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Molecular Determinants of Beta-adrenergic Signaling to Voltage-gated K+ Channels in the Cerebral Circulation

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Abstract

Voltage-gated K^+ (K_v) channels are major determinants of membrane potential in vascular smooth muscle cells (VSMCs), and regulate the diameter of small cerebral arteries and arterioles. However, the intracellular structures that govern the expression and function of vascular K_v channels are poorly understood. Scaffolding proteins including postsynaptic density 95 (PSD95) recently were identified in rat cerebral VSMCs. Primarily characterized in neurons, the PSD95 scaffold has more than 50 known binding partners and it can mediate macromolecular signaling between cell surface receptors and ion channels. In cerebral arteries, *Shaker*-type K_v1 channels appear to associate with the PSD95 molecular scaffold, and PSD95 is required for the normal expression and vasodilator influence of members of this K^+ channel gene family. Furthermore, recent findings suggest that the β1-subtype adrenergic receptor is expressed in cerebral VSMCs and forms a functional vasodilator complex with K_v1 channels on the PSD95 scaffold. Activation of β1-subtype adrenergic receptors in VSMCs enables protein kinase A-dependent phosphorylation and opening of K_v1 channels in the PSD95 complex; the subsequent K^+ efflux mediates membrane hyperpolarization and vasodilation of small cerebral arteries. Early evidence from other studies suggests that other families of K_v channels and scaffolding proteins are expressed in VSMCs. Future investigations into these macromolecular complexes that modulate the expression and function of K_v channels may reveal unknown signaling cascades that regulate VSMC excitability and provide novel targets for ion channel–based medications to optimize vascular tone.

Introduction

Cerebral arteries regulate blood flow to the brain by reducing their diameter in response to increased intraluminal pressure – an event also known as the myogenic response.[1, 2] One important mechanism of vasoregulation is the activity of potassium (K^+) channels in the cerebral vascular smooth muscle cells (cVSMCs).[2–4] The closing of K^+ channels causes membrane depolarization, opening of voltage-sensitive Ca^{2+} channels, and vasoconstriction.[2, 3] Thus, voltage-gated K^+ (K_v) channels represent a major K^+ channel superfamily that contributes to the resting diameter of small cerebral arteries.[5–14] There are twelve gene

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families (K_v1 to K_v12) in the K_v channel superfamily.[15] At least three gene families, K_v1, K_v2, and K_v7 channels, are expressed in cVSMCs and regulate myogenic tone.[8, 11, 14]

The open-state probability of K_v channels increases in response to a depolarized membrane potential, and multiple phosphorylation sites also regulate channel activity.[16–23] In this review, we will focus mainly on the *Shaker*-type K_v1 channels and expand our discussion to other K_v channel gene families when appropriate. The K_v1 channels consist of four pore-forming α-subunits and auxiliary β-subunits.[8, 10] In the rat cerebral circulation, the α1.2 and α1.5 subunits minimally form the heterotetrameric pore structure.[8] Seemingly contradicting reports describe how phosphorylation of the α-subunits regulates the function of K_v1 channels. For example, the adenylyl cyclase activator, forskolin, elicits protein kinase A (PKA)-dependent phosphorylation and increases delayed rectifier-type K^+ current in smooth muscle cells from rabbit portal vein, suggesting that PKA can signal effectively to open K_v1 channels in native VSMCs.[24] In contrast, several studies using forskolin or cAMP analogues to elicit PKA phosphorylation of recombinant $K_v1.2$ channels report an increase in $K_v1.2$ protein expression, but little change in K_v1 channel current.[20, 23, 25, 26] Instead, the direct application of protein kinase A (PKA) catalytic subunits is required to activate cloned $K_v1.2$ channels.[21] This apparent dichotomy infers that a signaling complex in native VSMCs closely associates PKA and K_v1 channels to enable PKA phosphorylation of K_v1 channel α-subunits; however, the close association of PKA and K_v1 channels is absent in transfected expression systems, necessitating the direct application of exogenous PKA for phosphorylation and activation of K_v1 channels. To date, the K_v1 channel signaling complexes in VSMCs are largely enigmatic. However, the topology and amino acid sequences of the K_v1 channel α-subunits provide important clues with regard to possible protein interaction domains. The K_v1 channel α-subunits have six transmembrane segments flanked by intracellular N- and C-termini. Of particular interest are the final four amino acids on the C-terminus, i.e., -LTDV for the α1.2 subunit and -ETDL for the α1.5 subunit. These sequences form so-called Class I “PDZ binding motifs”,[27, 28] which selectively bind to PDZ domains on scaffolding proteins as explained in the next section.

Postsynaptic density 95 (PSD95) scaffolding of K_v channels

Postsynaptic density 95 (PSD95),[29] also known as synapse associated protein 90 (SAP90),[30] was first characterized as a 95-kilodalton protein found in the postsynaptic density fraction of rat brain.[29] PSD95 belongs to a large family of scaffolding proteins known as MAGUKs (membrane-associated guanylate kinase homologues).[31] As the name suggests, MAGUKs are not transmembrane proteins *per se*, but associate with membrane-bound proteins to form clustered multimeric structures near the plasma membrane.[32, 33] Uniquely positioned to interact with ion channels and cell surface receptors, as well as cell adhesion molecules and cytoplasmic proteins, MAGUKs modulate the surface expression and function of ion channels and receptors.[28, 34–37] Several structural characteristics of MAGUKs enable them to play such a unique role in integrating signaling and effector molecules in a cell-specific manner.[31] The basic core of MAGUK proteins including PSD95 contains a Src homology 3 (SH3) domain,[38] a guanylate kinase (GK) domain, and one or more PDZ domains.[39] The PDZ domain refers to the ~90 amino acid residues that form a hydrophobic pocket commonly found in MAGUK proteins including PSD95,[29, 30] *Drosophila* septate junction

protein Discs-large,[40] and epithelial tight junction protein ZO-1.[41] The PSD95 protein has three PDZ domains followed by the SH3 and GK domains (Figure 1A). Although the PDZ domains were first identified in MAGUKs, they are present in a wide variety of proteins in diverse organisms including bacteria, yeast, and plants.[42] In the human genome, the PDZ domain family ranks as the 19th most abundant of domain families, suggesting a diverse and intricate role in protein-protein interactions.[43] The crystal structure of the pocket-like PDZ domain and the structure of the PDZ binding motif have been resolved for several proteins.[44–46] The PDZ domain contains a series of GLGF (Gly-Leu-Gly-Phe) repeats,[29] which can tightly bind to the hydrophobic PDZ binding motifs of its protein partners. Differences in the side chains on the pocket of the PDZ domains confer differential affinity for the various PDZ binding motifs found in receptors and ion channels.[28, 47–49]

The PDZ domains on the PSD95 scaffold bind to a subset of PDZ binding motifs recognized as X-S/T-X-V, where S/T is serine or threonine, V is valine, and X is any amino acid.[50, 51] Notably, more than 50 proteins associate with PSD95 in neurons and other cells.[28, 37] The best known binding partners of PSD95 include the N-methyl-D-aspartate receptors (NMDAR),[50] amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) receptors,[52] and neuronal nitric oxide synthase (nNOS).[53] Interestingly, some of the binding partners of PSD95 identified in neurons, also are expressed in rat cerebral arteries.[54] This fact drew attention to PSD95 as a potentially unappreciated molecular scaffold in cVSMCs. For example, inwardly rectifying K^+ (K_{ir}) channels and TWIK-related acid-sensitive K^+ channels are expressed in cerebral arteries and possess the PDZ binding motif for PSD95.[37, 55] G-protein coupled receptors such as the serotonin receptor subunit 5-HT2C[56, 57] and the β1-subtype adrenergic receptor (β1AR)[58] also associate with PSD95 as potential regulators of vascular tone. Additionally, PSD95 can bind to A-kinase anchoring protein 150 (AKAP150) via the SH3 and GK domains to pair PKA[59] and K_v1 channels on the PSD95 scaffold, and enable phosphorylation and opening of K_v1 channels.[60] Interestingly, a previous report of AKAP150 in cVSMCs illustrates a punctate structure near the plasma membrane reminiscent of scaffolding clusters by PSD95.[61] The authors indicate the AKAP150 scaffold facilitates protein kinase C (PKC)–elicited opening of voltage-gated calcium channels and vasoconstriction.[61] Collectively, these data suggest the existence of an intricate latticework of intracellular scaffolds near the plasma membrane of cVSMCs, which dynamically aligns different ion channels with cell-surface receptors and signaling molecules to optimally regulate cVSMC excitability and vessel diameter.

Among the different α-subunits of the K_v1 channel family, only the C-terminus of the K_v1 channel pore-forming α1.2 subunit (-LTDV) exactly matches the definition of the PDZ binding motif for PSD95, whereas its partner in pore formation, α1.5, has a slightly different C-terminus (-ETDL) that may confer less affinity for PSD95. Other K_v1 channel α-subunits (α1.3 and α1.4) abundant in neurons also bind to the first two PDZ domains of PSD95.[27, 51, 62–67] By means of this PDZ binding, PSD95 can regulate K_v1 channel clustering and surface expression. For example, postsynaptic clustering of K_v1 channels is abolished by mutation of the PSD95-homologue in *Drosophila*.[68] Additionally, PSD95 slows the internalization of K_v1 channels in heterologous expression systems.[65] Similarly, the surface expression of $K_v1.2$ channels increases when co-expressed with PSD95.[69] Since its first discovery in 1992,[29] PSD95 has been extensively studied as evidenced by more than

1,000 original research articles, but primarily it was regarded as a marker for postsynaptic density in neurons and rarely studied in non-neuronal tissues. However, we observed that PSD95 is abundantly expressed near the plasma membrane of rat cVSMCs to anchor K_v1 channels. Although mice with gene deletion of PSD95[104] or PSD95 mutants lacking the first two PDZ domains[105] have been designed, chronic global knockdown or mutation of PSD95 that is densely expressed in brain may trigger changes in the cerebral circulation unrelated to the loss of protein in the vasculature. For that reason, in our initial study we used an antisense-mediated knockdown of PSD95 in rat cerebral arteries, which caused a concomitant loss of K_v1 channel protein and its tonic vasodilator influence, leading to cVSMC depolarization and abnormal vasoconstriction.[54]

Notably, we also reported that a similar scaffolding protein, SAP97, is expressed abundantly in rat cerebral arteries, but it does not co-immunoprecipitate with K_v1 channels and is mainly found in the intracellular, juxtanuclear structures of cVSMCs under normal conditions.[54] SAP97 shares a structural similarity with PSD95,[70] but may have a different set of binding partners. For example, SAP97 in cardiac myocytes interacts with K_v4[71] and Kir[72] channels. Considering the robust mRNA and protein expression of SAP97 in rat cerebral arteries,[54] it would be interesting to investigate whether SAP97 is involved in the regulation of these additional K^+ channel types in cVSMCs, or potentially alters their expression and function under pathological conditions, possibly by binding with other PDZ motif-containing proteins. It is also possible that different scaffolding proteins are expressed in non-cerebral vascular beds, where they may play unrecognized roles in regulating ion channel expression and function. For example, in the mesenteric circulation of hypertensive mice, the Kir2 and Kir4 channel proteins are down-regulated as part of the extensive electrical remodeling of arterial K^+ channels.[73] In diabetic rats, an exaggerated impact of oxidative stress signaling on Kir1.1 and Kir2.1 channel function may contribute to the loss of renal afferent arteriolar tone that predisposes to kidney damage.[74]

Dynamic and reversible interaction of K_v1 channels and PSD95

The PDZ interaction between the pore-forming α1.2 subunit of K_v1 channels and PSD95 appears to be dynamic and reversible.[27, 51, 67, 69] In particular, the C-terminus of K_v1 channels is intrinsically disordered and can act as a molecular “fishing rod” to bind to PSD95 scaffolds.[67, 75] This property has been compared to the ball-and-chain model of channel inhibition, whereby the N-terminus of the K_v channel swings around to block the channel pore.[76] Interestingly, analysis of the unfoldability profiles of the C-terminal tails using a dataset of 70 K_v channel sequences revealed that 80% of all K_v channels contain intrinsically disordered C-terminal segments immediately adjacent to the terminal PDZ-binding motif.[75] Thus, the intrinsic instability of the C-terminus that promotes PDZ binding to K_v1 channels also is a feature of other K_v channel families.

This dynamic and reversible interaction between K_v1 channels and PSD95 can be taken advantage of to elucidate the physiological outcome of the interaction.[54] Because the PDZ domains of MAGUKs form unique interactions with their binding partners, “interfering peptides” that disrupt PDZ binding to its intended protein partners have been used as tools to selectively target unique scaffolding interactions without affecting the rest of the protein

complex.[50, 77] Using this approach, a pharmacological dose of short dominant negative peptide (~10 amino acids) containing the C-terminus PDZ binding motif of the protein is introduced into the cell to bind to PDZ domains, and thereby disrupt the PDZ-mediated interaction between PSD95 and its biological binding partner. In neurons, overloading cells with peptide sequences that mimic the C-terminus PDZ binding motif of NMDAR will serve as a PSD95 decoy, effectively uncoupling PSD95 from biological NMDAR to limit its access to nNOS. The end result is reduced excitotoxicity triggered by the NMDAR-nNOS signaling pathway.[50, 77] A similar strategy was extended to rodents and non-human primates *in vivo* as a therapeutic intervention to reduce cortical damage after experimental stroke.[78–81] In these studies, a cell-permeable HIV-tat sequence was coupled to the dominant negative peptide to ensure penetration into the cells of interest for disruption of NMDAR-nNOS signaling.

Separately, we devised a related strategy to elucidate whether a dynamic interaction with PSD95 is required for the vasodilator function of K_v1 channels in rat cerebral arteries.[82] Here, we used a cell-permeable, dominant-negative peptide ("K_v1-C peptide") that couples the HIV-tat sequence to the C-terminus PDZ binding motif of the $K_v1.2$ subunit to specifically disrupt the scaffolding interaction of PSD95 and native K_v1 channels (Figure 1B, 1C).[82] Compared to our earlier work using antisense-based knockdown of PSD95, which resulted in the concurrent loss of both PSD95 and K_v1 channel proteins,[54] this new strategy allowed us to evaluate the impact of disrupting the PSD95 - K_v1 channel interaction in rat cVSMCs without a loss of cell surface expression of K_v1 channels (Figure 1D).[82] Surprisingly, a profound vasoconstriction was elicited within 5 minutes of the application of the K_v1-C peptide to rat cerebral arteries *ex vivo* and *in vivo*. The magnitude of the vasoconstriction was equivalent to that caused by a K_v1 channel-selective blocker, Psora-4 (100 nmol/L).[54, 83, 84] A secondary binding site in the unique side pocket of K_v1 channels confers selectivity of submicromolar concentrations of Psora-4 for K_v1 channels compared to other K_v channel families.[83, 85] Finally, the constrictor effects of the K_v1-C peptide and Psora-4 were not additive when administered together, suggesting a shared mechanism of action.[82] The rapid constriction of rat cerebral arteries by K_v1-C peptide, presumed to be caused by loss of PKA phosphorylation of K_v1 channels, was blunted by phosphatase inhibitors okadaic acid and calyculin-A.[82] Collectively, these results indicate that the "dynamic" PSD95 scaffolding of K_v1 channels is a critical requirement for K_v1 channel α-subunit phosphorylation and opening, hyperpolarization, and subsequent dilation of rat cerebral arteries.[82] Because PKA does not directly bind to PSD95, these results raise the possibility that PKA indirectly associates with PSD95 through AKAP-PSD95 interaction to provide a functional macromolecular scaffolding complex in cVSMCs. In neurons, AKAP-PSD95 interaction is required for the PKA-dependent phosphorylation of AMPA receptors,[59] and it appears a similar AKAP-PSD95 complex also may be required for PKA phosphorylation of vascular K_v1 channels. Potassium channels in the K_v superfamily other than K_v1 and K_v4 are not known to interact directly with PDZ binding domains. However, these K_v channels may indirectly associate with multi-protein complexes that include the PSD95 scaffold. As one possibility, K_v7 channels encoded by the KCNQ1 gene associate with an AKAP9 protein, Yotiao, and are phosphorylated by PKA.[86] Similar associations that form molecular networks of interacting receptors, ion channels and signaling molecules may

exist in VSMCs and underlie the complex interaction between K_v channels and other proteins involved in the regulation of vascular tone.

Interestingly, the PDZ-mediated interaction of PSD95 with its binding partners appears to be dynamic and reversible as shown in similar studies in which application of a competing peptide disrupts NMDAR-PSD95 binding in cultured neurons[77] and in neurons *in vivo*.[79–81] In Canada and the United States, clinical trials using cell-permeable interfering peptides targeting NMDAR-PSD95 interaction (dubbed “PSD95 inhibitors”) are underway to explore PSD95 inhibitors as medications for ischemic brain damage.[87] Considering that the K_v1 channel α1.2 subunit and NMDAR share the ability to bind to the PDZ1 and PDZ2 domains of PSD95,[79] therapeutic peptides that bind to the PDZ domains of PSD95 to prevent NMDAR-PSD95 interaction, also would make PSD95 unavailable to its other biological binding partners, including K_v1 channels in cVSMCs. The loss of functional K_v1 channels caused by “unscaffolding” from PSD95 would favor cerebral vasoconstriction. Thus, it may be important to consider the broader pharmacological implications of the PSD95 inhibitors[79, 80] and constriction of cerebral arteries could be a potential off-target effect.

Activation of K_v1 channels by the β1-subtype adrenergic receptor

Adrenergic receptors (ARs) mediate sympathetic responses to norepinephrine (NE) released locally by nerve endings, or to circulating epinephrine and NE released by the adrenal medulla. The typical response of most systemic arterioles and arteries to sympathetic activation is vasoconstriction mediated by α1-subtype adrenergic receptors (α1AR), although vasodilation of skeletal muscle arteries can result from activation of β2-subtype adrenergic receptors (β2AR).[88] Traditionally, the final response of an artery to sympathetic activation was thought to depend on the relative expression levels of α1AR and β2AR in the VSMCs, and the effectiveness of their signaling pathways. However, newer studies using subtype-specific β1- and β2- adrenergic receptor knockout mice have revealed a mixed distribution of functional β1AR and β2AR in many arteries, challenging initial dogma of predominant β2AR expression in VSMCs.[89] For example, in pulmonary and femoral arteries, β1ARs instead of β2ARs mediate the vasodilator response to isoproterenol, a nonselective β-AR agonist.[89]

The precise contribution of sympathetic influences to the regulation of cerebral blood flow still is debated. The cerebral arteries of several species including human[90, 91] and rat[92] express both α-AR and β-AR. However, some early studies in cat, dog and monkey failed to detect direct diameter responses to cervical nerve stimulation[93] despite heavy sympathetic innervation of cerebral arteries.[94] Regardless, later studies in cerebral arteries from several species including rat and cat suggested that β-AR activation induces cerebral vasodilation.[94–98] Interestingly, studies dating back to the 1980’s report β1AR-mediated dilation in cerebral arteries of rats and other mammals, instead of prevalent dilation by β2AR.[99, 100] Realizing that β1AR has a C-terminus PDZ binding motif (-ESKV) that binds to the PDZ3 domain of PSD95,[54, 82, 101] this receptor has surfaced as a prime candidate to be a member of the PSD95-K_v1 vasodilator complex in cVSMCs (Figure 2).[54, 82, 101] The predominance of β1AR in cVSMCs has important relevance for the regulation of cerebral blood flow, because: 1) β1AR but not β2AR contains the C-terminus PDZ binding motif required for

binding to PSD95;[58, 102, 103] 2) NE as an endogenous mixed adrenergic agonist has high affinity for α-AR and β1AR, but not for β2AR; and 3) the most widely used β-AR blocking drug, metoprolol, selectively blocks β1AR over β2AR.[104]

Considering these facts, we recently proposed that a β1AR-PSD95-K_v1 complex in cVSMCs enables β1AR to signaling through PKA-mediated phosphorylation of K_v1 channels, which mediates NE-induced dilation of rat cerebral arteries.[101] Notably, the expression of β1AR and its association with PSD95 in rat cerebral arteries were confirmed by real-time RT-PCR, Western blots, co-immunoprecipitation and confocal microscopy of cVSMCs.[101] Indeed, NE and isoproterenol (Iso) dilated rat cerebral arteries *in vivo* (Figure 3A–B), and similarly dilated and hyperpolarized rat cerebral arteries *in vitro* (Figure 3C–D). The vasodilator responses were inhibited by either the β1AR-selective blocker CGP20712 (Figure 3E–F)[105, 106] or the K_v1 channel blocker Psora-4, but not the β2AR-selective blocker, ICI18551. Additionally, isoproterenol-induced dilation was inhibited by the K_v1-C peptide that disrupts PSD95-K_v1 channel association and by a PKA inhibitor.[88] Collectively, these results suggest that a β1AR-PSD95-K_v1 signalosome (Figure 2) mediates the vasodilator response of cerebral arteries and arterioles to endogenous catecholamines or exogenous β-AR agonists.[101]

It is interesting then to consider whether β-AR blocking drugs (i.e., beta blockers); which are commonly administered to block cardiac β1ARs and thereby lower heart rate, slow atrioventricular conduction, and reduce blood pressure; could potentially compromise cerebral blood flow by disabling the β1AR-PSD95-K_v1 signalosome in cVSMCs. In this regard, a meta-analysis comparing β-AR blockers to other antihypertensive drugs showed that the relative risk of stroke was 16% to 26% higher in patients taking β-AR blockers compared to other antihypertensive drugs with similar blood pressure reduction.[107] Also, post-surgical patients with atherosclerotic risk administered a β-AR blocker for 30 days had a significantly higher incidence of stroke compared to a placebo group.[108] Furthermore, the new guidelines by the Eighth Joint National Committee (JNC 8) removed β-AR blockers as a first-line antihypertensive drug option,[109] reversing a previous recommendation by JNC 7.[110] This decision was based on evidence of increased stroke incidence after β-AR blocker use.[111] Alarmingly, β-AR blockers represent one of the most frequently prescribed drug classes. For example, metoprolol, a β1AR-selective blocker, is dispensed in more than 80 million prescriptions annually. It ranked 4th for medicines prescribed in the United States in 2013.[104] With growing recognition of the vasodilator function of β1AR in the cerebral circulation, and its association with other signaling molecules including K_v1 channels via PSD95, the impact of β-AR blockers and other medications that modify macromolecular signaling in cVSMCs will have to be considered when conducting risk:benefit assessment.

Future directions

The β1AR-PSD95-K_v1 signalosome described in this chapter represents only one of many potential receptor-ion channel interactions mediated by PSD95 and other MAGUK scaffolds in the cerebral vasculature. Considered alone, PSD95 has more than 50 known binding partners, most of which have not been explored as components of the PSD95 signalosome in cVSMCs. Future studies are needed to identify the upstream signals that regulate signaling

molecules in cVSMCs and the downstream effectors including ion channels that impact vascular tone. Additionally, the function of other MAGUK proteins in the cerebral circulation, for example, SAP97, is completely unexplored. SAP97 is known to interact with Kir channels[72] that contribute to neurovascular coupling, and impaired function of Kir channels has been implicated in a poor blood flow response to transient global cerebral ischemia.[112] The initial reports detailing scaffolding proteins in the rat cerebral circulation, including AKAP150 scaffolding of voltage-gated Ca^{2+} channels,[61] and PSD95-mediated association of β1AR and K_v1 channels,[101] may provide impetus to explore MAGUK-scaffolded signalosomes in other vascular beds. A clear definition of these vascular macromolecular complexes may represent an immediate opportunity to better decipher the complex networks that regulate vascular tone and affect pharmacological responses to medications, thereby impacting the lives of millions of patients with cardiovascular disorders.

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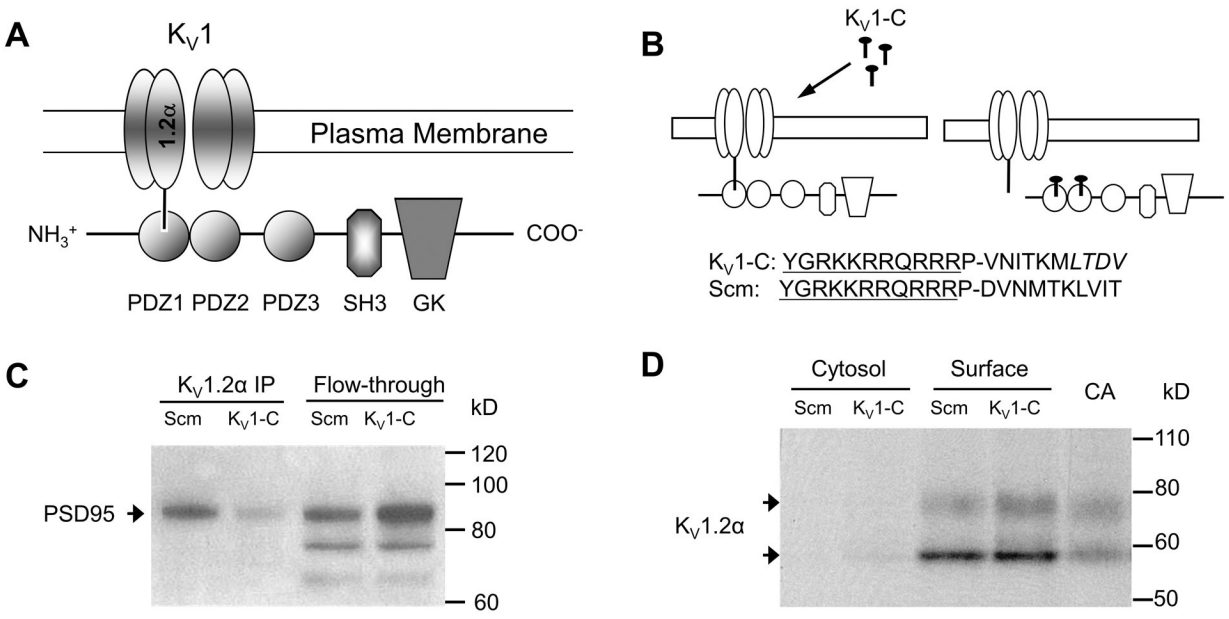

Figure 1. PSD95 interaction with the K_V1 channel and disruption by K_V1-C peptide.

A) Schematic of the association of the K_V1 channel α1.2 subunit with the PSD95 scaffold via the PDZ1 binding domain. PSD95 contains three PDZ binding domains (PDZ1–3), and Src-homology (SH3) and guanylate kinase (GK) domains. **B)** The K_V1-C dominant negative peptide was designed to compete for the PDZ binding domain on PSD95. The last 10 amino acids of the C-terminus of the α1.2 pore protein were conjugated to HIV-tat (YGRKKRRQRRR) to confer cell-permeability. P is a spacer. *LTDV* is a class-1 PDZ binding motif on the α1.2 subunit. A peptide with same amino acid composition in a scrambled order (Scm) was used as control. **C)** Immunoprecipitation using anti-K_Vα1.2 of rat cerebral arterial lysate treated with Scm or K_V1-C peptide for 30 min. The K_Vα1.2 immunoprecipitate and column flow-through (Flow-through) were probed for PSD95 on a Western blot. Depicted is a representative scan from three similar experiments showing that K_V1-C peptide disrupted PSD95 association with α1.2. **D)** Biotinylation of rat cerebral arteries treated with Scm or K_V1-C peptide for 30 min. Cytosolic and surface fractions were probed for the K_V1 channel α1.2 subunit. Control lysate from freshly isolated cerebral arteries (CA) was loaded for size comparison. Depicted is a representative blot from five similar experiments. K_V1-C did not alter the surface expression of K_V1 channel α1.2 subunits, which appear as a doublet band at ~ 58 kD and 80 kD; the upper band represents the glycosylated form. Figures and legend from reference 82.

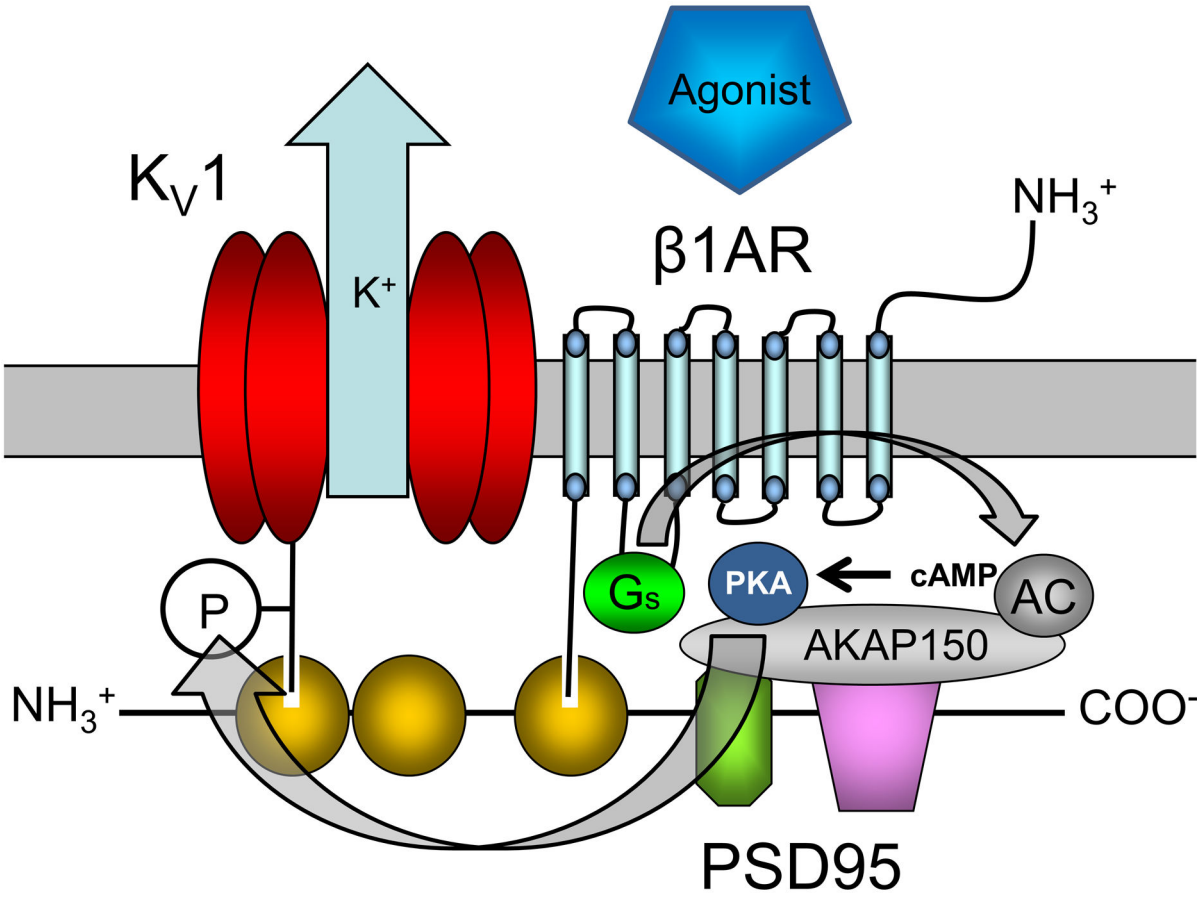

Figure 2. Proposed association of the β1-subtype adrenergic receptor (β1AR) and K_v1 channel on a PSD95 scaffold to form a "vasodilator signalosome" in cVSMCs.

The carboxyl termini of the β1AR and α1.2 pore protein of the K_v1 channel bind to PDZ domains on the PSD95 scaffold to create a receptor-effector signaling complex or "signalosome". Agonist binding to β1AR initiates the G_S-protein, adenylyl cyclase (AC), protein kinase A (PKA) cascade. This second-messenger cascade in cVSMCs culminates in PKA-phosphorylation (P) and activation of K_v1 channels, which promotes vasodilation of cerebral arteries. PDZ domains (yellow), SH3 domain (green octagon), guanylate kinase domain (purple), A-kinase anchoring protein (AKAP150), cyclic adenosine monophosphate (cAMP). Involvement of gray-colored proteins has not been tested directly in cVSMCs, but is speculated based on reported associations in other cell types. For simplicity, PSD95 is depicted linearly to illustrate proposed association of the receptor, signaling proteins and K_v1 channel. This two-dimensional schematic may not represent the actual relative positions of components in three-dimensional space. Figures and legend from reference 101.

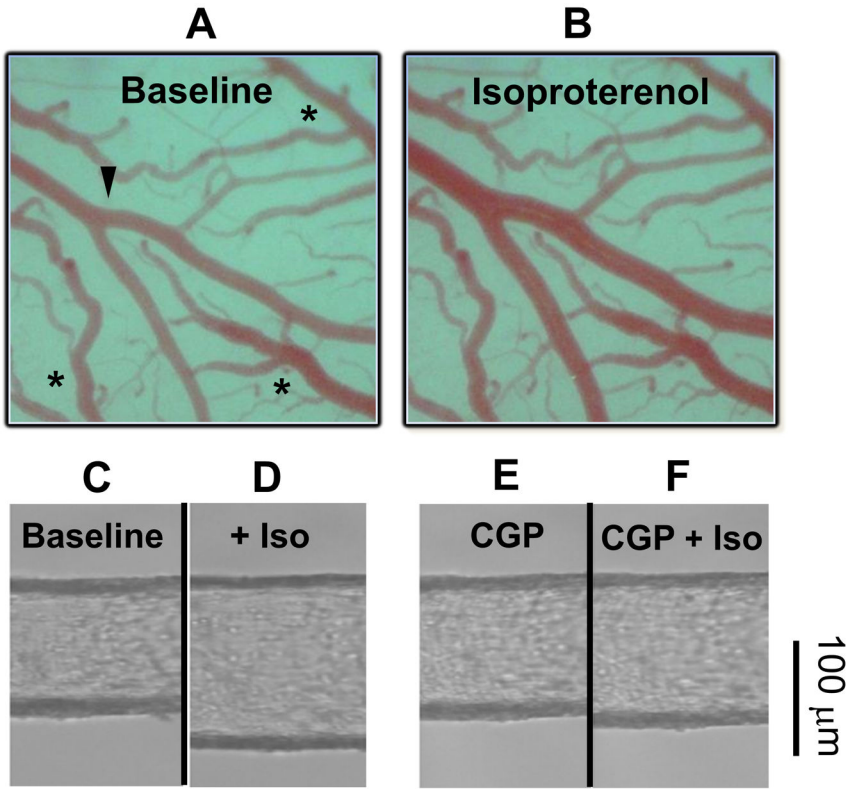

Figure 3. Isoproterenol-induced dilation of cerebral arteries *in vivo*.

A-B) Representative images of a rat middle cerebral artery branch (arrowhead) in a cranial window at (A) baseline and (B) in response to 10 μmol/L isoproterenol. Veins (asterisks) are unresponsive to isoproterenol. Figures and legend from reference 101. **C-F)** Image of a rat superior cerebellar artery cannulated and pressurized to 80 mmHg as performed in reference 101. After 1 hour of equilibration (C, Baseline), isoproterenol (Iso) was added to the bath in half-log concentrations between 1 nM and 10 μmol/L. The diameter response to the highest Iso concentration of 10 μmol/L is shown (D, Iso). After washout, the β1AR blocker CGP20712 was applied for 10 minutes (C, CGP) and then Iso was added cumulatively up to 10 μmol/L (D, CGP+Iso). Scale bar, 100 μm.