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Abstract Title

A Novel Selective Activator of Adenylyl Cyclase Isoform 6 With Pulmonary Relaxation Properties

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Background

Adenylyl cyclase (AC) is crucial for pulmonary vasodilation in persistent pulmonary hypertension of the newborn (PPHN). Hypoxia inhibits AC activity due to cysteine nitrosylation of AC6, the main pulmonary AC isoform. Forskolin (FSK) is a non-selective activator of AC isoforms that binds at the allosteric site to stimulate ATP catalysis. We tested a series of FSK derivatives with modifications at various carbon positions against AC isoforms 3, 5, 6, 7, and 9 to determine whether selective AC6 activation is feasible.

Objective

Design and synthesize FSK derivatives to activate the AC6 isoform in the pulmonary vascular system selectively.

Methods

A library of forskolin derivatives was synthesized by modifications at C1, C6, C7, C9, C11, and/or C14/15 positions and screened against AC isoforms 3, 5, 6, 7, or 9 stably expressed in HEK293T cells. We measured AC catalytic activity using a terbium-norfloxacin fluorescence assay. End-point and real-time cAMP generation were measured using a live-cell cAMP biosensor assay (cADDIS, Molecular Montana, CA). The derivatives were tested *ex vivo* in 200µm precision-cut lung slices from mice using PhysioWare (Scireq, Montreal).

Results

AC6 catalytic activity is uniquely inhibited by hypoxia. We identified two FSK derivatives (C13, C20) that selectively activate AC6, generating cAMP in HEK293T cells overexpressing AC6, but not other AC isoforms. C13 exhibited greater potency for pulmonary vasorelaxation than C20. Molecular docking and simulations indicated that both derivatives interact with AC6 through amino acids similar to those involved in FSK binding. Alanine substitution of the predicted interacting residues markedly reduced derivative-induced cAMP generation to near-basal levels, supporting their contribution to AC6 activation.

Conclusion

AC6 serves as a druggable target for several cardiopulmonary diseases, including PPHN. Hypoxia inhibits AC6 activity in the pulmonary system, but pharmacological stimulation of hypoxia-inhibited AC6 remains feasible. This study identifies FSK derivatives with selective AC6 activation and provides a foundation for developing isoform-selective AC-targeted therapeutics to restore cAMP signaling in pulmonary vascular disease.