUSCLE CALCIUM CHANNELS

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Earlier experiments with animal and human arteries have shown that farnesol, a natural 15-carbon (C15) isoprenoid, is an inhibitor of vasoconstriction. Farnesol reduced KCl- and norepinephrine-dependent cytosolic Ca^{2+} transients in fura-2- loaded intact arteries and in cultured aortic smooth muscle cells (A10 and A7r5 cells). Perforated patch-clamp experiments further showed in two vascular smooth muscle cell lines (A10 and A7r5), a reversible, dose-dependent inhibitory effect of farnesol on L-type Ca^{2+} currents (IC50 = 2.2 mM). Current inhibition by farnesol was prominent over the whole voltage range without changes in the current-voltage. Shorter (C10, geraniol) and longer (C20, geranylgeraniol) isoprenols were inactive. Furthermore, L-type Ca^{2+} channel blockade also occurred under cell-attached conditions and single-channel analysis, thus suggesting a possible action of farnesol from within the intracellular space. It was shown that farnesol is an inhibitor of vascular smooth muscle Ca^{2+} signaling with plasma membrane Ca^{2+} channel blocker properties (Roullet et al. J. Clin. Invest. 97: 2384–2390, 1996, Roullet et al. JBC 272 (51): 32240–32246, 1997).

To explore whether the action of farnesol is restricted to the complex Ca^{2+} channel we sought to characterize the effects of farnesol on vascular smooth muscle L-type Ca^{2+} channels. L-type Ca^{2+} channels are heteroligomeric complexes consisting of five subunits (α_1, α_2, β, γ, δ). The α_1 subunit functions as a voltage sensor, a drug receptor, and a Ca^{2+} selective pore. The other subunits serve regulatory purposes (Singer et al. Science, 253:1553-1557, 1991). To investigate farnesol's site of action, we transfected *Xenopus* oocytes to express pore-forming $\alpha_{1C\text{-}b}$ and α_{1G} subunits, which display L-type and T-type Ca^{2+} currents despite the lack of regulatory subunits, respectively (Bosse et al. EMBO J., 11:2033-2038, 1992, Gollasch et al. Am J Physiol., 271:C842-C850, 1996).

For that purpose, ovarian tissue was obtained from female *Xenopus* laevis (NASCO, Modesto, CA). Oocytes were enzymatically isolated and maintained at 18°C for several hours before cRNA injection. Plasmides carrying α_{1G} (pSP72α1G) and $\alpha_{1C\text{-}b}$ (pSP72α1Cb) were

linearized with ClaI and Acc65I, respectively. Expression of various types of voltage-dependent Ca^{2+} channels in oocytes was achieved by injection of the appropriate α_1 cRNA species dissolved in water.

For Ca^{2+} current measurements, oocytes were placed in 5 mM Ba^{2+} recording solution without chloride salts to minimize the currents through endogenous chloride channels present in *Xenopus* oocytes.

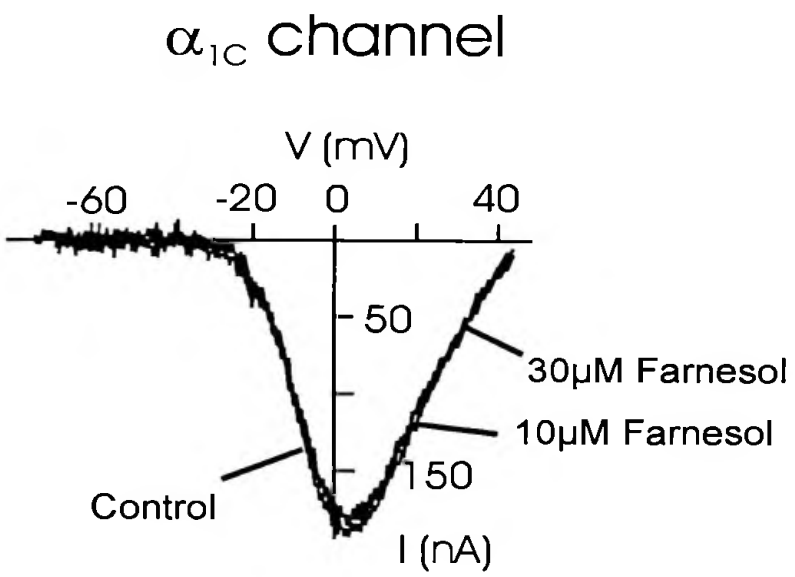

Fig. 1 Effect of farnesol on the current of L-type Ca^{2+} channel $\alpha_{1C\text{-}b}$ subunit. Current-voltage relationship of the L-type Ca^{2+} channel $\alpha_{1C\text{-}b}$ subunit expressed in Xenopus oocytes. Application of farnesol (10-30μM) to the cells did not result in a significant inhibition of the Ba^{2+} current through L-type Ca^{2+} channel $\alpha_{1C\text{-}b}$ subunits.

Whole cell Ba^{2+} currents in oocytes were recorded using a two-electrode voltage-clamp amplifier (TEC-10CX, NPI Electronic, Tamm, Germany).

L-type Ca^{2+} channel $\alpha_{1C\text{-}b}$ subunits expressed in *Xenopus* oocytes exhibited a typical, slowly inactivating Ca^{2+} current (L-type), and no fast inactivating (T-type) current (n = 33). The recorded IV curves (Fig. 1) were U-shaped with a maximum current at a potential of 4 ± 5 mV, an apparent threshold potential at -32 ± 6 mV, and a reversal potential at 49 ± 6 mV (n = 33). Ba^{2+} currents were reversibly blocked by 1 μM nimodipine (n = 4) or 100 μM Cd^{2+} (n = 4), and were reversibly increased by 1 μM (±)-Bay K 8644 (n =7, data not shown). Application of farnesol (10-30μM) to the cells did not result in a significant inhibition of the Ba^{2+} current through L-type Ca^{2+} channel $\alpha_{1C\text{-}b}$ subunits (n=8).

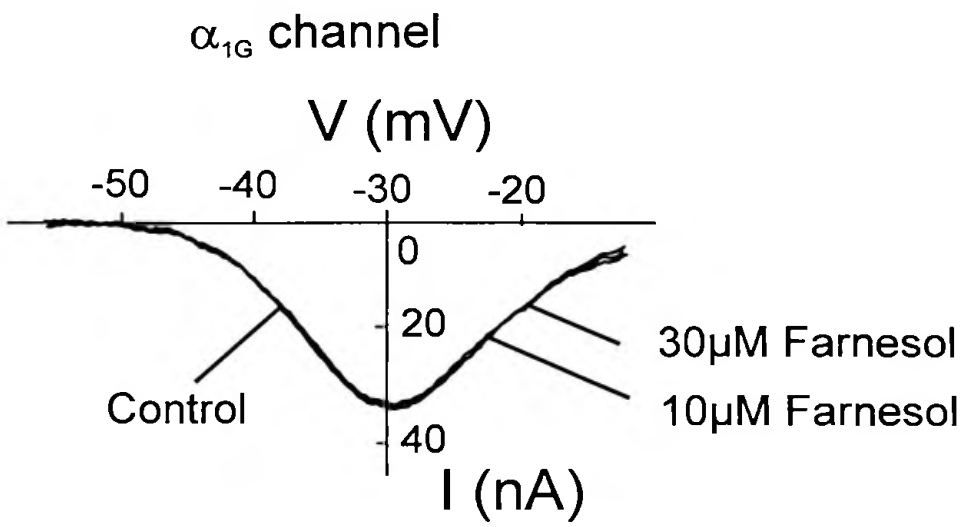

Fig. 2 Effect of farnesol on the currents of T-type Ca^{2+} channel α_{1G} subunit. Current-voltage relationship of the T-type Ca^{2+} channel α_{1G} subunit expressed in Xenopus oocytes. Application of farnesol (10-30μM) to the cells did not result in an inhibition of the Ba^{2+} current through T-type Ca^{2+} channel α_{1G} subunits.

T-type Ca^{2+} channel α_{1G} subunits in *Xenopus* oocytes exhibited a typical, fast inactivating Ca^{2+} current (T-type), and no slowly inactivating (L-type) current (n = 14). The recorded IV curves were U-shaped with a maximum current at a potential of -30 ± 4 mV, an apparent threshold potential at -50 ± 6 mV, and a reversal potential at > -21 ± 6 mV (n = 14) (Fig. 2). Ba^{2+} currents were blocked by 100 μM Cd^{2+} (n = 4) but not affected by 1 μM nimodipine (n = 8) or 1 μM (±)-Bay K 8644 (n = 4, data not shown). Fig. 2 shows that farnesol (10μM and 30 μM) did not affect α_{1G} subunit currents (n = 7).

Application of farnesol into the cytoplasm by injection of farnesol during the voltage-clamp measurements did also not result in an inhibition of the Ba^{2+} currents through eighter L-type Ca^{2+} channel $\alpha_{1C\text{-}b}$ or T-type Ca^{2+} channel α_{1G} subunits. Thus, single a-subunits of the vascular smooth muscle Ca^{2+} channel do not seem to represent the molecular target for farnesol.

We conclude that farnesol may represent an endogenous smooth muscle L-type Ca^{2+} channel antagonist targeting the complex multi-subunit Ca^{2+} channel directly and/or involving a second messenger molecule characteristic for mammalian cells. Support from the Mount Desert Island Biological Laboratory New Investigator Award and through the NSF number DBI 9820400.