SVEP1 as a regulator of GPCR-mediated vasoconstriction

Introduction

Cardiovascular diseases (CVDs) are a leading cause of mortality worldwide, and vasoconstriction plays a crucial role in the pathophysiology of hypertension, a major risk factor for CVDs. The interactions between G protein-coupled receptors (GPCRs) and integrins are known to regulate vasoconstriction, and the integrin ligand SVEP1 has recently been identified as a key regulator of this process. SVEP1 is a protein of the extracellular matrix (ECM) that surrounds and supports cells. Integrins are adhesion molecules that facilitate interactions between neighboring cells or between cells and the ECM. Integrins are made up of an α and a β subunit.

The contractile behavior of vascular smooth muscle cells (VSMCs) is involved in the regulation of vascular tone and blood pressure. While mutations in SVEP1 and ITAG9, the gene that encodes the α9 integrin subunit, are associated with elevated blood pressure, there is little understanding of their roles in regulating smooth muscle cell contraction. In coronary artery disease (CAD), the contractile behavior of smooth muscle cells is impacted by the build-up of atherosclerotic plaques at the site of vascular damage.

An early event in plaque formation is the recruitment of monocytes to locations of endothelial damage, followed by monocyte migration across the endothelial layer and differentiation into macrophages. Two conflicting reports were published which highlighted different effects of a loss of SVEP1 on monocyte recruitment and plaque maturation. Given the previous demonstration of the interaction of SVEP1 with integrins $\alpha4\beta1$ / $\alpha9\beta1$, researchers built off their existing publications to investigate the role of SVEP1 in coronary artery disease (CAD) progression.[2] This duo of papers from the Webb Lab at the University of Leicester highlights the newly identified roles of the proteins SVEP1, integrin $\alpha9\beta1$, and integrin $\alpha4\beta1$ in cardiovascular function and disease.

Genetic variants and clinical implications

In the first paper, the authors demonstrated the importance of SVEP1 in regulating blood pressure through its association with $\alpha4\beta1$ / $\alpha9\beta1$ integrins.[1] Building from this work, the authors then demonstrated how mutations in SVEP1, known to be associated with an increased risk of CAD, drive the recruitment and differentiation of macrophages to atherosclerotic plaques. Increased contractile signaling and/or contractile activity was seen in cell models and animal vessels deficient in SVEP1, integrin $\alpha4$, or integrin $\alpha9$ (including in an SVEP1-/- iPSC line engineered at Revvity, previously known as Horizon Discovery), implying that these proteins are involved in reducing arterial contraction. Based on results from the interrogation of signaling pathways to determine how the SVEP1-integrin $\alpha4/\alpha9$ achieves this effect, the authors proposed a mechanism by which SVEP1 acts on a voltage-gated calcium channel (VGCC) signaling cascade to lower VSMC contractility.

The SVEP1-/- iPSC line (generated in collaboration with Revvity) enabled the researchers to use complementary methods to investigate the role of SVEP1 in arterial contraction. Using this cell line, in combination with other models, they found that loss of SVEP1 led to increased contractile signaling (a greater influx of intracellular Ca2+) than in wild-type controls.

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Mechanism of action

The mechanism of action behind contraction involves calcium ion influx through VGCCs and myosin light chain kinase (MLCK) activation. The phosphorylation of MLCK is triggered by calcium-bound calmodulin, and contraction is prolonged due to the inhibition of myosin light chain phosphatase, which regulates the interaction of actin and myosin with smooth muscle. Integrins, including integrin $\alpha9\beta1$, have been shown to modulate VSMC contraction by regulating calcium influx through VGCCs. SVEP1 is a ligand for integrin $\alpha9\beta1$ that is required for normal development of lymphatic vessels and epidermal differentiation.[1] A genetic variant in SVEP1 (rs111245230) associated with elevated blood pressure (BP) and CAD is adjacent to the binding motif for integrin $\alpha9\beta1$. The reduced expression of ITGA9, which encodes the $\alpha9$ subunit of integrin $\alpha9\beta1$, is also associated with increased BP. However, the role of SVEP1 and integrin $\alpha9\beta1$ in vasoregulation and smooth muscle contraction is not yet fully understood.

Data interpretation

The first studies found that SVEP1 and integrin $\alpha9\beta1$ inhibition reduced the contraction of VSMCs in response to $G\alpha q$-GPCR activation.[1] The reduction in contraction was associated with decreased calcium influx through VGCCs and reduced MLCK phosphorylation. The inhibition of SVEP1 and integrin $\alpha9\beta1$ also reduced MLCP inhibition, which shortened the duration of contraction. These results suggest that SVEP1 and integrin $\alpha9\beta1$ regulate VSMC contraction and blood vessel tone.

The study conducted complied with the recommended experimental design and statistical analysis standards in pharmacology set by the British Journal of Pharmacology. The group size for each experimental group was based on the number of independent values designed to be equal, but outliers were excluded using pre-defined criteria. For instance, in the single-cell Ca2+ human iPSC-derived vascular smooth muscle cells (iVSMC) imaging studies, cells that did not respond to vasoconstrictor application within a field of view were excluded, and in the wire myography studies, vessels that contracted with an amplitude less than 2 mN were excluded.[1]

The study investigated the binding of integrin $\alpha4\beta1$ and $\alpha9\beta1$ to SVEP1 with immunoblots, and evaluated the binding efficiency of HEK293A cells overexpressing integrin $\alpha4\beta1$ or $\alpha9\beta1$. SVEP1 inhibition increased iVSMC [Ca2+]i to different vasoconstrictors, including ET-1, carbachol, and U46619.[1] iVSMCs were treated with either non-targeting control (NTC) or SVEP1 siRNA, and the maximal fluorescence signal (F/F0) was recorded. The study also investigated the effect of simultaneous inhibition of SVEP1 and integrin $\alpha4$ or $\alpha9$, which did not induce additional [Ca2+]i elevation in iVSMCs. iVSMCs were treated with NTC, ITGA4, ITGA9, or SVEP1 siRNA, or the dual integrin $\alpha4\beta1$-$\alpha9\beta1$ inhibitor BOP prior to ET-1 challenge.

The genetic variant in SVEP1 (rs111245230) associated with elevated BP and CAD disrupted the binding of SVEP1 to integrin $\alpha9\beta1$. The variant SVEP1 had a reduced ability to inhibit VSMC contraction and calcium influx through VGCCs.[1] These results suggest that the genetic variant in SVEP1 may contribute to elevated BP and CAD by altering the regulation of VSMC contraction through integrin $\alpha9\beta1$.

While the first publication focused on the role of SVEP1 in vascular smooth muscle cells, the second publication investigated the role of SVEP1 on monocytes. Researchers used an SVEP-/- THP-1 cell line (generated in collaboration with Revvity) which functions as a proxy for monocytes and can differentiate into a macrophage-like cell with stimulation. They found that SVEP1 expression rises eight-fold during the differentiation process.[2] This increase in expression facilitates the adhesion and migration of monocytes through SVEP1 binding to $\alpha4\beta1$ / $\alpha9\beta1$ integrins. With the SVEP-/- THP1 cells, they observed reduced monocyte adhesion, migration, and cell spreading.

Cell spreading is attributed to several signaling pathways involved in actin cytoskeleton remodeling. The loss of SVEP1 specifically impacted the Rac1 and Rho-A pathways, which are known to drive morphological changes in cell migration and spreading. Rac1 and Rho-A activity was decreased in SVEP-/-THP-1 cells.[2]

Together, the studies provide evidence that SVEP1 and integrin $\alpha9\beta1$ regulate VSMC contraction and blood vessel tone. The genetic variant in SVEP1 (rs111245230) associated with elevated BP and CAD disrupts the binding of SVEP1 to integrin $\alpha9\beta1$, which may contribute to the pathogenesis of these diseases.[1] The studies highlight the potential of targeting SVEP1 and integrin $\alpha9\beta1$ for the development of novel therapeutic strategies for hypertension and CAD. However, further studies are needed to investigate the mechanisms underlying the effects of SVEP1 and integrin $\alpha9\beta1$ on VSMC contraction and blood vessel tone in vivo and their potential as therapeutic targets for vascular diseases.

Limitations

One potential limitation of the study is the use of ex vivo wire myography to investigate the functional effects of SVEP1 deficiency. While this technique is useful for assessing vascular reactivity, it may not fully reflect the in vivo effects of SVEP1 deficiency. Therefore, future studies using in vivo models of hypertension will be necessary to confirm the therapeutic potential of SVEP1 inhibition.

Another potential limitation of the study is the focus on SVEP1 as a therapeutic target for the treatment of hypertension. While hypertension is a major risk factor for CVDs, it is only one aspect of cardiovascular physiology. Therefore, future studies will be necessary to investigate the role of SVEP1 in other cardiovascular diseases, such as atherosclerosis, heart failure, and stroke, and to determine whether SVEP1 inhibition may be a viable therapeutic strategy for these conditions.

Additionally, the study highlights the importance of integrins in regulating vascular tone, and future research may investigate the potential of other integrin ligands as therapeutic targets for CVDs. Overall, this study provides valuable insights into the regulation of vasoconstriction and identifies SVEP1 as a potential therapeutic target for the treatment of hypertension.

Conclusion

The data provides valuable insights into the precise role of SVEP1 in regulating GPCR-mediated vasoconstriction via integrins $\alpha 9\beta 1$ and $\alpha 4\beta 1$.[1] The authors demonstrate that SVEP1 is necessary for the regulation of vasoconstriction and that it activates the RhoA/ROCK pathway, leading to increased phosphorylation of the myosin light chain and subsequent smooth muscle contraction.[1] SVEP1 is important in regulating vascular tone and blood pressure and researchers identified SVEP1 as a potential therapeutic target for the treatment of hypertension. SVEP1, which serves a potential atheroprotective role in vascular smooth muscle cells, plays a different role in monocytes, where it promotes the formation of early atherosclerotic plaques.[2] This body of work, supported by Revvity, previously known as Horizon Discovery, demonstrates the complicated and nuanced balance of arterial health and the varied roles the key cell adhesion molecules can play.

However, further studies are needed to confirm the therapeutic potential of SVEP1 inhibition in vivo and to investigate its role in other cardiovascular diseases. Overall, these studies contribute to our understanding of the molecular mechanisms underlying vasoconstriction and suggest new avenues for the development of therapeutics to treat hypertension and other cardiovascular diseases. The findings have implications for both basic research and clinical practice, and further research in this area is warranted.

References

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