International Journal of
Molecular Sciences

MDPI

Review

Functional Interaction among K_{Ca} and TRP Channels for Cardiovascular Physiology: Modern Perspectives on Aging and Chronic Disease

Erik J. Behringer * and Md A. Hakim

Department of Basic Sciences, 11041 Campus Street, Risley Hall, Loma Linda University, Loma Linda, CA 92350, USA; mhakim@llu.edu

* Correspondence: ebehringer@llu.edu; Tel.: +1-909-651-5334; Fax: +1-909-558-0119

Received: 7 February 2019; Accepted: 15 March 2019; Published: 19 March 2019

Abstract: Effective delivery of oxygen and essential nutrients to vital organs and tissues throughout the body requires adequate blood flow supplied through resistance vessels. The intimate relationship between intracellular calcium ($[Ca^{2+}]_i$) and regulation of membrane potential (V_m) is indispensable for maintaining blood flow regulation. In particular, Ca^{2+}-activated K^+ (K_{Ca}) channels were ascertained as transducers of elevated $[Ca^{2+}]_i$ signals into hyperpolarization of V_m as a pathway for decreasing vascular resistance, thereby enhancing blood flow. Recent evidence also supports the reverse role for K_{Ca} channels, in which they facilitate Ca^{2+} influx into the cell interior through open non-selective cation (e.g., transient receptor potential; TRP) channels in accord with robust electrical (hyperpolarization) and concentration (~20,000-fold) transmembrane gradients for Ca^{2+}. Such an arrangement supports a feed-forward activation of V_m hyperpolarization while potentially boosting production of nitric oxide. Furthermore, in vascular types expressing TRP channels but deficient in functional K_{Ca} channels (e.g., collecting lymphatic endothelium), there are profound alterations such as downstream depolarizing ionic fluxes and the absence of dynamic hyperpolarizing events. Altogether, this review is a refined set of evidence-based perspectives focused on the role of the endothelial K_{Ca} and TRP channels throughout multiple experimental animal models and vascular types. We discuss the diverse interactions among K_{Ca} and TRP channels to integrate Ca^{2+}, oxidative, and electrical signaling in the context of cardiovascular physiology and pathology. Building from a foundation of cellular biophysical data throughout a wide and diverse compilation of significant discoveries, a translational narrative is provided for readers toward the treatment and prevention of chronic, age-related cardiovascular disease.

Keywords: Ca^{2+}-activated K^+ channels; transient receptor potential channels; intracellular Ca^{2+} homeostasis; endothelium-derived hyperpolarization; cardiovascular disease; aging

1. Introduction

Endothelial cells lining the lumen of resistance arteries command changes in vascular diameter as needed to meet the metabolic demand of vital organs and tissues throughout the body. In particular, the relationship of intracellular calcium ($[Ca^{2+}]_i$) to the hyperpolarization of membrane potential (V_m) is essential for a key signaling pathway underlying blood flow control, known as endothelium-derived hyperpolarization (EDH; i.e., activation of small- and intermediate-Ca^{2+}-activated K^+ (SK_{Ca}/IK_{Ca}) channels). In such a manner, endothelial cells coordinate with their surrounding smooth muscle cells via gap junctions for arterial relaxation and increased blood flow [1]. Physiological stimulation of EDH entails stimulation of G_q-protein-coupled receptors (G_qPCRs) and then an increase in $[Ca^{2+}]_i$ typically defined by initial Ca^{2+} release from the endoplasmic reticulum (ER) followed by a "plateau" phase

of extracellular Ca^{2+} influx into the cellular interior through open transient receptor potential (TRP) channels [2,3]. Vascular TRP channels may be recruited by Orai for Ca^{2+} influx in response to cytosolic stromal interaction molecule (STIM) oligomers that form as a result of ER Ca^{2+} depletion [4–7]. The influx of Ca^{2+} contributes serves a dual role to (i) sustain activation of intracellular enzymes and plasma membrane ion channels for prolonged cellular function, and (ii) to refill ER Ca^{2+} (via smooth endoplasmic reticulum Ca^{2+} ATPase (SERCA) pump activity) to maintain consistent and repetitive physiological function. During elevation of overall $[Ca^{2+}]_i$, SK_{Ca}/IK_{Ca} channels are activated, and the cell interior local to the inner leaflet of the plasma membrane increases in negative charge ("hyperpolarization") due to K^+ efflux through open SK_{Ca}/IK_{Ca} channel pores [8,9]. Thus, the intimate relationship of endothelial $[Ca^{2+}]_i$ and V_m is integral to blood flow regulation and, therefore, indispensable for cardiovascular function.

The aim of this review is to resolve the interaction of K_{Ca} and TRP channel function with $[Ca^{2+}]_i$ and electrical signaling during physiology and pathology. The current working paradigm of the "microanatomy" of the $[Ca^{2+}]_i$-to-electrical signaling interface underlying EDH is located to endothelial projections that traverse through the internal elastic lamina to contact smooth muscle cells (Figure 1). Endothelial projections contain spatially proximal myoendothelial gap junctions, IK_{Ca} channels, TRP channels, and inositol 1,4,5-trisphosphate receptors (IP_3Rs) of the endoplasmic reticulum [10–12]. When K_{Ca} channel function is present, robust V_m hyperpolarization occurs in response to sequential $[Ca^{2+}]_i$ release and Ca^{2+} influx, notably during treatment with a G_qPCR agonist (e.g., acetylcholine (ACh) or adenosine triphosphate (ATP)) or hydrogen peroxide (H_2O_2), which mimics conditions of oxidative stress during old age. When K_{Ca} channel function is absent, an otherwise rapid V_m hyperpolarization response converts into slow V_m depolarization due to accumulation of intracellular positive charges in the form of Na^+ and Ca^{2+} having entered through activated non-selective cation channels. With perspectives for future directions, we examine recent evidence demonstrating the functional contrasts among "normal" or "healthy" vascular aging and the development of chronic disease (e.g., hypertension, diabetes, heart failure, and coronary artery disease).

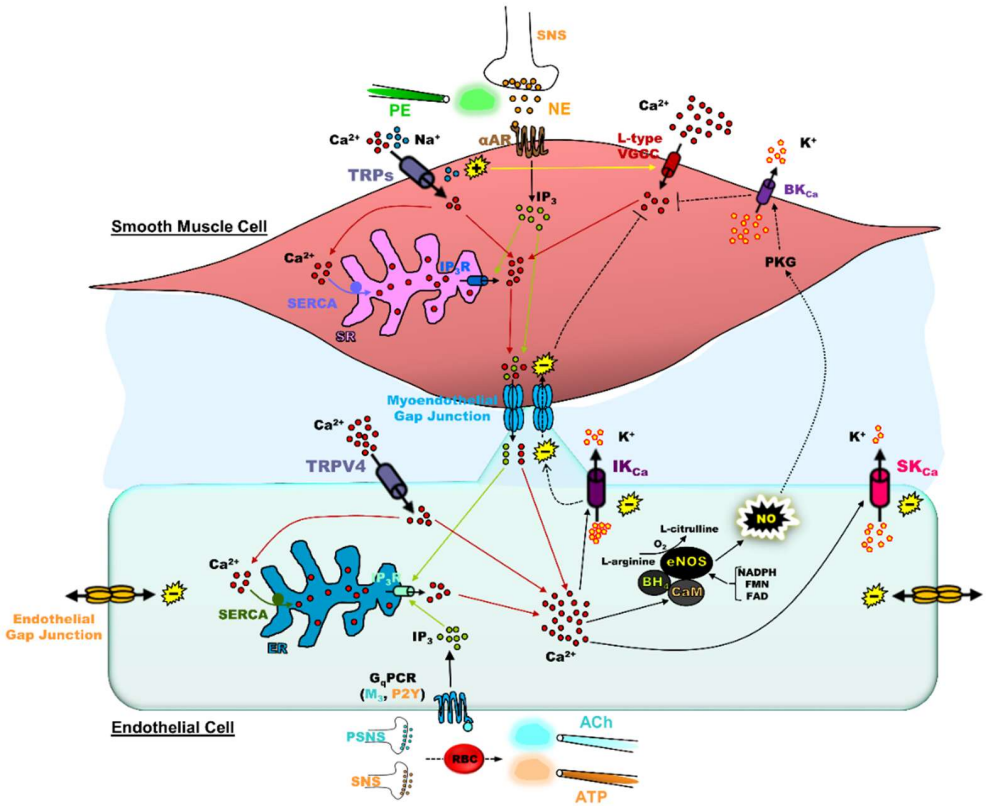

Figure 1. Anatomy of endothelium-derived hyperpolarization and myoendothelial coupling. Endothelium: Physiological stimulation of endothelial G_q-protein-coupled receptors (G_qPCRs; muscarinic

(M_3), purinergic (P2Y)) occurs through neurotransmitter secretion (parasympathetic nervous system (PSNS), acetylcholine (ACh); sympathetic nervous system (SNS), adenosine triphosphate (ATP)) or ATP release from red blood cells (RBCs) as primary examples. ACh and ATP are pharmacologically applied using bulk flow or focal delivery via pipettes (iontophoresis and pressure ejection). Following endothelial G_qPCR activation, inositol 1,4,5-trisphosphate (IP_3) is produced, which in turn activates IP_3 receptors (IP_3Rs) to release Ca^{2+} from the endoplasmic reticulum (ER) into the cytosol. ER Ca^{2+} release is observed as the initial "peak" response in $\Delta[Ca^{2+}]_i$. The ER Ca^{2+} stores are filled or refilled through uptake of Ca^{2+} from the cytosol into the ER through smooth endoplasmic reticulum Ca^{2+} ATPase (SERCA) pumps to sustain repetitive physiological signaling. The influx of Ca^{2+} occurs through TRP channels (e.g., vanilloid class, TRPV4) to help refill ER Ca^{2+} stores while integral to the secondary "plateau" phase of the $\Delta[Ca^{2+}]_i$ following G_qPCR stimulation. The increase in $[Ca^{2+}]_i$ leads to production of nitric oxide (NO) and/or activation of small- and intermediate-conductance Ca^{2+}-activated K^+ (SK_{Ca} and IK_{Ca}) channels. The production of NO is dependent on the conversion of L-arginine to L-citrulline in the presence of O_2 via endothelial NO synthase (eNOS). Endothelial NOS contains an oxygenase domain to bind L-arginine, heme, Zn^{2+}, and an essential cofactor tetrahydrobiopterin (BH_4); a calmodulin (CaM) domain to bind Ca^{2+}; and a reductase domain that binds to reducing agents nicotinamide adenine dinucleotide phosphate (NADPH), flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD). Activation of SK_{Ca} and IK_{Ca} channels generates hyperpolarization that is transmitted concomitantly along endothelial cells and to smooth muscle cells through myoendothelial gap junctions. Note that, regardless of endothelial Ca^{2+} mobilizing mechanism, endothelial $[Ca^{2+}]_i$ increases can regulate vascular tone ranging from normalization of vasoconstriction for basal tone (steady-state blood flow) to net decreases in vascular resistance (increased blood flow). Smooth muscle: Endothelium-derived hyperpolarization deactivates L-type voltage-gated Ca^{2+} channels (VGCCs) to prevent Ca^{2+} entry into smooth muscle cells (see broken lines with flat ends). Additionally, endothelial production of NO diffuses to smooth muscle and increases the activity of K^+ channels such as the large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel via cGMP-dependent protein kinase (PKG) for hyperpolarization, another signaling input for deactivation of L-type VGCCs. Although not covered in detail in this review, TRP channels (TRPs; e.g., TRPM4) are also expressed in smooth muscle cells and play a general role for depolarization of V_m, thereby activating L-type VGCCs for myogenic constriction. Note that, regardless of smooth muscle Ca^{2+} mobilizing mechanism, smooth muscle $[Ca^{2+}]_i$ increases can regulate vascular tone ranging from normalization of vasoconstriction for basal tone (steady-state blood flow) to net increases in vascular resistance (decreased blood flow). Myoendothelial feedback: Activation of α-adrenergic receptors (αARs) on smooth muscle by norepinephrine (NE) secreted by the SNS or the pharmacological α_1R agonist phenylephrine (PE) results in IP_3 production to elicit Ca^{2+} release through IP_3Rs in the sarcoplasmic reticulum (SR) to evoke vascular contraction. When elevated in smooth muscle, IP_3 and Ca^{2+} diffuse through myoendothelial gap junctions into the endothelium to activate SK_{Ca}/IK_{Ca} channels and/or NO production, providing negative feedback to smooth muscle contraction (see broken lines indicating signaling from endothelium back to smooth muscle). The (−) and (+) symbols indicate V_m hyperpolarization and depolarization respectively while the respective color of lines corresponds to Ca^{2+} (red), IP_3 (lime green), or Na^+ (light blue) signaling.

2. Significance of the Relationship of $[Ca^{2+}]_i$ and V_m in the Vascular Wall of Blood Vessels

Whether influenced by hormones, transmitters, or the shear forces of blood flow itself, the interplay between smooth muscle and endothelium of the vascular wall regulates resistance to blood flow in the form of vascular dilation or constriction [1]. Myoendothelial gap junctions between the two cell layers allow for the transmission of IP_3, Ca^{2+}, cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), or the general distribution of electrogenic ions (e.g., Na^+, K^+, or Cl^-) from endothelium to smooth muscle in response to stimulation of endothelial G_qPCRs (e.g., muscarinic, purinergic) [13,14], or vice versa following smooth muscle α-adrenergic receptor stimulation [15]. Fundamentally, the central mediator underlying coordination of vascular reactivity is Ca^{2+}, which activates endothelial nitric oxide synthase (eNOS) and the production of

nitric oxide (NO) concomitant with stimulation of SK_{Ca}/IK_{Ca} channels for EDH. The oxidoreductase activity of eNOS is described by the conversion of L-arginine to NO and L-citrulline with additional requirements of the Ca^{2+} sensor calmodulin, the co-factor tetrahydrobiopterin (BH_4; composes the oxygenase domain with heme), and nicotinamide adenine dinucleotide phosphate (NADPH; reductase domain) [16]; deficiency in L-arginine or BH_4 results in eNOS "uncoupling", whereby reactive oxygen species are produced [17]. Smooth muscle relaxation to eNOS-derived NO occurs via protein kinase G (PKG)-dependent phosphorylation of multiple ion pumps and channels (e.g., SERCAs or voltage-gated K^+ channels) in a manner that establishes a hyperpolarized smooth muscle V_m, reduced smooth muscle $[Ca^{2+}]_i$ and, thereby, reduced myosin light-chain phosphorylation [18,19]. Calmodulin also plays a primary role in SK_{Ca}/IK_{Ca} channel activation via the sensing of $[Ca^{2+}]_i$ using C- and N-lobe EF hand domains to open channel pores and allow K^+ efflux from the endothelial cell. However, in studies using *Xenopus* oocytes and the inside-out patch clamp configuration to examine intracellular regulation of SK_{Ca} channels, it was found that the C-lobe may play a dispensable role for modulating Ca^{2+} affinity, whereas the N-lobe in particular constitutively stabilizes K_{Ca} subunits for activation [20]. The resulting hyperpolarization of endothelial V_m transmits to the smooth muscle via myoendothelial gap junctions [21,22], whereby L-type voltage-gated Ca^{2+} channels are deactivated, and in like fashion with the NO/cGMP/PKG pathway, smooth muscle $[Ca^{2+}]_i$ is ultimately reduced to promote vasodilation [23].

Original investigations of the structural resolution of myoendothelial gap junctions [24,25] and functional determinations of myography and electrophysiology [26] altogether revealed regional contributions of EDH vs. NO to vasodilation along the vascular network. In particular, myoendothelial gap junctions are composed of connexins (Cxns) Cx37, Cx40, and Cx43 [11,27,28] as required for the spread of EDH from the endothelium to the smooth muscle, a mechanism that plays a prominent role in small arteries and arterioles [29]. Shimokawa et al. showed that the contribution of EDH to endothelium-dependent relaxations rises as vessel size (diameter) decreases in six- to eight-month-old male rats [26]. In particular, the range of the contribution of EDH was >2-fold when extending from aorta (~30%) to the proximal (~46%) and then to the distal (~72%) mesenteric arteries, whereas trends in NO-dependent vasodilation were the opposite (aorta: ~56%, proximal: ~17%, distal: ~20%). It is also worth noting that the contribution of prostacyclin (PGI_2) was negligible regardless of blood vessel size. Thus, when examining Ca^{2+} and electrical signaling underlying EDH or NO, it is important to consider the anatomical position of the arterial segment throughout the conduit and resistance blood vessel network feeding into each organ in the body.

Altogether, regardless of source (intracellular release or plasma membrane entry), increased $[Ca^{2+}]_i$ plays a dichotomous role in the smooth muscle vs. endothelial cell layers (See Figure 1 Legend; smooth muscle $[Ca^{2+}]_i$ increase → depolarization → L-type Ca^{2+} channel activation → myosin light-chain phosphorylation → vasoconstriction vs. endothelial $[Ca^{2+}]_i$ increase → SK_{Ca}/IK_{Ca} channel activation → hyperpolarization → myosin light-chain dephosphorylation → vasodilation) and maintains a narrow window of effective blood flow regulation [30,31] while preventing vascular rupture or ischemia. With some exception (e.g., direct PKG activation of myosin light-chain phosphatase and subsequent dephosphorylation of myosin light chain [32]), the cross-talk between $[Ca^{2+}]_i$ and V_m is the "master regulator" for the coordination of blood flow throughout vascular resistance networks regardless of the mode of the upstream cellular signaling pathway. The most direct bridge between these two physiological variables is EDH with SK_{Ca}/IK_{Ca} channels as the transducers of increased $[Ca^{2+}]_i$ to hyperpolarization of the V_m throughout the vascular wall.

Recent perspective points to an initial rapid role for EDH during vasodilation following the onset of physical activity and skeletal muscle contraction, whereas NO signaling underlies a secondary prolonged but slower vasorelaxation for sustained blood flow per lumenal sheer stress [1]. It is also worth noting that the spatial domain of signaling for NO is on the order of hundreds of microns vs. thousands of microns for EDH along the vascular wall encompassing from large extraparenchymal arteries to capillaries. Furthermore, a phenomenon of G_qPCR-stimulated "slow"

Ca^{2+} waves (~100 µm/s vs. cm/s for electrical conduction) among and along the endothelial cell layer may govern the spatial activation of both NO and EDH [33,34]. Although, as described, Ca^{2+} waves occur within an order of timing most consistent with the production and signaling of NO. It is possible that the amplitude, distance, and/or speed of a Ca^{2+} wave can be enhanced by direct opening of SK_{Ca}/IK_{Ca} channels to hyperpolarize V_m, thereby increasing the electrical gradient for Ca^{2+} to move into cells through open TRP channels, a phenomenon known as "hyperpolarization-induced Ca^{2+} entry" [3]. In its entirety for intra- and intercellular signaling, this hypothesis was proposed >5 years ago [34], and now has support with findings of enhancement in NO production [35] and the feed-forward activation of EDH [3,36]. Of course, there are limits to the effectiveness of SK_{Ca}/IK_{Ca} channel activation, vasodilation, and the delivery of blood flow, as we found that hyperpolarization of V_m >−60 mV results in current leak that reduces the spread of hyperpolarization among endothelial cells by more than half [37,38]. In addition to the diminishment of sufficient resting vascular tone [10], the consequence of the "over-activation" of SK_{Ca}/IK_{Ca} channels entails significant charge loss through electrically "leaky" membranes, thereby impairing effective cell-to-cell vasoreactivity coordination of blood flow along vascular resistance networks [1,21]. Accordingly, as the relationship between V_m and the increase in $[Ca^{2+}]_i$ is only linear from ~−25 to −60 mV [3], and as only 10 to 15 mV of hyperpolarization from resting (~−30 to −40 mV) is needed for maximal vasodilation [39,40], "more" is certainly not "better" with respect to K_{Ca} channel function.

3. Significance of SK_{Ca}/IK_{Ca} and TRP Channels in the Blood Vasculature

Properties of transmembrane ion channel activity include the electrical and driving forces on the ionic species that are permeant through the channel pore, channel conductance for ease of current flow, and the number of channels (*n*) multiplied by their individual probability of opening (P_O). The SK_{Ca}/IK_{Ca} channels are permeant to K^+ ions which are favored for outward movement in accordance with the concentration gradient ($[K^+]_o/[K^+]_i$: ~0.030) despite an opposing electrical gradient (the intracellular side of the plasma membrane is negatively charged and attracts cations). The V_m must be as high as −90 mV at physiological temperature (37 °C) in order to bring transmembrane K^+ flux to equilibrium (E_K). For reference, vascular endothelial cells typically have a V_m of ~−30 to −40 mV during rest, and activation of SK_{Ca}/IK_{Ca} channels alone using NS309 can increase V_m to E_K [37,41]. As a dominant form of K^+ channel expressed in the endothelial membrane, the SK_{Ca}/IK_{Ca} channels are tetrameric (four subunits), voltage-independent (from V_m ~−80 to +10 mV [37]), and are constitutively bound to calmodulin binding sites for $[Ca^{2+}]_i$ [42]. The physiological "agonist" is Ca^{2+}, whereby the P_O of the channels is determined by the $[Ca^{2+}]_i$ needed to produce half-maximal activation for opening channel pores ($K_{0.5}$) and cooperativity among the four subunits of the channel per number of Ca^{2+} ions (Hill coefficient; HC).

The SK_{Ca} channels were first identified for their role in the "after hyperpolarization" phase following the firing of individual action potentials in central neurons of the mammalian brain [43]. SK_{Ca} channels ($K_{Ca}2.1$, $K_{Ca}2.2$, and $K_{Ca}2.3$, or SK1, SK2, and SK3), have a relatively "small" conductance (~5 to 20 pS), a high affinity for Ca^{2+} ($K_{0.5}$: ~400 to 700 nM), and a steep dependence on Ca^{2+}, whereby at least four Ca^{2+} ions (HC = 3.9 to 4.8) are involved in the cooperativity of channels subunits for gating the pore ([43]; SK messenger RNA (mRNA) extracted from human (SK1) and rat (SK2 and SK3) brain and cloned in *Xenopus* oocytes). Later, the $K_{Ca}2.3$ (or SK3) channels in particular were demonstrated to play a role in endothelial cell regulation of vascular tone in mesenteric arteries and systemic blood pressure [44] using a $SK3^{T/T}$ mouse model, whereby the $K_{Ca}2.3$ gene promoter is governed by a tetracycline-sensitive transactivator protein [45]. Relative to wild type, the mesenteric arteries of $SK3^{T/T}$ mice contained elevated expression of $K_{Ca}2.3$ channels that were suppressed upon treatment with the tetracycline derivative doxycycline (DOX) to the extent that SK_{Ca} currents were ~25-fold lower in endothelial cells; thus, resting endothelial V_m was depolarized by ~14 mV. Also, vasoconstriction due to intravascular pressure (20 or 100 mmHg) or direct stimulation of smooth muscle α_1-adrenergic receptors using phenylephrine was enhanced by >20% with blocking SK_{Ca}

channels via apamin or removal of the endothelium in $SK3^{T/T}$ mice (no DOX) or by genetically suppressing SK_{Ca} channel expression in $SK3^{T/T}$ mice fed DOX. Furthermore, genetic deletion of SK_{Ca} channels increased diastolic (~94 to 118 mmHg) and systolic (~128 to 147 mmHg) blood pressure (mean arterial: ~105 to 128 mmHg) [44].

Relative to SK_{Ca} channels, IK_{Ca} (K_{Ca} 3.1, SK4, or IK1) channels have an "intermediate" conductance (=39 pS), reduced cooperativity of individual subunits per Ca^{2+} ion (>50%; HC = 1.7), and a similarly high Ca^{2+} affinity with a $K_{0.5}$ of ~300 nM ([46]; IK1 mRNA extracted from human pancreas and cloned in *Xenopus* oocytes). The IK_{Ca} channels are generally absent in excitable cells such as neurons and cardiac myocytes [46–48] with an originally defined role characterized in immune cells [49]. However, similar to the role of SK_{Ca} channels for hyperpolarizing cellular V_m, the importance of the IK_{Ca} channel is now well established for endothelium-dependent vascular function [50]. A comprehensive demonstration of the cardiovascular role of endothelial $K_{Ca}3.1$ channels was achieved using a $K_{Ca}3.1^{-/-}$ (or $IK^{-/-}$) mouse model with a genetic deletion of exon 4 coding the channel pore [51]. As a result, the overall endothelial K_{Ca} current densities were reduced by ~50%, while endothelial V_m hyperpolarization in response to ACh decreased by ~60%. Accordingly, measurements of maximal EDH-dependent vasodilation in response to ACh in isolated, pressurized carotid arteries ex vivo and cremaster arteries in vivo were less by ~30 to 50%. The physiological consequence entailed increased systolic and diastolic blood pressure by ~13 mmHg and ~12 mmHg, respectively (Δ mean arterial: ~14 mmHg) and mild left ventricular hypertrophy in $K_{Ca}3.1^{-/-}$ mice [51]. Thus, endothelial IK_{Ca} channels are fundamental for endothelial hyperpolarization underlying regulation of vascular tone, blood flow, and blood pressure, determinants that can altogether impact chronic cardiac remodeling as well.

Following thorough characterization of individual genetic models for SK_{Ca} and IK_{Ca}, further studies utilized $SK3^{T/T}IK^{-/-}$ offspring generated by interbreeding $SK3^{T/T}$ and $K_{Ca}3.1^{-/-}$ mice, respectively [52]. This approach allowed for the delineation of the individual roles of SK_{Ca} and IK_{Ca} to EDH and regulation of blood pressure in a combinatorial manner. As expected, the combined genetic SK_{Ca} and IK_{Ca} deficiency eliminated endothelial K^+ currents and substantially impaired (>50%) smooth muscle hyperpolarization of carotid arteries from $IK1^{-/-}/SK3^{T/T}$ mice (+DOX). Consequently, IK_{Ca} deficiency ($IK^{-/-}/SK^{+/+}$ mice) reduced ACh-induced EDH-mediated vasodilation by ~75%, whereas deficit of both channels ($IK^{-/-}/SK3^{T/T}$ + DOX mice) eliminated responses altogether (by ~99%). A reduction in ACh-induced vasodilation in either carotid arteries ex vivo or cremasteric arteries in vivo was not apparent in mice with a SK_{Ca} deficiency alone ($IK^{+/+}/SK^{T/T}$ + DOX). The mean arterial pressure relative to wild-type mice (~100 mmHg) modestly increased with SK_{Ca} deficiency (to ~106 mmHg), IK_{Ca} deficiency (to ~108 mmHg), or combined SK_{Ca}/IK_{Ca} genetic deletion (to ~110 mmHg) [52]. An additional study of the double transgenic study concluded that SK_{Ca} channels, but not IK_{Ca} channels, played an integral role for vasodilation of cremasteric arterioles in response to tetanic muscle stimulation [53]. Also, it is also worth noting that the SK_{Ca} channel-driven vasodilation requires Cx40 endothelial and myoendothelial containing gap junctions [53], whereas IK_{Ca}-dependent responses do not require Cx40 during hyperemia of the mouse cremaster tissue [54]. These general findings of the endothelial role of SK_{Ca} vs. IK_{Ca} channels were further reinforced using an endothelium-specific SK3 knock-out mouse model generated from crossing a floxed SK3 mouse with another that expresses endothelial Cre recombinase driven by the endothelial receptor-specific tyrosine kinase (or Tie2) promoter [55].

The majority of the work delineating the individual contributions of SK_{Ca} and IK_{Ca} channels to vasodilation and tissue hyperemia was performed using mesenteric (gut) arteries as an accessible and abundant source of resistance arteries in the body [56–58]. However, to a lesser extent, studies also resolved clear contributions of respective K_{Ca} channel subtypes in the microcirculation of the eye [59], brain [60–62], heart [63,64], lung [65,66], skeletal muscle [8,67], and kidney [68–70]. While it is clear that both SK_{Ca} and IK_{Ca} channels are integral to overall cardiovascular health (systemic vascular resistance and blood pressure control), respective K_{Ca} contributions underlying hyperemia

throughout organs may vary. A common feature at the biophysical level in isolated endothelium is that IK_{Ca} (vs. SK_{Ca}) channels play a predominant role in the regulation of V_m and membrane resistance during steady-state and pharmacological conditions in the presence of direct (e.g., NS309 and SKA-31) or indirect (e.g., ACh and ATP) K_{Ca} openers [8,71]. At the level of vasoreactivity and regulation of blood flow, evidence supports SK_{Ca} channels as the "affinity" component (sensitivity of vasodilation/hyperemia per unit stimulus), whereas IK_{Ca} channels define "efficacy" (amplitude of vasodilation/hyperemia per unit stimulus) [51,52,72].

Equipped with an enhanced electrical gradient via hyperpolarization [3] in accordance with a ~20,000-fold transmembrane concentration gradient [73], Ca^{2+} influx through open Ca^{2+}-permeant TRP channels may serve at least two purposes: (i) to sustain the duration of elevated $[Ca^{2+}]_i$ for prolonged control of blood flow, and (ii) to facilitate Ca^{2+} refilling of the endoplasmic reticulum to maintain continued, repetitive vascular function [8]. Note that endothelial TRP channels involved in EDH can be homo- or heteromeric among families and respective isoforms: canonical (TRPC; isoforms 1, 3, 4, 5, and 6), vanilloid (TRPV; isoforms 1, 3, and 4), ankyrin (TRPA; isoform 1), and polycystin (TRPP; isoform 2) [74,75]. Functional examples of heteromeric configurations of vascular TRPs include TRPC3-C4 [76], TRPV4-C1 [77–79], and TRPV4-C1-P2 [80]. Due to recent breakthroughs in pharmacological and genetic tools [81,82], the role of TRPV4-containing channels is the most widely studied thus far [3,83,84].

In general, most experimental evidence demonstrates that TRP channel function produces EDH via $[Ca^{2+}]_i$ activation of SK_{Ca} and IK_{Ca} channels governing vascular dilation, thereby promoting increases in blood flow (Figure 2A). However, TRP channels can regulate EDH in a positive or negative manner based on relatively permeability of Ca^{2+} to Na^+ ions of a particular TRP channel configuration. Whereas TRPC1-containing channels are relatively low conductance (~16 pS) and non-selective (P_{Ca}/P_{Na} ~1:1; [85]), TRPC6 (~35 pS, P_{Ca}/P_{Na} ~5:1; [86]) or TRPV4 (~90 pS, P_{Ca}/P_{Na} ~6:1; [87]) channels are examples of high conductance, dominant for mediating Ca^{2+} (vs. Na^+) influx. Consequently, TRPC1-containing channels in mouse resistance arteries mediate a net depolarizing influence on V_m, counteracting EDH, whereas TRPC6- [88] and TRPV4-containing channels [83] augment EDH in the presence of functional SK_{Ca}/IK_{Ca} channels. Depolarization of endothelial V_m is due to relatively higher Na^+ permeability and influx, whereas net hyperpolarization is a result of prominent influx of Ca^{2+} because, unlike highly electrogenic Na^+ ions, Ca^{2+} ions are primarily second messengers that rapidly bind with intracellular proteins such as calmodulin to activate SK_{Ca}/IK_{Ca} channels (as one example). Similar to endothelial TRPC1, smooth muscle TRP melastatin (TRPM, isoform 4; ~25pS, not Ca^{2+} permeant [89]) channels also permit Na^+ influx to depolarize smooth muscle V_m, thereby activating L-type voltage Ca^{2+} channels for myogenic constriction [90]. Remarkably, blocking TRPM4 channels in rat mesenteric arteries using 9-phenanthrol also hyperpolarizes endothelial V_m via apparent enhancement of IK_{Ca} channel function [91]. Also, in mouse collecting lymphatic endothelium, where TRPV4 channels are present but functional SK_{Ca}/IK_{Ca} channels are absent (i.e., no EDH), depolarization of V_m in response to TRPV4 channel activation occurs almost exclusively due to Na^+ influx [92] (Figure 2B). Subtle variations of positive or negative regulation of EDH will depend on oligomeric configurations of the TRP channel to govern permeability of one form of cationic species vs. another, and, in this regard, much remains to be resolved in physiological study models vs. heterologous cell culture systems.

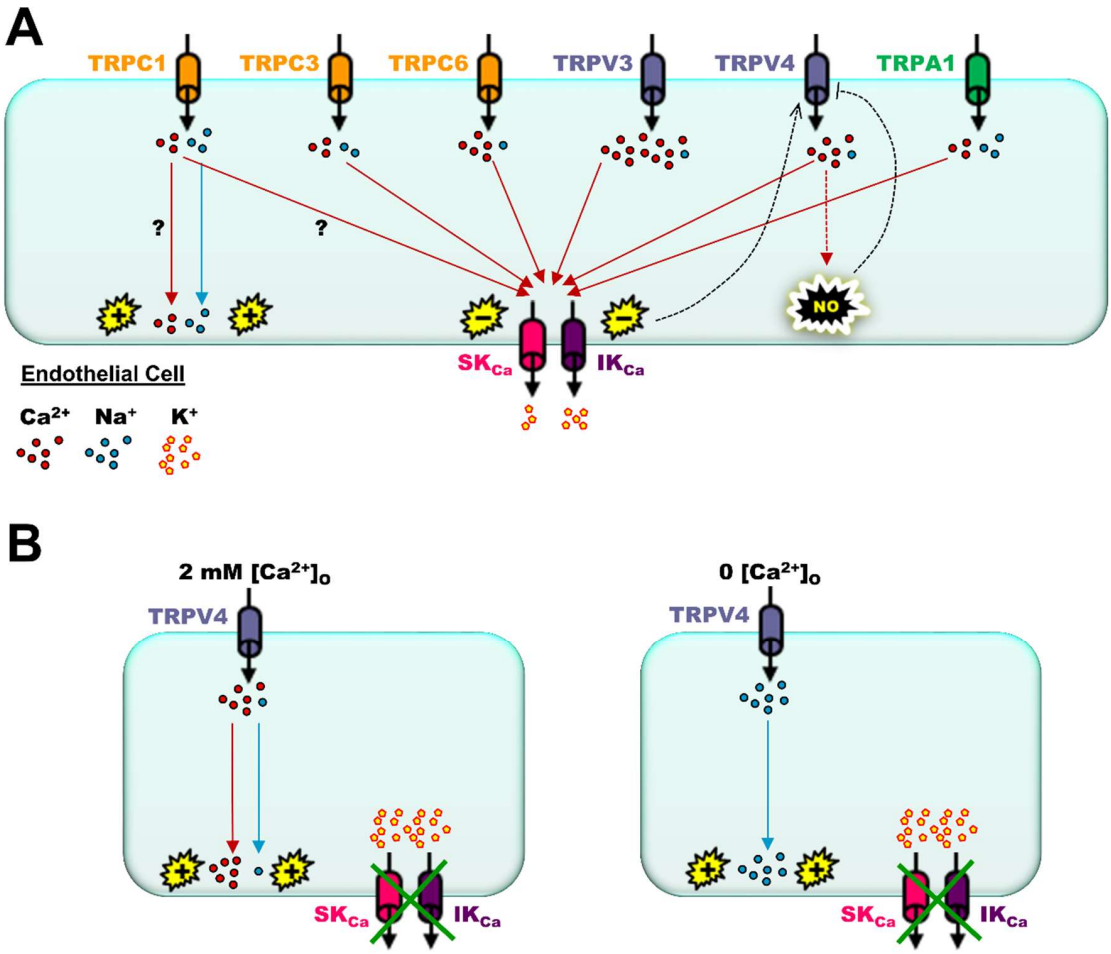

Figure 2. Functional contribution of major transient receptor potential (TRP) channel isoforms to activation of small- and intermediate-Ca^{2+}-activated K^+ (SK_{Ca} and IK_{Ca}) channels and endothelium-derived hyperpolarization. (**A**) Permeability of Ca^{2+} to Na^+ ions (P_{Ca}/P_{Na}) varies across the canonical (TRPC), vanilloid (TRPV), and ankyrin families of TRP channels. However, with the exception of TRPC1, endothelial TRP channels underlie significant influx of Ca^{2+} to activate SK_{Ca} and IK_{Ca} channels. As discussed in the text, note that these individual TRP isoforms can form tetrameric TRP channels via heteromeric combinations (e.g., TRPV4–TRPC1, TRPC3–TRPC4) in a physiological setting that may not be representative of determinations of homomeric channels expressed in heterologous culture systems. As it relates to the competition with Na^+ influx, the "?" symbol indicates the unknown contribution of Ca^{2+} influx through TRPC1-containing channels for V_m depolarization vs. activation of SK_{Ca} and IK_{Ca} channels for V_m hyperpolarization as both are theoretically possible. Hyperpolarization via activation of SK_{Ca}/IK_{Ca} channels may stimulate Ca^{2+} influx through TRPV4-containing channels for further activation of SK_{Ca} and IK_{Ca} channels and production of NO ("positive" feedback; broken black arrow). Although, nitric oxide (NO) may inhibit TRPV4-containing channels ("negative" feedback) via *S*-nitrosylation (broken black line with flat end). (**B**) In the absence of functional SK_{Ca} and IK_{Ca} channels, Ca^{2+} and Na^+ influx (P_{Ca}/P_{Na} ~6:1; extracellular Ca^{2+} ($[Ca^{2+}]_o$) present) through TRPV4-containing channels leads to endothelial depolarization (**left panel**). In the absence of $[Ca^{2+}]_o$, Na^+ influx through TRPV4 channels is robust, and depolarization occurs regardless of SK_{Ca} and IK_{Ca} channel presence and function (**right panel**). Green crosses over SK_{Ca} and IK_{Ca} channels denote their functional absence in the plasma membrane (e.g., collecting lymphatic endothelium). For all panels, (−) and (+) symbols indicate V_m hyperpolarization and depolarization respectively while the respective color of lines corresponds to Ca^{2+} (red) or Na^+ (light blue) signaling. All lines with arrow ends indicate positive signaling of respective ions to ultimately effect a change in V_m (hyperpolarization or depolarization) or production of NO.

Since original myoendothelial investigations around the turn of the 21st century [24,25], the use of transgenic animal models and confocal microscopy greatly advanced cardiovascular studies of "microdomain" signaling among spatially localized K_{Ca} and TRP channels. The Taylor laboratory group in particular developed auto-detection and analysis algorithms to quantitate discrete Ca^{2+} events throughout cellular interior with primary data outputs of $\Delta[Ca^{2+}]_i$ event amplitude, frequency, duration, and spatial spread [93–95]. Throughout such studies, mesenteric arterial endothelium was examined using an en face study model, whereby experimentally feasible arteries (diameter: ~300 μm) for this protocol were cut open longitudinally and pinned down into silicone with the endothelium facing up toward the microscope objective. In comparison to wild type, the number of ACh-induced Ca^{2+} events during "plateau" responses (predominant TRP channel activity) are reduced by ≥60% in genetic elimination of IK1 ($IK1^{-/-}$), whereas basal Ca^{2+} dynamics are similar [95]. In tandem with elimination of IK1, genetic SK3 suppression ($IK1^{-/-}/SK3^{T/T}$ + DOX) or SK3 overexpression ($IK1^{-/-}/SK3^{T/T}$ − DOX) has minimal effects on the occurrence of Ca^{2+} events under both basal and ACh-stimulated conditions [95]. In addition to removal of extracellular Ca^{2+}, pharmacological corroboration of these findings was demonstrated with a block of SK_{Ca} and IK_{Ca} channels (apamin + charybdotoxin) or TRPV4 channel block with HC-067047, whereby ACh Ca^{2+} dynamics in wild-type arteries were reduced to the level of mice genetically deficient for both SK3 and IK1 ($IK1^{-/-}/SK3^{T/T}$ + DOX) [95]. A follow-up study characterized endothelial dynamic Ca^{2+} signals during basal and conditions of Substance P-stimulated vasorelaxation in swine coronary endothelial cells. This investigation detected endothelial spatial and temporal events governing arterial tone that were not apparent using averaged, whole-field measurements of $[Ca^{2+}]_i$ [96]. With the use of an endothelium-specific SK3 knock-out mouse model, it was found that the coupling of SK3 and TRPV4 channels in mesenteric arteries appears to elicit large-amplitude and slow-decay Ca^{2+} kinetics that may be consistent with relatively long-term physiological delivery of blood flow in response to metabolic demand of active tissues [55].

Another foundational study demonstrated how small (diameter: ~100 μm) mesenteric endothelial TRPV4 channel-mediated Ca^{2+} "sparklets" activate SK_{Ca} and IK_{Ca} channels using wild-type, endothelial genetic Ca^{2+} sensor (GCaMP2Cx40) and $TRPV4^{-/-}$ mice and customized Ca^{2+} event detection software; only a few open TRPV4 channels are required for maximal vasodilation [83]. Later, it was found that this coupling between endothelial K_{Ca} and TRP channels was due, at least in part, to the presence of protein kinase C (PKC) and PKC-anchoring protein AKAP150 or, otherwise, hypertension would ensue [12]. However, this scenario can change depending on vessel type as, in mouse pulmonary arteries, TRPV4 Ca^{2+} "sparklets" preferentially activate eNOS in a negative feedback manner, whereby NO-activated PKG inhibits cooperative opening of TRPV4 channels [66] (Figure 2A). Remarkably, a decrease in intralumenal pressure on its own (from 80 mmHg to ≤50 mmHg of rat cremasteric arterioles) can significantly increase TRPV4-mediated Ca^{2+} events that selectively activate IK_{Ca} vs. SK_{Ca} channels, leading to a loss of myogenic tone and blood flow autoregulation [10]. Also, using a novel gradient index (or GRIN) fluorescence microendoscopy method positioned inside of rat carotid arteries, ACh-induced widefield Ca^{2+} events (summation of IP_3R release and TRP Ca^{2+} influx) decreased with increasing pressure (60, 110, and 160 mmHg), an effect that was diminished during late middle age (18 mo) vs. youth (3 mo) [97].

Although studied to a lesser extent vs. TRPV4 channels, the activation of SK_{Ca} and IK_{Ca} channels occurs downstream of other novel families and/or isoforms of TRP channels as well. In particular, TRPV3 channels contain ~2-fold the unitary conductance and Ca^{2+} permeability (~150 to 200 pS, P_{Ca}/P_{Na} ~12:1; [98]) of TRPV4 channels [87] and, thus, may be more pertinent for activating eNOS and/or EDH. With use of total internal fluorescence (TIRF) microscopy and the oregano monoterpenoid carvacrol (TRPV3 activator; [99]), TRPV3-to-SK_{Ca}/IK_{Ca} channel coupling was characterized in rat cerebral parenchymal arterioles [100]. Notably, carvacrol-induced vasodilation was not sensitive to block of NO and cyclooxygenase signaling and was almost completely abolished upon conditions of either SK_{Ca} or IK_{Ca} block alone [100]. Another TRP channel of the ankyrin family (TRPA1;

activated by mustard oil, P_{Ca}/P_{Na} ~1:1; [101]) was found to contribute to endothelial SK_{Ca} and IK_{Ca} channel activation as well and in a manner insensitive to NO and cyclooxygenase signaling [102]. Despite very modest Ca^{2+}-permeant properties vs. TRPV3- and TRPV4-containing channels, TRPA1 channel function may be involved in microdomain myoendothelial signaling for activation of K_{Ca} channels during physiology [103] and conditions of enhanced oxidative signaling [104]. Finally, in similar fashion to TRPV3, TRPV4, and TRPA1, the role of TRPC3 (~70 pS, P_{Ca}/P_{Na} ~1.5:1; [105]) was thoroughly characterized for its coupling to SK_{Ca}/IK_{Ca} channels in mouse cerebral arteries [71] and rat mesenteric arteries [106] using a selective antagonist as the pyrazole compound Pyr3.

When interpreting studies on TRP channel expression and function, it is important to bear in mind that TRP channels are tetrameric in their physiological form and may contain only one isoform (homotetrameric) or two to four different isoforms (heterotetrameric). Parameters of conductance and Ca^{2+} permeability are primarily determined in heterologous cell culture systems that only express homotetrameric TRP channels to determine parameters of individual TRP isoforms. Furthermore, genetic tools (e.g., TRP knock-out animals) do not necessarily manifest results consistent with pharmacological agents (e.g., TRP blockers) [3,107], as the former method prevents genetic expression of the TRP isoform and perhaps decreases the total number of functional TRP channels overall, whereas the latter strategy modulates a post-translational TRP channel product. Again, for pharmacological interventions, heterotetrameric TRP channel configurations were considered and, thus, potential insensitivity to agonists or antagonists were demonstrated to be effective for homotetrameric channels formed in a heterologous system.

4. Impact of Adrenergic Tone on SK_{Ca}/IK_{Ca} and TRP Channels & Significance of In Vivo vs. Ex Vivo Observations

A central (and extremely complex) research topic entails how the stimulation of smooth muscle α-adrenergic receptors (α1ARs) influences the interaction between endothelial K_{Ca} and TRP channels during "myoendothelial feedback" [24,30,31]. In isolated mouse mesenteric arteries, activation of smooth muscle α1ARs elicits vasoconstriction for ~2 min followed by a slow dilation induced by increases in endothelial TRPV4-mediated Ca^{2+} influx and activation of K_{Ca} channels, a mechanism that may cease prolonged vascular resistance and hypertension as observed in $TRPV4^{-/-}$ mice [108]. Whether the ionic species directly passing from smooth muscle to endothelial cells through gap junctions is in the form of IP_3 (activator of IP_3Rs and Ca^{2+} release from endothelial ER) [109], Ca^{2+} [14], or perhaps both [110], in the context of animal species (e.g., rat vs. mouse), blood vessel type (e.g., mesenteric vs. skeletal muscle) and/or experimental conditions (e.g., ex vivo vs. in vivo) remains a topic of active investigation. In addition, there is a complex interplay among the relative contributions of endothelial NO, SK_{Ca} channels, and IK_{Ca} channels as it pertains to the coordination of local and conducted vasodilation signaling. As a key example, a recent study demonstrated that isolated rat mesenteric arteries in the presence of norepinephrine mediate myoendothelial feedback primarily through activation of IK_{Ca} channels, whereas, with ongoing physiological sheer stress to blood flow, feedback is in the form of SK_{Ca} channel activation and NO production in the intact mesenteric network [111].

Despite efforts for experimental controls and proposals for simplified working models across vascular types and methods of pre-constricted tone, studies of myoendothelial feedback remain empirically convoluted by nature. To begin with, it is inherently difficult to delineate Ca^{2+} signaling events among respective smooth muscle and endothelial cell layers with common molecular targets (e.g., TRP channels and ER IP_3Rs) of interest. Next, there are factors of interventional drug delivery (ablumenal vs. lumenal), permeability (cell-permeant vs. extracellular), and target selectivity to contend with. In addition, whether via means of a chemical fluorescent dye or genetic sensor, compaction or dilution of Ca^{2+} sensor molecules can alter with cellular mechanical movement regardless of distinct stimuli or pharmacological interventions. Also, as in the case of in vivo experiments, halogenated (e.g., isoflurane) or injectable (e.g., pentobarbital) anesthetics can influence vascular function on their own toward the alteration of endothelial function [112,113]. Furthermore, even agents that are

commonly used for cell/tissue-specific genetic manipulations (e.g., tamoxifen (estrogen receptor partial agonist/antagonist) and DOX (tetracycline antibiotic)) and, interestingly, the choice of vehicle solvent (e.g., sunflower vs. peanut oil) [114] may introduce experimental artefacts. Thus, scientific corroboration among complementary methods such as a vascular co-culture method [115], pressure myography [116], microendoscopy imaging [117], and intravital microscopy [118] helps to clarify myoendothelial signaling events during health and disease.

5. Role of Familial Mutations in K_{Ca} and TRP Channels in the Emergence of Cardiovascular Disease

The cardiovascular consequences of deliberate experimental mutations of major components of EDH (K_{Ca} and TRP channels, and gap junction Cxns) were discussed, but genetic mutations are also present during inheritable diseases. A single autosomal dominant mutation in *KCNN3* (guanine (mutant) substitution for cytosine (wild type) at nucleotide 1348 in coding DNA (or c.1348 C>G) and the K_{Ca}2.3 channel subunit (leucine (mutant) substitution for valine (wild type) at amino acid 450 (or V450L)) underlies development of idiopathic non-cirrhotic portal hypertension [119]. Also, a variety of single-nucleotide polymorphisms for *KCNN3* (chromosome 1q21 locus) are involved in lone atrial fibrillation [120], presumably due to dysregulation in the repolarization of action potentials in cardiac atria. As cardiac myocytes are also rich in gap junctions for coordinating electrical activity, it is not surprising that germline heterozygous missense mutations in *GJA5* (Cx40; e.g., phenylalanine for isoleucine, I75F [121]) also underlie lone atrial fibrillation. Autosomal dominant *KCNN4* (K_{Ca}3.1; "Gardos" channel) mutations on chromosome 19 lead to decreases in erythrocyte production ([122]; Diamond–Blackfan anemia) and health ([123]; xerocytosis, dehydration due to excess K^+ and H_2O loss). TRP "channelopathy" mutations are linked to an assortment of disorders throughout the entire body, including cardiovascular maladies of ischemic heart disease, hypertension, and atherosclerosis (see review [124]). However, there are a few adequately described cases of familial mutations accompanying cardiovascular pathology, including *TRPC3* (Williams–Beuren syndrome, deletion of 26 to 28 genes in 7q11.23; blood vessel stenosis [125]) and *TRPM4* (progressive familial heart block type I, missense mutations in 19q13.32, and gain of function; atrioventricular block [126]).

Although not discussed here, inheritable mutations in K_{Ca} channels, TRP channels, and Cxns also involve prominent neurological (e.g., Parkinson's) and developmental (e.g., skeletal dysplasia) disorders. Further investigation is needed to delineate de novo germline mutations from post-zygotic mutations that arise and provide susceptibility to development of cardiovascular pathology (e.g., arrhythmias or hypertension) with advancing age and select conditions of environmental exposure. In addition, albeit indirectly, the functional efficiency of EDH may also be affected by germline and somatic mutations of the upstream (e.g., *CHRM3*; muscarinic type 3 G_qPCR, M_3R [127]) and/or downstream (e.g., *MYLK*; myosin light-chain kinase, MLCK [128]) signaling components of vascular function. As of the present, human genetic mutations in key components involved in EDH appear extremely rare while not selective toward cardiovascular impairments alone. As a clearer foundation for understanding mammalian cardiovascular function and pathology, focus on the impact of aging and associated pathology (primarily using rodent models) is discussed next.

6. Endothelial SK_{Ca} and IK_{Ca} during Aging and Chronic Pathology

The leading cause of death in the United States is cardiovascular disease [129] with aging [130,131] and age-related vascular endothelial dysfunction [132,133] as key risk factors. A central mechanism of endothelial function for vasodilation and coordination of blood flow along and among vascular networks is EDH [8,134]. The physiological consequences of enhancement or diminishment of EDH during development of disease relative to young, healthy cardiovascular conditions remain altogether unclear. It is worth noting that IK_{Ca} function was found to increase in coronary arteries [135] and saphenous arteries [136] of obese rats, and mesenteric arteries of hypertensive [137] and diabetic [138] rats. At least in part, EDH compensates for reduced NO bioavailability during diabetic conditions in coronary arteries of dogs [139] and subcutaneous arteries of humans [140]. In aortae of apolipoprotein

E knock-out mice, basal K_{Ca} channel function appears to be reduced with enhancement of peak hyperpolarization to indirect (ACh) and direct (SKA-31 or NS1619) stimulation of the channels [141]. Remarkably, EDH may also emerge as a prominent contributor to the dilation of cremaster arterioles in developing mice of 15 weeks of age fed a high-fat diet continuously, beginning from at least four weeks prior to conception vs. control or temporally split control/high-fat diet treatments [142]. With respect to aging, increased enhanced IK_{Ca} function was observed in the aortae of 75- to 100-week-old mice [143] and skeletal muscle feed arteries of 24- to 26-month-old mice [38,144]. Remarkably, large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels also emerge in vascular endothelium as a result of conditions associated with chronic hypoxia (48 h), forming microdomain arrangements among Caveolin-1, TRPV4 channels, and BK_{Ca} channels (rat gracilis arteries) [145]. It is worth noting that, either on its own or in combination with deficient gap junction transmission, K_{Ca} over-activation may significantly diminish conducted hyperpolarization and/or vasodilation during old age (≥24-month-old mouse skeletal muscle arteries [38], and ≥64-year-old human coronary arteries; [63]), hypertension (Cx40-deficient mice [146]), hyperhomocysteinemia (cystathionine β-synthase mouse gluteus maximus arterioles [147]), and chronic hyperglycemia (diabetic mouse mesenteric arteries [148]). Currently, the upregulation of EDH during aging and development of chronic pathology is best explained by enhanced oxidative signaling (Figure 3). In brief, superoxide ($O_2^{\bullet-}$) production emerges from a variety of intracellular sources (NADPH oxidases, mitochondria, uncoupled NO synthase, and xanthine oxidase) which inactivates NO to peroxynitrite ($ONOO^-$) [149,150] and, via superoxide dismutase, $O_2^{\bullet-}$ is rapidly converted to H_2O_2 with spontaneous breakdown products such as hydroxyl radicals ($OH^{\bullet}$) [151] which increase EDH [8,38,152]. Thus, with this mechanistic basis in mind, SK_{Ca}/IK_{Ca} channel function may "compensate" for sustaining local vasodilation [153] at the expense of a restricted spatial domain of the spread of coordinated blood flow due to "leaky" endothelial plasma membranes [8].

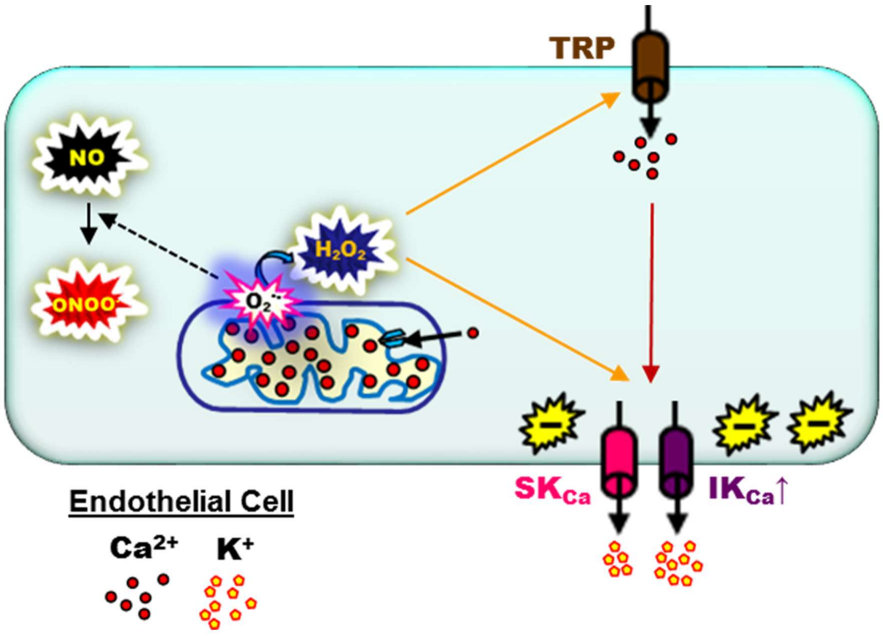

Figure 3. Working model for the upregulation of endothelium-derived hyperpolarization during aging and development of chronic pathology per enhanced oxidative signaling. Cardiovascular aging and the development of chronic disease is associated with a progressive increase in endothelial oxidative signaling. Mitochondrial respiration is a primary source of superoxide ($O_2^{\bullet-}$) which inactivates NO to peroxynitrite ($ONOO^-$) and, via superoxide dismutase, $O_2^{\bullet-}$ is rapidly converted to H_2O_2. $H_2O_2/OH^{\bullet}$ activates SK_{Ca} and IK_{Ca} (primarily IK_{Ca}) channels directly and/or indirectly (Ca^{2+} influx through TRP channels). Thus, SK_{Ca}/IK_{Ca} channel function may "compensate" for decreased NO bioavailability to sustain local vasodilation. The (−) symbol indicates V_m hyperpolarization while the respective color of lines corresponds to $O_2^{\bullet-}$ (black), H_2O_2 (orange), or Ca^{2+} (red) signaling.

On the flip side, there is support that the contribution of EDH may also decrease during conditions of cardiovascular disease. In rats, respective contributions of NO and EDH to the dilation of mesenteric arteries are reduced during diabetes [154] or of kidney arterioles at 18 months of age relative to 3 months [155]. It was also determined that physiological contribution of SK_{Ca}/IK_{Ca} channel function of saphenous arteries was less in 34- and 64-week-old (vs. 12-week-old) mice [156]. Finally, recent reports also conclude that EDH-dependent dilation of mesenteric arteries decreases in genetically hypertensive rats [157] or in 32-week-old rats fed a high-fat and/or high-fructose diet beginning at four weeks of age [158]. Discrepancies in conclusions for remodeled EDH function can be attributed to several differences among studies (e.g., diet application protocol or vascular study model). However, with consideration for "healthy" aging alone, perhaps impaired vascular IK_{Ca} channel function is not a consistent observation for rodents >20 months of age.

Recent support of SK_{Ca}/IK_{Ca} and TRP channel function as a "double-edged sword" that promotes the gain or loss of blood flow control is intriguing and important for a modern understanding of acute and chronic cardiovascular disease. The extent of SK_{Ca}/IK_{Ca} channel activation coincident with $V_m \geq -60$ mV may be excessive [3,38] as it pertains to the loss of myogenic tone and significant impairment of blood flow control [10]. Remarkably, pharmacological block or genetic deletion of IK_{Ca} channels may actually serve as protection as demonstrated in $K_{Ca}3.1^{-/-}$ mice that avoid an otherwise fatal pulmonary circulatory collapse in response to the activation of Ca^{2+}-permeant TRPV4 channels with GSK1016790A (high dose: 10 nM [159]; see Reference [160] for a thorough review on the interaction of pulmonary K_{Ca} and TRPV4 channels). Furthermore, direct genetic or pharmacological inhibition of TRPV4 prevents inflammatory cytokine signaling and endothelial dysfunction in septic mice (via cecal ligation and puncture or injection of tumor necrosis factor α or lipopolysaccharide) [161] and cardiac left ventricular cell/tissue damage in aged mice (24 to 27 months) exposed to hypoosmotic conditions (250 mOsm vs. physiological, ~300 mOsm) representative of ischemia–reperfusion injury [162]. Theoretically, enhanced smooth muscle α-adrenergic receptor activation via perivascular sympathetic nerves during aging and chronic vascular disorders (e.g., hypertension) may further overstimulate endothelial IK_{Ca} channels in response to high IP_3 and Ca^{2+} concentrations transmitted from smooth muscle through myoendothelial gap junctions [1,21]. Thus, in conditions of excessive stimulation of G_qPCRs, such as adrenergic receptors or TRPV4 channels that ultimately cause supraphysiological increases in vascular wall $[Ca^{2+}]_i$, a deleted or diminished role for endothelial IK_{Ca} channels may actually alleviate the consequences of pathological burden of $[Ca^{2+}]_i$ associated with the development of acute and chronic disease.

7. What May Be Next for Investigative Studies of Endothelial Function: Novel Physiological and Pharmacological Molecular Signaling Pathways

The number of "endothelium" publications recognized by the National Library of Medicine significantly increased from ~5000 in the early 1990s [163] to the present with >150,000. Under this heading, studies of EDH as they relate to the interaction of K_{Ca} and TRP channels are a rapidly growing research landscape for resolving cardiovascular physiology (rest and exercise) and the development of chronic disease. In general, characterizations of the essential components of EDH (G_qPCRs, IP_3Rs, K_{Ca}, and TRP channels) were thoroughly investigated across animal species and blood vessel types as discussed in this review. Finally, as a key intracellular organelle for the integration of Ca^{2+} and oxidative signaling in the vasculature, a physiological role for endothelial mitochondria is being investigated during ATP-dependent control of Ca^{2+} homeostasis [164], enhanced EDH during vascular aging [144], and metabolic stress during conditions of hyperglycemia [165] as a few recent examples. Also, additional clarification regarding endothelial mitochondrial K_{ATP} channels [166] and BK_{Ca} channels [167], and delineation of their actions of depolarization of the inner mitochondrial membrane vs. the well-characterized plasma membrane K^+ channels (hyperpolarization of the plasma membrane) is required.

In addition to NO, there are other gases now recognized to play a role during blood flow control including hydrogen sulfide (H_2S) and carbon monoxide (CO). As a reducing agent (vs. oxidizing H_2O_2), H_2S is generated from substrates homocysteine (via cystathionine β-synthase), cysteine (3-mercaptopyruvate sulfurtransferase), and thiosulfate (cystathionine γ-lyase), [168]. Overall evidence supports H_2S as a vasodilator that increases TRPV4 channel-dependent Ca^{2+} events and activation of endothelial BK_{Ca} channels of rat mesenteric arteries [169]. H_2S generated by cystathionine γ-lyase in particular may activate endothelial SK_{Ca} and IK_{Ca} channels for vasodilation of mouse mesenteric arteries [170]. CO is produced from heme via the actions of heme oxygenase [171]. Although there is some evidence to suggest that CO is integral to endothelial function [167] and vasodilation [172], further investigation is needed.

There are several other endogenous and therapeutic molecules (lipids and polyphenols) that operate via endothelium-dependent vasodilation. In particular, legalization and social acceptance for the recreational and medicinal use of cannabis among American populations increased since 2007 [173]. Thus, the investigation of cannabinoids is perhaps currently among the highest of priorities in biomedical research. Although reports are presently scarce, there is evidence to support that the endocannabinoid 2-arachidonoylglycerol induces vasorelaxation of rat mesenteric arteries as dependent on K_{Ca} and TRPV channels [174]. In human mesenteric arteries, this vasodilation appears to be dependent on cyclooxygenase metabolism and prostanoid signaling [175]. Recent evidence also suggests that endothelial cannabinoid receptors are not existent, and vasodilatory effects are directly mediated via BK_{Ca} channels [176]. As representative of studies testing cannabis Δ^9-tetrahydrocannabinol (THC; CB1 receptor agonist) on vasoreactivity, there is evidence demonstrating the opposite as well, whereby the application of THC reduces EDH-dependent vasorelaxation in mouse arteries [177]. Regardless, a consensus is presently lacking across the mechanisms of action of various cannabinoids, respective pharmacological kinetics, presence/classification of vascular cannabinoid receptors, and contribution of K_{Ca} channels vs. NO vs. cyclooxygenases among animal species and blood vessel types. Other studies support the role of endothelial K_{Ca} channel function during arterial treatment with omega-3 polyunsaturated acids [178], fruit-derived polyphenols [179], *Rhododendron* flavonoids [180], or sex hormones (testosterone [181] and estrogen [182]). All of such studies and therapeutic strategies that are either ancillary to, or perhaps circumvent, classical G_qPCR pathways (e.g., purinergic and muscarinic) are in need of further investigation.

8. Summary and Conclusions

Cardiovascular disease is the number one killer of Americans [129] with age-related vascular endothelial dysfunction at the center [132,133]. Coordinated blood flow and the perfusion of organs depend on the endothelial lining of major arteries and respective microcirculatory networks (arterioles and capillaries) that travel ≥100 km throughout the body [183,184]. The signaling pathway integral to the promotion of blood flow is endothelium-derived hyperpolarization (EDH). EDH sequentially entails G_q-protein-coupled receptor stimulation in intracellular Ca^{2+} release from the endoplasmic reticulum and Ca^{2+} influx through transient receptor potential (TRP) channels to activate Ca^{2+}-activated K^+ (K_{Ca}) channels and produce hyperpolarization [8]. The structural and functional arrangement among TRP and K_{Ca} channels in the blood vasculature is diverse across animal species, vessel type, and modes of local vs. conducted signaling to tune blood flow. Under- or over-activation of EDH is associated with aging and/or the development of chronic cardiovascular disease.

The future of cardiovascular physiology will likely entail progressive recruitment of state-of-the-art methods in the form of genetics, pharmacology, and engineering (e.g., stem-cell therapy [185], nanoparticle delivery of antioxidant molecules [186], and "blood–brain barrier on a chip" [187]) to examine classical components of genetics, structure, and function using cell/tissue/animal models of aging and disease. However, a present priority includes comprehensively defining a role of endothelial mitochondria as a nexus for vascular Ca^{2+} and oxidative signaling and

modulatory input of novel molecules in the form of gases, lipids, hormones, and phytochemicals. Regardless of physiological examination and therapeutic strategies, we anticipate that fully understanding mechanisms underlying blood flow regulation as it relates to the aging cardiovascular system will ultimately lead to the prevention of chronic disease.

Author Contributions: E.J.B. and M.A.H. interpreted results of the studies cited; E.J.B. and M.A.H. prepared figures; E.J.B. and M.A.H. drafted manuscript; E.J.B. edited and revised manuscript; E.J.B. and M.A.H. approved final version of the manuscript.

Funding: The authors' contributions to this review were supported by National Institutes of Health Grants R00AG047198 and R56AG062169 to Erik J. Behringer. The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Acknowledgments: The authors would like to thank John N. Buchholz for kindly going through the manuscript and providing an overall assessment of its quality.

Conflicts of Interest: The authors declare no conflicts of interest

References

1. Socha, M.J.; Segal, S.S. Microvascular mechanisms limiting skeletal muscle blood flow with advancing age. *J. Appl. Physiol. (1985)* **2018**, *125*, 1851–1859. [CrossRef] [PubMed]
2. Himmel, H.M.; Whorton, A.R.; Strauss, H.C. Intracellular calcium, currents, and stimulus-response coupling in endothelial cells. *Hypertension* **1993**, *21*, 112–127. [PubMed]
3. Behringer, E.J.; Segal, S.S. Membrane potential governs calcium influx into microvascular endothelium: Integral role for muscarinic receptor activation. *J. Physiol.* **2015**, *593*, 4531–4548. [CrossRef] [PubMed]
4. Di Giuro, C.M.L.; Shrestha, N.; Malli, R.; Groschner, K.; van Breemen, C.; Fameli, N. Na^+/Ca^{2+} exchangers and Orai channels jointly refill endoplasmic reticulum (ER) Ca^{2+} via ER nanojunctions in vascular endothelial cells. *Pflug. Arch.* **2017**, *469*, 1287–1299. [CrossRef] [PubMed]
5. Avila-Medina, J.; Mayoral-Gonzalez, I.; Dominguez-Rodriguez, A.; Gallardo-Castillo, I.; Ribas, J.; Ordonez, A.; Rosado, J.A.; Smani, T. The Complex Role of Store Operated Calcium Entry Pathways and Related Proteins in the Function of Cardiac, Skeletal and Vascular Smooth Muscle Cells. *Front. Physiol.* **2018**, *9*, 257.
6. Trebak, M. STIM/Orai signalling complexes in vascular smooth muscle. *J. Physiol.* **2012**, *590*, 4201–4208. [CrossRef]
7. Zhang, X.; Gueguinou, M.; Trebak, M. Store-Independent Orai Channels Regulated by STIM. In *Calcium Entry Channels in Non-Excitable Cells*; Kozak, J.A., Putney, J.W., Jr., Eds.; CRC Press: Boca Raton, FL, USA, 2018; pp. 197–214.
8. Behringer, E.J. Calcium and electrical signaling in arterial endothelial tubes: New insights into cellular Physiol.ogy and cardiovascular function. *Microcirculation* **2017**, *24*. [CrossRef]
9. Ledoux, J.; Taylor, M.S.; Bonev, A.D.; Hannah, R.M.; Solodushko, V.; Shui, B.; Tallini, Y.; Kotlikoff, M.I.; Nelson, M.T. Functional architecture of inositol 1,4,5-trisphosphate signaling in restricted spaces of myoendothelial projections. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 9627–9632. [CrossRef]
10. Bagher, P.; Beleznai, T.; Kansui, Y.; Mitchell, R.; Garland, C.J.; Dora, K.A. Low intravascular pressure activates endothelial cell TRPV4 channels, local Ca^{2+} events, and IK_{Ca} channels, reducing arteriolar tone. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 18174–18179. [CrossRef]
11. Sandow, S.L.; Neylon, C.B.; Chen, M.X.; Garland, C.J. Spatial separation of endothelial small- and intermediate-conductance calcium-activated potassium channels (K_{Ca}) and connexins: Possible relationship to vasodilator function? *J. Anat.* **2006**, *209*, 689–698. [CrossRef]
12. Sonkusare, S.K.; Dalsgaard, T.; Bonev, A.D.; Hill-Eubanks, D.C.; Kotlikoff, M.I.; Scott, J.D.; Santana, L.F.; Nelson, M.T. AKAP150-dependent cooperative TRPV4 channel gating is central to endothelium-dependent vasodilation and is disrupted in hypertension. *Sci. Signal.* **2014**, *7*, ra66. [CrossRef]
13. Emerson, G.G.; Segal, S.S. Electrical coupling between endothelial cells and smooth muscle cells in hamster feed arteries: Role in vasomotor control. *Circ. Res.* **2000**, *87*, 474–479. [CrossRef]
14. Garland, C.J.; Bagher, P.; Powell, C.; Ye, X.; Lemmey, H.A.L.; Borysova, L.; Dora, K.A. Voltage-dependent Ca^{2+} entry into smooth muscle during contraction promotes endothelium-mediated feedback vasodilation in arterioles. *Sci. Signal.* **2017**, *10*, eaal3806. [PubMed]

15. Billaud, M.; Lohman, A.W.; Johnstone, S.R.; Biwer, L.A.; Mutchler, S.; Isakson, B.E. Regulation of cellular communication by signaling microdomains in the blood vessel wall. *Pharm. Rev.* **2014**, *66*, 513–569. [CrossRef] [PubMed]
16. Garcia, V.; Sessa, W.C. Endothelial NOS: Perspective and recent developments. *Br. J. Pharm.* **2019**, *176*, 189–196. [CrossRef] [PubMed]
17. Forstermann, U.; Sessa, W.C. Nitric oxide synthases: Regulation and function. *Eur. Heart J.* **2012**, *33*, 829–837. [PubMed]
18. Feil, R.; Lohmann, S.M.; de Jonge, H.; Walter, U.; Hofmann, F. Cyclic GMP-dependent protein kinases and the cardiovascular system: Insights from genetically modified mice. *Circ. Res.* **2003**, *93*, 907–916. [CrossRef] [PubMed]
19. Irvine, J.C.; Favaloro, J.L.; Kemp-Harper, B.K. NO- activates soluble guanylate cyclase and Kv channels to vasodilate resistance arteries. *Hypertension* **2003**, *41*, 1301–1307. [CrossRef] [PubMed]
20. Li, W.; Halling, D.B.; Hall, A.W.; Aldrich, R.W. EF hands at the N-lobe of calmodulin are required for both SK channel gating and stable SK-calmodulin interaction. *J. Gen. Physiol.* **2009**, *134*, 281–293. [CrossRef]
21. Behringer, E.J.; Segal, S.S. Spreading the signal for vasodilatation: Implications for skeletal muscle blood flow control and the effects of ageing. *J. Physiol.* **2012**, *590*, 6277–6284. [CrossRef]
22. Emerson, G.G.; Neild, T.O.; Segal, S.S. Conduction of hyperpolarization along hamster feed arteries: Augmentation by acetylcholine. *Am. J. Physiol. Heart Circ. Physiol.* **2002**, *283*, H102–H109. [CrossRef]
23. Nelson, M.T.; Quayle, J.M. Physiological roles and properties of potassium channels in arterial smooth muscle. *Am. J. Physiol.* **1995**, *268 Pt 1*, C799–C822. [CrossRef]
24. Dora, K.A.; Doyle, M.P.; Duling, B.R. Elevation of intracellular calcium in smooth muscle causes endothelial cell generation of NO in arterioles. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 6529–6534. [CrossRef]
25. Sandow, S.L.; Hill, C.E. Incidence of myoendothelial gap junctions in the proximal and distal mesenteric arteries of the rat is suggestive of a role in endothelium-derived hyperpolarizing factor-mediated responses. *Circ. Res.* **2000**, *86*, 341–346. [CrossRef]
26. Shimokawa, H.; Yasutake, H.; Fujii, K.; Owada, M.K.; Nakaike, R.; Fukumoto, Y.; Takayanagi, T.; Nagao, T.; Egashira, K.; Fujishima, M.; et al. The importance of the hyperpolarizing mechanism increases as the vessel size decreases in endothelium-dependent relaxations in rat mesenteric Circulation. *J. Cardiovasc. Pharm.* **1996**, *28*, 703–711. [CrossRef]
27. Haas, T.L.; Duling, B.R. Morphology favors an endothelial cell pathway for longitudinal conduction within arterioles. *Microvasc. Res.* **1997**, *53*, 113–120. [CrossRef]
28. Hakim, C.H.; Jackson, W.F.; Segal, S.S. Connexin isoform expression in smooth muscle cells and endothelial cells of hamster cheek pouch arterioles and retractor feed arteries. *Microcirculation* **2008**, *15*, 503–514. [CrossRef]
29. Sandow, S.L.; Tare, M.; Coleman, H.A.; Hill, C.E.; Parkington, H.C. Involvement of myoendothelial gap junctions in the actions of endothelium-derived hyperpolarizing factor. *Circ. Res.* **2002**, *90*, 1108–1113. [CrossRef]
30. Nagaraja, S.; Kapela, A.; Tran, C.H.; Welsh, D.G.; Tsoukias, N.M. Role of microprojections in myoendothelial feedback—A theoretical study. *J. Physiol.* **2013**, *591*, 2795–2812. [CrossRef]
31. Tran, C.H.; Taylor, M.S.; Plane, F.; Nagaraja, S.; Tsoukias, N.M.; Solodushko, V.; Vigmond, E.J.; Furstenhaupt, T.; Brigdan, M.; Welsh, D.G. Endothelial Ca^{2+} wavelets and the induction of myoendothelial feedback. *Am. J. Physiol.-Cell Physiol.* **2012**, *302*, C1226–C1242. [CrossRef]
32. Cole, W.C.; Welsh, D.G. Role of myosin light chain kinase and myosin light chain phosphatase in the resistance arterial myogenic response to intravascular pressure. *Arch. Biochem. Biophys.* **2011**, *510*, 160–173. [CrossRef] [PubMed]
33. Uhrenholt, T.R.; Domeier, T.L.; Segal, S.S. Propagation of calcium waves along endothelium of hamster feed arteries. *Am. J. Physiol. Heart Circ. Physiol.* **2007**, *292*, H1634–H1640. [CrossRef]
34. Socha, M.J.; Behringer, E.J.; Segal, S.S. Calcium and electrical signalling along endothelium of the resistance vasculature. *Basic Clin. Pharm. Toxicol.* **2012**, *110*, 80–86. [CrossRef] [PubMed]
35. Kerr, P.M.; Wei, R.; Tam, R.; Sandow, S.L.; Murphy, T.V.; Ondrusova, K.; Lunn, S.E.; Tran, C.H.; Welsh, D.G.; Plane, F. Activation of endothelial IK_{Ca} channels underlies NO-dependent myoendothelial feedback. *Vasc. Pharm.* **2015**, *74*, 130–138. [CrossRef]

36. Stankevicius, E.; Dalsgaard, T.; Kroigaard, C.; Beck, L.; Boedtkjer, E.; Misfeldt, M.W.; Nielsen, G.; Schjorring, O.; Hughes, A.; Simonsen, U. Opening of small and intermediate calcium-activated potassium channels induces relaxation mainly mediated by nitric-oxide release in large arteries and endothelium-derived hyperpolarizing factor in small arteries from rat. *J. Pharm. Exp.* **2011**, *339*, 842–850. [CrossRef] [PubMed]
37. Behringer, E.J.; Segal, S.S. Tuning electrical conduction along endothelial tubes of resistance arteries through Ca^{2+}-activated K^+ channels. *Circ. Res.* **2012**, *110*, 1311–1321. [CrossRef] [PubMed]
38. Behringer, E.J.; Shaw, R.L.; Westcott, E.B.; Socha, M.J.; Segal, S.S. Aging impairs electrical conduction along endothelium of resistance arteries through enhanced Ca^{2+}-activated K^+ channel activation. *Arter. Thromb. Vasc. Biol.* **2013**, *33*, 1892–1901. [CrossRef] [PubMed]
39. Emerson, G.G.; Segal, S.S. Endothelial cell pathway for conduction of hyperpolarization and vasodilation along hamster feed artery. *Circ. Res.* **2000**, *86*, 94–100. [CrossRef]
40. Wolfle, S.E.; Chaston, D.J.; Goto, K.; Sandow, S.L.; Edwards, F.R.; Hill, C.E. Non-linear relationship between hyperpolarisation and relaxation enables long distance propagation of vasodilatation. *J. Physiol.* **2011**, *589 Pt 10*, 2607–2623. [CrossRef]
41. Hakim, M.A.; Buchholz, J.N.; Behringer, E.J. Electrical dynamics of isolated cerebral and skeletal muscle endothelial tubes: Differential roles of G-protein-coupled receptors and K^+ channels. *Pharm. Res. Perspect.* **2018**, *6*, e00391. [CrossRef]
42. Stocker, M. Ca^{2+}-activated K^+ channels: Molecular determinants and function of the SK family. *Nat. Rev. Neurosci.* **2004**, *5*, 758–770. [CrossRef]
43. Kohler, M.; Hirschberg, B.; Bond, C.T.; Kinzie, J.M.; Marrion, N.V.; Maylie, J.; Adelman, J.P. Small-conductance, calcium-activated potassium channels from mammalian brain. *Science* **1996**, *273*, 1709–1714. [CrossRef] [PubMed]
44. Taylor, M.S.; Bonev, A.D.; Gross, T.P.; Eckman, D.M.; Brayden, J.E.; Bond, C.T.; Adelman, J.P.; Nelson, M.T. Altered expression of small-conductance Ca^{2+}-activated K^+ (SK3) channels modulates arterial tone and blood pressure. *Circ. Res.* **2003**, *93*, 124–131. [CrossRef]
45. Bond, C.T.; Sprengel, R.; Bissonnette, J.M.; Kaufmann, W.A.; Pribnow, D.; Neelands, T.; Storck, T.; Baetscher, M.; Jerecic, J.; Maylie, J.; et al. Respiration and parturition affected by conditional overexpression of the Ca^{2+}-activated K^+ channel subunit, SK3. *Science* **2000**, *289*, 1942–1946. [CrossRef]
46. Ishii, T.M.; Silvia, C.; Hirschberg, B.; Bond, C.T.; Adelman, J.P.; Maylie, J. A human intermediate conductance calcium-activated potassium channel. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 11651–11656. [CrossRef]
47. Wei, A.D.; Gutman, G.A.; Aldrich, R.; Chandy, K.G.; Grissmer, S.; Wulff, H. International Union of Pharmacology. LII. Nomenclature and molecular relationships of calcium-activated potassium channels. *Pharm. Rev.* **2005**, *57*, 463–472. [CrossRef]
48. Kohler, R.; Ruth, P. Endothelial dysfunction and blood pressure alterations in K^+-channel transgenic mice. *Pflug Arch. Eur. J. Phys.* **2010**, *459*, 969–976. [CrossRef]
49. Logsdon, N.J.; Kang, J.; Togo, J.A.; Christian, E.P.; Aiyar, J. A novel gene, hKCa4, encodes the calcium-activated potassium channel in human T lymphocytes. *J. Biol. Chem.* **1997**, *272*, 32723–32726. [CrossRef]
50. Grgic, I.; Kaistha, B.P.; Hoyer, J.; Kohler, R. Endothelial Ca^{2+}-activated K^+ channels in normal and impaired EDHF-dilator responses–relevance to cardiovascular pathologies and drug discovery. *Br. J. Pharm.* **2009**, *157*, 509–526. [CrossRef]
51. Si, H.; Heyken, W.T.; Wolfle, S.E.; Tysiac, M.; Schubert, R.; Grgic, I.; Vilianovich, L.; Giebing, G.; Maier, T.; Gross, V.; et al. Impaired endothelium-derived hyperpolarizing factor-mediated dilations and increased blood pressure in mice deficient of the intermediate-conductance Ca^{2+}-activated K^+ channel. *Circ. Res.* **2006**, *99*, 537–544. [CrossRef]
52. Brahler, S.; Kaistha, A.; Schmidt, V.J.; Wolfle, S.E.; Busch, C.; Kaistha, B.P.; Kacik, M.; Hasenau, A.L.; Grgic, I.; Si, H.; et al. Genetic deficit of SK3 and IK1 channels disrupts the endothelium-derived hyperpolarizing factor vasodilator pathway and causes hypertension. *Circulation* **2009**, *119*, 2323–2332. [CrossRef] [PubMed]
53. Milkau, M.; Kohler, R.; de Wit, C. Crucial importance of the endothelial K^+ channel SK3 and connexin40 in arteriolar dilations during skeletal muscle contraction. *FASEB J.* **2010**, *24*, 3572–3579. [CrossRef] [PubMed]

54. Radtke, J.; Schmidt, K.; Wulff, H.; Kohler, R.; de Wit, C. Activation of K_{Ca}3.1 by SKA-31 induces arteriolar dilatation and lowers blood pressure in normo- and hypertensive connexin40-deficient mice. *Br. J. Pharm.* **2013**, *170*, 293–303. [CrossRef] [PubMed]
55. Yap, F.C.; Weber, D.S.; Taylor, M.S.; Townsley, M.I.; Comer, B.S.; Maylie, J.; Adelman, J.P.; Lin, M.T. Endothelial SK3 channel-associated Ca^{2+} microdomains modulate blood pressure. *Am. J. Physiol. Heart Circ. Physiol.* **2016**, *310*, H1151–H1163. [PubMed]
56. Doughty, J.M.; Plane, F.; Langton, P.D. Charybdotoxin and apamin block EDHF in rat mesenteric artery if selectively applied to the endothelium. *Am. J. Physiol.* **1999**, *276 Pt 2*, H1107–H1112. [CrossRef]
57. Sandow, S.L.; Haddock, R.E.; Hill, C.E.; Chadha, P.S.; Kerr, P.M.; Welsh, D.G.; Plane, F. What's where and why at a vascular myoendothelial microdomain signalling complex. *Clin. Exp. Pharm. Physiol.* **2009**, *36*, 67–76. [CrossRef] [PubMed]
58. Garland, C.J.; Dora, K.A. EDH: Endothelium-dependent hyperpolarization and microvascular signalling. *Acta Physiol. (Oxf.)* **2017**, *219*, 152–161. [CrossRef] [PubMed]
59. Otani, S.; Nagaoka, T.; Omae, T.; Tanano, I.; Kamiya, T.; Ono, S.; Hein, T.W.; Kuo, L.; Yoshida, A. Histamine-Induced Dilation of Isolated Porcine Retinal Arterioles: Role of Endothelium-Derived Hyperpolarizing Factor. *Investig. Ophthalmol. Vis. Sci.* **2016**, *57*, 4791–4798. [CrossRef] [PubMed]
60. Hannah, R.M.; Dunn, K.M.; Bonev, A.D.; Nelson, M.T. Endothelial SK_{Ca} and IK_{Ca} channels regulate brain parenchymal arteriolar diameter and cortical cerebral blood flow. *J. Cereb. Blood Flow Metab.* **2011**, *31*, 1175–1186. [CrossRef] [PubMed]
61. Marrelli, S.P.; Eckmann, M.S.; Hunte, M.S. Role of endothelial intermediate conductance K_{Ca} channels in cerebral EDHF-mediated dilations. *Am. J. Physiol. Heart Circ. Physiol.* **2003**, *285*, H1590–H1599. [PubMed]
62. McNeish, A.J.; Sandow, S.L.; Neylon, C.B.; Chen, M.X.; Dora, K.A.; Garland, C.J. Evidence for involvement of both IK_{Ca} and SK_{Ca} channels in hyperpolarizing responses of the rat middle cerebral artery. *Stroke* **2006**, *37*, 1277–1282.
63. Feher, A.; Broskova, Z.; Bagi, Z. Age-related impairment of conducted dilation in human coronary arterioles. *Am. J. Physiol. Heart Circ. Physiol.* **2014**, *306*, H1595–H1601. [CrossRef]
64. Liu, Y.; Terata, K.; Chai, Q.; Li, H.; Kleinman, L.H.; Gutterman, D.D. Peroxynitrite inhibits Ca^{2+}-activated K^+ channel activity in smooth muscle of human coronary arterioles. *Circ. Res.* **2002**, *91*, 1070–1076. [CrossRef] [PubMed]
65. Lin, M.T.; Jian, M.Y.; Taylor, M.S.; Cioffi, D.L.; Yap, F.C.; Liedtke, W.; Townsley, M.I. Functional coupling of TRPV4, IK, and SK channels contributes to Ca^{2+}-dependent endothelial injury in rodent lung. *Pulm. Circ.* **2015**, *5*, 279–290. [CrossRef]
66. Marziano, C.; Hong, K.; Cope, E.L.; Kotlikoff, M.I.; Isakson, B.E.; Sonkusare, S.K. Nitric Oxide-Dependent Feedback Loop Regulates Transient Receptor Potential Vanilloid 4 (TRPV4) Channel Cooperativity and Endothelial Function in Small Pulmonary Arteries. *J. Am. Heart Assoc.* **2017**, *6*, e007157.
67. Sinkler, S.Y.; Segal, S.S. Rapid versus slow ascending vasodilatation: Intercellular conduction versus flow-mediated signalling with tetanic versus rhythmic muscle contractions. *J. Physiol.* **2017**, *595*, 7149–7165. [CrossRef] [PubMed]
68. Bussemaker, E.; Popp, R.; Binder, J.; Busse, R.; Fleming, I. Characterization of the endothelium-derived hyperpolarizing factor (EDHF) response in the human interlobar artery. *Kidney Int.* **2003**, *63*, 1749–1755. [CrossRef]
69. Salomonsson, M.; Brasen, J.C.; Sorensen, C.M. Role of renal vascular potassium channels in Physiology and pathophysiology. *Acta Physiol. (Oxf.)* **2017**, *221*, 14–31. [CrossRef]
70. Waeckel, L.; Bertin, F.; Clavreul, N.; Damery, T.; Kohler, R.; Paysant, J.; Sansilvestri-Morel, P.; Simonet, S.; Vayssettes-Courchay, C.; Wulff, H.; et al. Preserved regulation of renal perfusion pressure by small and intermediate conductance K_{Ca} channels in hypertensive mice with or without renal failure. *Pflug. Arch.* **2015**, *467*, 817–831.
71. Kochukov, M.Y.; Balasubramanian, A.; Abramowitz, J.; Birnbaumer, L.; Marrelli, S.P. Activation of endothelial transient receptor potential C3 channel is required for small conductance calcium-activated potassium channel activation and sustained endothelial hyperpolarization and vasodilation of cerebral artery. *J. Am. Heart Assoc.* **2014**, *3*, e000913. [CrossRef]

72. Chaston, D.J.; Baillie, B.K.; Grayson, T.H.; Courjaret, R.J.; Heisler, J.M.; Lau, K.A.; Machaca, K.; Nicholson, B.J.; Ashton, A.; Matthaei, K.I.; et al. Polymorphism in endothelial connexin40 enhances sensitivity to intraluminal pressure and increases arterial stiffness. *Arter. Thromb. Vasc. Biol.* **2013**, *33*, 962–970. [CrossRef] [PubMed]
73. Clapham, D.E. Calcium signaling. *Cell* **2007**, *131*, 1047–1058. [CrossRef] [PubMed]
74. Dietrich, A.; Gudermann, T. TRP channels in the cardiopulmonary vasculature. *Adv. Exp. Med. Biol.* **2011**, *704*, 781–810. [PubMed]
75. Yue, Z.; Xie, J.; Yu, A.S.; Stock, J.; Du, J.; Yue, L. Role of TRP channels in the cardiovascular system. *Am. J. Physiol. Heart Circ. Physiol.* **2015**, *308*, H157–H182. [CrossRef]
76. Poteser, M.; Graziani, A.; Rosker, C.; Eder, P.; Derler, I.; Kahr, H.; Zhu, M.X.; Romanin, C.; Groschner, K. TRPC3 and TRPC4 associate to form a redox-sensitive cation channel. Evidence for expression of native TRPC3-TRPC4 heteromeric channels in endothelial cells. *J. Biol. Chem.* **2006**, *281*, 13588–13595. [CrossRef]
77. Ma, X.; Cao, J.; Luo, J.; Nilius, B.; Huang, Y.; Ambudkar, I.S.; Yao, X. Depletion of intracellular Ca^{2+} stores stimulates the translocation of vanilloid transient receptor potential 4-c1 heteromeric channels to the plasma membrane. *Arter. Thromb. Vasc. Biol.* **2010**, *30*, 2249–2255. [CrossRef]
78. Zhang, P.; Mao, A.Q.; Sun, C.Y.; Zhang, X.D.; Pan, Q.X.; Yang, D.T.; Jin, J.; Tang, C.L.; Yang, Z.Y.; Yao, X.Q.; et al. Translocation of PKG1alpha acts on TRPV4-C1 heteromeric channels to inhibit endothelial Ca^{2+} entry. *Acta Pharm. Sin.* **2016**, *37*, 1199–1207. [CrossRef]
79. Greenberg, H.Z.E.; Carlton-Carew, S.R.E.; Khan, D.M.; Zargaran, A.K.; Jahan, K.S.; Vanessa Ho, W.S.; Albert, A.P. Heteromeric TRPV4/TRPC1 channels mediate calcium-sensing receptor-induced nitric oxide production and vasorelaxation in rabbit mesenteric arteries. *Vasc. Pharm.* **2017**, *96–98*, 53–62. [CrossRef]
80. Du, J.; Ma, X.; Shen, B.; Huang, Y.; Birnbaumer, L.; Yao, X. TRPV4, TRPC1, and TRPP2 assemble to form a flow-sensitive heteromeric channel. *FASEB J.* **2014**, *28*, 4677–4685. [CrossRef]
81. Thorneloe, K.S.; Sulpizio, A.C.; Lin, Z.; Figueroa, D.J.; Clouse, A.K.; McCafferty, G.P.; Chendrimada, T.P.; Lashinger, E.S.; Gordon, E.; Evans, L.; et al. N-((1S)-1-{[4-((2S)-2-{[(2,4-dichlorophenyl)sulfonyl]amino}-3-hydroxypropanoyl)-1 -piperazinyl]carbonyl}-3-methylbutyl)-1-benzothiophene-2-carboxamide (GSK1016790A), a novel and potent transient receptor potential vanilloid 4 channel agonist induces urinary bladder contraction and hyperactivity: Part I. *J. Pharm. Exp.* **2008**, *326*, 432–442.
82. Cheung, M.; Bao, W.; Behm, D.J.; Brooks, C.A.; Bury, M.J.; Dowdell, S.E.; Eidam, H.S.; Fox, R.M.; Goodman, K.B.; Holt, D.A.; et al. Discovery of GSK2193874: An Orally Active, Potent, and Selective Blocker of Transient Receptor Potential Vanilloid 4. *ACS Med. Chem. Lett.* **2017**, *8*, 549–554. [CrossRef]
83. Sonkusare, S.K.; Bonev, A.D.; Ledoux, J.; Liedtke, W.; Kotlikoff, M.I.; Heppner, T.J.; Hill-Eubanks, D.C.; Nelson, M.T. Elementary Ca^{2+} signals through endothelial TRPV4 channels regulate vascular function. *Science* **2012**, *336*, 597–601. [CrossRef]
84. Bubolz, A.H.; Mendoza, S.A.; Zheng, X.; Zinkevich, N.S.; Li, R.; Gutterman, D.D.; Zhang, D.X. Activation of endothelial TRPV4 channels mediates flow-induced dilation in human coronary arterioles: Role of Ca^{2+} entry and mitochondrial ROS signaling. *Am. J. Physiol. Heart Circ. Physiol.* **2012**, *302*, H634–H642. [CrossRef]
85. Zitt, C.; Zobel, A.; Obukhov, A.G.; Harteneck, C.; Kalkbrenner, F.; Luckhoff, A.; Schultz, G. Cloning and functional expression of a human Ca^{2+}-permeable cation channel activated by calcium store depletion. *Neuron* **1996**, *16*, 1189–1196. [CrossRef]
86. Hofmann, T.; Obukhov, A.G.; Schaefer, M.; Harteneck, C.; Gudermann, T.; Schultz, G. Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. *Nature* **1999**, *397*, 259–263. [CrossRef]
87. Strotmann, R.; Schultz, G.; Plant, T.D. Ca^{2+}-dependent potentiation of the nonselective cation channel TRPV4 is mediated by a C-terminal calmodulin binding site. *J. Biol. Chem.* **2003**, *278*, 26541–26549. [CrossRef]
88. Schmidt, K.; Dubrovska, G.; Nielsen, G.; Fesus, G.; Uhrenholt, T.R.; Hansen, P.B.; Gudermann, T.; Dietrich, A.; Gollasch, M.; de Wit, C.; et al. Amplification of EDHF-type vasodilatations in TRPC1-deficient mice. *Br. J. Pharm.* **2010**, *161*, 1722–1733. [CrossRef]
89. Launay, P.; Fleig, A.; Perraud, A.L.; Scharenberg, A.M.; Penner, R.; Kinet, J.P. TRPM4 is a Ca^{2+}-activated nonselective cation channel mediating cell membrane depolarization. *Cell* **2002**, *109*, 397–407. [CrossRef]
90. Earley, S.; Waldron, B.J.; Brayden, J.E. Critical role for transient receptor potential channel TRPM4 in myogenic constriction of cerebral arteries. *Circ. Res.* **2004**, *95*, 922–929. [CrossRef]

91. Garland, C.J.; Smirnov, S.V.; Bagher, P.; Lim, C.S.; Huang, C.Y.; Mitchell, R.; Stanley, C.; Pinkney, A.; Dora, K.A. TRPM4 inhibitor 9-phenanthrol activates endothelial cell intermediate conductance calcium-activated potassium channels in rat isolated mesenteric artery. *Br. J. Pharm.* **2015**, *172*, 1114–1123. [CrossRef]
92. Behringer, E.J.; Scallan, J.P.; Jafarnejad, M.; Castorena-Gonzalez, J.A.; Zawieja, S.D.; Moore, J.E., Jr.; Davis, M.J.; Segal, S.S. Calcium and electrical dynamics in lymphatic endothelium. *J. Physiol.* **2017**, *595*, 7347–7368. [CrossRef] [PubMed]
93. Francis, M.; Qian, X.; Charbel, C.; Ledoux, J.; Parker, J.C.; Taylor, M.S. Automated region of interest analysis of dynamic Ca^{2+} signals in image sequences. *Am. J. Physiol. Cell Physiol.* **2012**, *303*, C236–C243. [CrossRef] [PubMed]
94. Francis, M.; Waldrup, J.; Qian, X.; Taylor, M.S. Automated analysis of dynamic Ca^{2+} signals in image sequences. *J. Vis. Exp.* **2014**. [CrossRef] [PubMed]
95. Qian, X.; Francis, M.; Kohler, R.; Solodushko, V.; Lin, M.; Taylor, M.S. Positive feedback regulation of agonist-stimulated endothelial Ca^{2+} dynamics by $K_{Ca}3.1$ channels in mouse mesenteric arteries. *Arter. Thromb. Vasc. Biol.* **2014**, *34*, 127–135. [CrossRef] [PubMed]
96. Francis, M.; Waldrup, J.R.; Qian, X.; Solodushko, V.; Meriwether, J.; Taylor, M.S. Functional Tuning of Intrinsic Endothelial Ca^{2+} Dynamics in Swine Coronary Arteries. *Circ. Res.* **2016**, *118*, 1078–1090. [CrossRef] [PubMed]
97. Wilson, C.; Saunter, C.D.; Girkin, J.M.; McCarron, J.G. Advancing Age Decreases Pressure-Sensitive Modulation of Calcium Signaling in the Endothelium of Intact and Pressurized Arteries. *J. Vasc. Res.* **2016**, *53*, 358–369. [CrossRef]
98. Chung, M.K.; Lee, H.; Mizuno, A.; Suzuki, M.; Caterina, M.J. 2-aminoethoxydiphenyl borate activates and sensitizes the heat-gated ion channel TRPV3. *J. Neurosci.* **2004**, *24*, 5177–5182. [CrossRef] [PubMed]
99. Earley, S.; Gonzales, A.L.; Garcia, Z.I. A dietary agonist of transient receptor potential cation channel V3 elicits endothelium-dependent vasodilation. *Mol. Pharm.* **2010**, *77*, 612–620. [CrossRef] [PubMed]
100. Pires, P.W.; Sullivan, M.N.; Pritchard, H.A.; Robinson, J.J.; Earley, S. Unitary TRPV3 channel Ca^{2+} influx events elicit endothelium-dependent dilation of cerebral parenchymal arterioles. *Am. J. Physiol. Heart Circ. Physiol.* **2015**, *309*, H2031–H2041. [CrossRef] [PubMed]
101. Story, G.M.; Peier, A.M.; Reeve, A.J.; Eid, S.R.; Mosbacher, J.; Hricik, T.R.; Earley, T.J.; Hergarden, A.C.; Andersson, D.A.; Hwang, S.W.; et al. ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell* **2003**, *112*, 819–829. [CrossRef]
102. Earley, S.; Gonzales, A.L.; Crnich, R. Endothelium-dependent cerebral artery dilation mediated by TRPA1 and Ca^{2+}-Activated K^+ channels. *Circ. Res.* **2009**, *104*, 987–994. [CrossRef] [PubMed]
103. Qian, X.; Francis, M.; Solodushko, V.; Earley, S.; Taylor, M.S. Recruitment of dynamic endothelial Ca^{2+} signals by the TRPA1 channel activator AITC in rat cerebral arteries. *Microcirculation* **2013**, *20*, 138–148. [CrossRef] [PubMed]
104. Sullivan, M.N.; Gonzales, A.L.; Pires, P.W.; Bruhl, A.; Leo, M.D.; Li, W.; Oulidi, A.; Boop, F.A.; Feng, Y.; Jaggar, J.H.; et al. Localized TRPA1 channel Ca^{2+} signals stimulated by reactive oxygen species promote cerebral artery dilation. *Sci. Signal.* **2015**, *8*, ra2. [CrossRef] [PubMed]
105. Kiselyov, K.; Xu, X.; Mozhayeva, G.; Kuo, T.; Pessah, I.; Mignery, G.; Zhu, X.; Birnbaumer, L.; Muallem, S. Functional interaction between InsP3 receptors and store-operated Htrp3 channels. *Nature* **1998**, *396*, 478. [PubMed]
106. Senadheera, S.; Kim, Y.; Grayson, T.H.; Toemoe, S.; Kochukov, M.Y.; Abramowitz, J.; Housley, G.D.; Bertrand, R.L.; Chadha, P.S.; Bertrand, P.P.; et al. Transient receptor potential canonical type 3 channels facilitate endothelium-derived hyperpolarization-mediated resistance artery vasodilator activity. *Cardiovasc. Res.* **2012**, *95*, 439–447. [CrossRef] [PubMed]
107. Jackson, W.F. Endothelial cell ion channel expression and function in arterioles and resistance arteries. In *Vascular Ion Channels in Physiology and Disease*; Levitan, I., Dopico, A.M., Eds.; Springer: Cham, Switzerland, 2016; pp. 3–36.
108. Hong, K.; Cope, E.L.; DeLalio, L.J.; Marziano, C.; Isakson, B.E.; Sonkusare, S.K. TRPV4 (Transient Receptor Potential Vanilloid 4) Channel-Dependent Negative Feedback Mechanism Regulates Gq Protein-Coupled Receptor-Induced Vasoconstriction. *Arter. Thromb. Vasc. Biol.* **2018**, *38*, 542–554. [CrossRef] [PubMed]

109. Nausch, L.W.; Bonev, A.D.; Heppner, T.J.; Tallini, Y.; Kotlikoff, M.I.; Nelson, M.T. Sympathetic nerve stimulation induces local endothelial Ca^{2+} signals to oppose vasoconstriction of mouse mesenteric arteries. *Am. J. Physiol. Heart Circ. Physiol.* **2012**, *302*, H594–H602. [CrossRef] [PubMed]
110. Isakson, B.E.; Ramos, S.I.; Duling, B.R. Ca^{2+} and inositol 1,4,5-trisphosphate-mediated signaling across the myoendothelial junction. *Circ. Res.* **2007**, *100*, 246–254. [CrossRef]
111. Wei, R.; Lunn, S.E.; Tam, R.; Gust, S.L.; Classen, B.; Kerr, P.M.; Plane, F. Vasoconstrictor stimulus determines the functional contribution of myoendothelial feedback to mesenteric arterial tone. *J. Physiol.* **2018**, *596*, 1181–1197.
112. Aguirre, J.A.; Lucchinetti, E.; Clanachan, A.S.; Plane, F.; Zaugg, M. Unraveling Interactions between Anesthetics and the Endothelium: Update and Novel Insights. *Anesth. Analg.* **2016**, *122*, 330–348. [CrossRef]
113. Tran, C.H.; Gordon, G.R. Astrocyte and microvascular imaging in awake animals using two-photon microscopy. *Microcirculation* **2015**, *22*, 219–227. [CrossRef] [PubMed]
114. Suarez-Martinez, A.D.; Peirce, S.M.; Isakson, B.E.; Nice, M.; Wang, J.; Lounsbury, K.M.; Scallan, J.P.; Murfee, W.L. Induction of microvascular network growth in the mouse mesentery. *Microcirculation* **2018**, *25*, e12502. [PubMed]
115. Biwer, L.A.; Lechauve, C.; Vanhoose, S.; Weiss, M.J.; Isakson, B.E. A Cell Culture Model of Resistance Arteries. *J. Vis. Exp.* **2017**. [CrossRef] [PubMed]
116. Pires, P.W.; Dabertrand, F.; Earley, S. Isolation and Cannulation of Cerebral Parenchymal Arterioles. *J. Vis. Exp.* **2016**. [CrossRef]
117. Wilson, C.; Saunter, C.D.; Girkin, J.M.; McCarron, J.G. Pressure-dependent regulation of Ca^{2+} signalling in the vascular endothelium. *J. Physiol.* **2015**, *593*, 5231–5253. [PubMed]
118. Bagher, P.; Segal, S.S. The mouse cremaster muscle preparation for intravital imaging of the microCirc.ulation. *J. Vis. Exp.* **2011**. [CrossRef] [PubMed]
119. Koot, B.G.; Alders, M.; Verheij, J.; Beuers, U.; Cobben, J.M. A de novo mutation in KCNN3 associated with autosomal dominant idiopathic non-cirrhotic portal hypertension. *J. Hepatol.* **2016**, *64*, 974–977. [CrossRef] [PubMed]
120. Ellinor, P.T.; Lunetta, K.L.; Glazer, N.L.; Pfeufer, A.; Alonso, A.; Chung, M.K.; Sinner, M.F.; de Bakker, P.I.; Mueller, M.; Lubitz, S.A.; et al. Common variants in KCNN3 are associated with lone atrial fibrillation. *Nat. Genet.* **2010**, *42*, 240–244. [CrossRef] [PubMed]
121. Sun, Y.; Yang, Y.Q.; Gong, X.Q.; Wang, X.H.; Li, R.G.; Tan, H.W.; Liu, X.; Fang, W.Y.; Bai, D. Novel germline GJA5/connexin40 mutations associated with lone atrial fibrillation impair gap junctional intercellular communication. *Hum. Mutat.* **2013**, *34*, 603–609.
122. Ghanshani, S.; Coleman, M.; Gustavsson, P.; Wu, A.C.; Gargus, J.J.; Gutman, G.A.; Dahl, N.; Mohrenweiser, H.; Chandy, K.G. Human calcium-activated potassium channel gene KCNN4 maps to chromosome 19q13.2 in the region deleted in diamond-blackfan anemia. *Genomics* **1998**, *51*, 160–161. [CrossRef]
123. Rapetti-Mauss, R.; Lacoste, C.; Picard, V.; Guitton, C.; Lombard, E.; Loosveld, M.; Nivaggioni, V.; Dasilva, N.; Salgado, D.; Desvignes, J.P.; et al. A mutation in the Gardos channel is associated with hereditary xerocytosis. *Blood* **2015**, *126*, 1273–1280. [CrossRef] [PubMed]
124. Nilius, B.; Szallasi, A. Transient receptor potential channels as drug targets: From the Science of basic research to the art of medicine. *Pharm. Rev.* **2014**, *66*, 676–814. [CrossRef] [PubMed]
125. Letavernier, E.; Rodenas, A.; Guerrot, D.; Haymann, J.P. Williams-BEur.en syndrome hypercalcemia: Is TRPC3 a novel mediator in calcium homeostasis? *Pediatrics* **2012**, *129*, e1626–e1630. [PubMed]
126. Daumy, X.; Amarouch, M.Y.; Lindenbaum, P.; Bonnaud, S.; Charpentier, E.; Bianchi, B.; Nafzger, S.; Baron, E.; Fouchard, S.; Thollet, A.; et al. Targeted resequencing identifies TRPM4 as a major gene predisposing to progressive familial heart block type I. *Int. J. Cardiol.* **2016**, *207*, 349–358. [CrossRef] [PubMed]
127. Deng, A.Y.; deBlois, D.; Laporte, S.A.; Gelinas, D.; Tardif, J.C.; Thorin, E.; Shi, Y.; Raignault, A.; Menard, A. Novel Pathogenesis of Hypertension and Diastolic Dysfunction Caused by M3R (Muscarinic Cholinergic 3 Receptor) Signaling. *Hypertension* **2018**, *72*, 755–764. [PubMed]

128. Wang, L.; Guo, D.C.; Cao, J.; Gong, L.; Kamm, K.E.; Regalado, E.; Li, L.; Shete, S.; He, W.Q.; Zhu, M.S.; et al. Mutations in myosin light chain kinase cause familial aortic dissections. *Am. J. Hum. Genet.* **2010**, *87*, 701–707. [CrossRef]
129. Benjamin, E.J.; Virani, S.S.; Callaway, C.W.; Chamberlain, A.M.; Chang, A.R.; Cheng, S.; Chiuve, S.E.; Cushman, M.; Delling, F.N.; Deo, R.; et al. Heart Disease and Stroke Statistics-2018 Update: A Report from the American Heart Association. *Circulation* **2018**, *137*, e67–e492. [PubMed]
130. Abete, P.; Della-Morte, D.; Gargiulo, G.; Basile, C.; Langellotto, A.; Galizia, G.; Testa, G.; Canonico, V.; Bonaduce, D.; Cacciatore, F. Cognitive impairment and cardiovascular diseases in the elderly. A heart-brain continuum hypothesis. *Ageing Res. Rev.* **2014**, *18*, 41–52. [CrossRef]
131. Berridge, M.J. Vitamin D, reactive oxygen species and calcium signalling in ageing and disease. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2016**, *371*, 20150434. [CrossRef]
132. Bugiardini, R.; Manfrini, O.; Pizzi, C.; Fontana, F.; Morgagni, G. Endothelial function predicts future development of coronary artery disease: A study of women with chest pain and normal coronary angiograms. *Circulation* **2004**, *109*, 2518–2523.
133. Schachinger, V.; Britten, M.B.; Zeiher, A.M. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* **2000**, *101*, 1899–1906. [CrossRef]
134. Segal, S.S. Integration and Modulation of Intercellular Signaling Underlying Blood Flow Control. *J. Vasc. Res.* **2015**, *52*, 136–157. [CrossRef]
135. Climent, B.; Moreno, L.; Martinez, P.; Contreras, C.; Sanchez, A.; Perez-Vizcaino, F.; Garcia-Sacristan, A.; Rivera, L.; Prieto, D. Upregulation of SK3 and IK1 channels contributes to the enhanced endothelial calcium signaling and the preserved coronary relaxation in obese Zucker rats. *PLoS ONE* **2014**, *9*, e109432. [CrossRef]
136. Chadha, P.S.; Haddock, R.E.; Howitt, L.; Morris, M.J.; Murphy, T.V.; Grayson, T.H.; Sandow, S.L. Obesity up-regulates intermediate conductance calcium-activated potassium channels and myoendothelial gap junctions to maintain endothelial vasodilator function. *J. Pharm. Exp.* **2010**, *335*, 284–293. [CrossRef]
137. Giachini, F.R.; Carneiro, F.S.; Lima, V.V.; Carneiro, Z.N.; Dorrance, A.; Webb, R.C.; Tostes, R.C. Upregulation of intermediate calcium-activated potassium channels counterbalance the impaired endothelium-dependent vasodilation in stroke-prone spontaneously hypertensive rats. *Transl. Res.* **2009**, *154*, 183–193. [CrossRef]
138. Schach, C.; Resch, M.; Schmid, P.M.; Riegger, G.A.; Endemann, D.H. Type 2 diabetes: Increased expression and contribution of IK_{Ca} channels to vasodilation in small mesenteric arteries of ZDF rats. *Am. J. Physiol. Heart Circ. Physiol.* **2014**, *307*, H1093–H1102. [CrossRef]
139. Yada, T.; Shimokawa, H.; Tachibana, H. Endothelium-dependent hyperpolarization-mediated vasodilatation compensates nitric oxide-mediated endothelial dysfunction during ischemia in diabetes-induced canine coronary collateral microcirculation in vivo. *Microcirculation* **2018**, *25*, e12456. [CrossRef]
140. Mokhtar, S.S.; Vanhoutte, P.M.; Leung, S.W.; Yusof, M.I.; Wan Sulaiman, W.A.; Mat Saad, A.Z.; Suppian, R.; Rasool, A.H. Endothelium dependent hyperpolarization-type relaxation compensates for attenuated nitric oxide-mediated responses in subcutaneous arteries of diabetic patients. *Nitric Oxide* **2016**, *53*, 35–44. [CrossRef]
141. Bondarenko, A.I.; Panasiuk, O.; Okhai, I.; Montecucco, F.; Brandt, K.J.; Mach, F. Ca^{2+}-dependent potassium channels and cannabinoid signaling in the endothelium of apolipoprotein E knockout mice before plaque formation. *J. Mol. Cell. Cardiol.* **2018**, *115*, 54–63. [CrossRef]
142. Stead, R.; Musa, M.G.; Bryant, C.L.; Lanham, S.A.; Johnston, D.A.; Reynolds, R.; Torrens, C.; Fraser, P.A.; Clough, G.F. Developmental conditioning of endothelium-derived hyperpolarizing factor-mediated vasorelaxation. *J. Hypertens.* **2016**, *34*, 452–463. [CrossRef]
143. Choi, S.; Kim, J.A.; Li, H.Y.; Shin, K.O.; Oh, G.T.; Lee, Y.M.; Oh, S.; Pewzner-Jung, Y.; Futerman, A.H.; Suh, S.H. $K_{Ca}3.1$ upregulation preserves endothelium-dependent vasorelaxation during aging and oxidative stress. *Aging Cell* **2016**, *15*, 801–810. [CrossRef] [PubMed]
144. Behringer, E.J.; Segal, S.S. Impact of Aging on Calcium Signaling and Membrane Potential in Endothelium of Resistance Arteries: A Role for Mitochondria. *J. Gerontol. A Biol. Sci. Med. Sci.* **2017**, *72*, 1627–1637. [CrossRef] [PubMed]
145. Naik, J.S.; Walker, B.R. Endothelial-dependent dilation following chronic hypoxia involves TRPV4-mediated activation of endothelial BK channels. *Pflug. Arch.* **2018**, *470*, 633–648. [CrossRef]

146. Jobs, A.; Schmidt, K.; Schmidt, V.J.; Lubkemeier, I.; van Veen, T.A.; Kurtz, A.; Willecke, K.; de Wit, C. Defective Cx40 maintains Cx37 expression but intact Cx40 is crucial for conducted dilations irrespective of hypertension. *Hypertension* **2012**, *60*, 1422–1429. [CrossRef]
147. Givvimani, S.; Narayanan, N.; Armaghan, F.; Pushpakumar, S.; Tyagi, S.C. Attenuation of conducted vasodilation in skeletal muscle arterioles during hyperhomocysteinemia. *Pharmacology* **2013**, *91*, 287–296. [CrossRef]
148. Lemmey, H.A.L.; Ye, X.; Ding, H.C.; Triggle, C.R.; Garland, C.J.; Dora, K.A. Hyperglycaemia disrupts conducted vasodilation in the resistance vasculature of db/db mice. *Vasc. Pharm.* **2018**, *103–105*, 29–35. [CrossRef] [PubMed]
149. Bachschmid, M.M.; Schildknecht, S.; Matsui, R.; Zee, R.; Haeussler, D.; Cohen, R.A.; Pimental, D.; Loo, B. Vascular aging: Chronic oxidative stress and impairment of redox signaling-consequences for vascular homeostasis and disease. *Ann. Med.* **2013**, *45*, 17–36. [CrossRef] [PubMed]
150. Muller-Delp, J.M.; Gurovich, A.N.; Christou, D.D.; Leeuwenburgh, C. Redox balance in the aging microCirc.ulation: New friends, new foes, and new clinical directions. *Microcirculation* **2012**, *19*, 19–28. [CrossRef]
151. Murphy, M.P. How mitochondria produce reactive oxygen species. *Biochem. J.* **2009**, *417*, 1–13. [CrossRef]
152. Chidgey, J.; Fraser, P.A.; Aaronson, P.I. Reactive oxygen species facilitate the EDH response in arterioles by potentiating intracellular endothelial Ca^{2+} release. *Free Radic. Biol. Med.* **2016**, *97*, 274–284. [CrossRef]
153. Feletou, M. Endothelium-Dependent Hyperpolarization and Endothelial Dysfunction. *J. Cardiovasc. Pharm.* **2016**, *67*, 373–387. [CrossRef] [PubMed]
154. Leo, C.H.; Hart, J.L.; Woodman, O.L. Impairment of both nitric oxide-mediated and EDHF-type relaxation in small mesenteric arteries from rats with streptozotocin-induced diabetes. *Br. J. Pharm.* **2011**, *162*, 365–377. [CrossRef] [PubMed]
155. Long, D.A.; Newaz, M.A.; Prabhakar, S.S.; Price, K.L.; Truong, L.D.; Feng, L.; Mu, W.; Oyekan, A.O.; Johnson, R.J. Loss of nitric oxide and endothelial-derived hyperpolarizing factor-mediated responses in aging. *Kidney Int.* **2005**, *68*, 2154–2163. [CrossRef] [PubMed]
156. Chennupati, R.; Lamers, W.H.; Koehler, S.E.; De Mey, J.G. Endothelium-dependent hyperpolarization-related relaxations diminish with age in murine saphenous arteries of both sexes. *Br. J. Pharm.* **2013**, *169*, 1486–1499.
157. Seki, T.; Goto, K.; Kiyohara, K.; Kansui, Y.; Murakami, N.; Haga, Y.; Ohtsubo, T.; Matsumura, K.; Kitazono, T. Downregulation of Endothelial Transient Receptor Potential Vanilloid Type 4 Channel and Small-Conductance of Ca^{2+}-Activated K^+ Channels Underpins Impaired Endothelium-Dependent Hyperpolarization in Hypertension. *Hypertension* **2017**, *69*, 143–153. [PubMed]
158. Gradel, A.K.J.; Salomonsson, M.; Sorensen, C.M.; Holstein-Rathlou, N.H.; Jensen, L.J. Long-term diet-induced hypertension in rats is associated with reduced expression and function of small artery SK_{Ca}, IK_{Ca}, and $K_{ir}2.1$ channels. *Clin. Sci. (Lond.)* **2018**, *132*, 461–474. [CrossRef] [PubMed]
159. Wandall-Frostholm, C.; Dalsgaard, T.; Bajoriunas, V.; Olivan-Viguera, A.; Sadda, V.; Beck, L.; Mogensen, S.; Stankevicius, E.; Simonsen, U.; Kohler, R. Genetic deficit of KCa 3.1 channels protects against pulmonary Circ.ulatory collapse induced by TRPV4 channel activation. *Br. J. Pharmacol.* **2015**, *172*, 4493–4505. [PubMed]
160. Simonsen, U.; Wandall-Frostholm, C.; Olivan-Viguera, A.; Kohler, R. Emerging roles of calcium-activated K channels and TRPV4 channels in lung oedema and pulmonary Circ.ulatory collapse. *Acta Physiol. (Oxf.)* **2017**, *219*, 176–187. [CrossRef] [PubMed]
161. Dalsgaard, T.; Sonkusare, S.K.; Teuscher, C.; Poynter, M.E.; Nelson, M.T. Pharmacological inhibitors of TRPV4 channels reduce cytokine production, restore endothelial function and increase survival in septic mice. *Sci. Rep.* **2016**, *6*, 33841. [PubMed]
162. Jones, J.L.; Peana, D.; Veteto, A.B.; Lambert, M.D.; Nourian, Z.; Karasseva, N.G.; Hill, M.A.; Lindman, B.R.; Baines, C.P.; Krenz, M.; et al. TRPV4 increases cardiomyocyte calcium cycling and contractility yet contributes to damage in the aged heart following hypoosmotic stress. *Cardiovasc. Res.* **2019**, *115*, 46–56.
163. Nachman, R.L.; Jaffe, E.A. Endothelial cell culture: Beginnings of modern vascular biology. *J. Clin. Investig.* **2004**, *114*, 1037–1040. [CrossRef] [PubMed]
164. Wilson, C.; Lee, M.D.; Heathcote, H.R.; Zhang, X.; Buckley, C.; Girkin, J.M.; Saunter, C.D.; McCarron, J.G. Mitochondrial ATP production provides long-range control of endothelial inositol trisphosphate-evoked calcium signaling. *J. Biol. Chem.* **2019**, *294*, 737–758. [CrossRef]

165. Durand, M.J.; Ait-Aissa, K.; Levchenko, V.; Staruschenko, A.; Gutterman, D.D.; Beyer, A.M. Visualization and Quantification of Mitochondrial Structure in the Endothelium of Intact Arteries. *Cardiovasc. Res.* **2018**. [CrossRef]
166. Busija, D.W.; Rutkai, I.; Dutta, S.; Katakam, P.V. Role of Mitochondria in Cerebral Vascular Function: Energy Production, Cellular Protection, and Regulation of Vascular Tone. *Compr. Physiol.* **2016**, *6*, 1529–1548. [PubMed]
167. Kaczara, P.; Motterlini, R.; Rosen, G.M.; Augustynek, B.; Bednarczyk, P.; Szewczyk, A.; Foresti, R.; Chlopicki, S. Carbon monoxide released by CORM-401 uncouples mitochondrial respiration and inhibits glycolysis in endothelial cells: A role for mitoBKCa channels. *Biochim. Biophys. Acta* **2015**, *1847*, 1297–1309. [CrossRef] [PubMed]
168. Kanagy, N.L.; Kevil, C.G. The pleiotropic effects of hydrogen sulfide. *Am. J. Physiol. Heart Circ. Physiol.* **2018**, *314*, H1–H2. [CrossRef] [PubMed]
169. Naik, J.S.; Osmond, J.M.; Walker, B.R.; Kanagy, N.L. Hydrogen sulfide-induced vasodilation mediated by endothelial TRPV4 channels. *Am. J. Physiol. Heart Circ. Physiol.* **2016**, *311*, H1437–H1444. [CrossRef] [PubMed]
170. Tang, G.; Yang, G.; Jiang, B.; Ju, Y.; Wu, L.; Wang, R. H_2S is an endothelium-derived hyperpolarizing factor. *Antioxid. Redox Signal.* **2013**, *19*, 1634–1646. [CrossRef] [PubMed]
171. Durante, W. Targeting heme oxygenase-1 in vascular disease. *Curr. Drug Targets* **2010**, *11*, 1504–1516. [CrossRef]
172. McRae, K.E.; Pudwell, J.; Peterson, N.; Smith, G.N. Inhaled carbon monoxide increases vasodilation in the microvascular circulation. *Microvasc. Res.* **2019**, *123*, 92–98. [CrossRef]
173. Rezkalla, S.; Kloner, R.A. Cardiovascular effects of marijuana. *Trends Cardiovasc. Med.* **2018**. [CrossRef] [PubMed]
174. Ho, W.S.; Zheng, X.; Zhang, D.X. Role of endothelial TRPV4 channels in vascular actions of the endocannabinoid, 2-arachidonoylglycerol. *Br. J. Pharm.* **2015**, *172*, 5251–5264. [CrossRef] [PubMed]
175. Stanley, C.P.; O'Sullivan, S.E. Cyclooxygenase metabolism mediates vasorelaxation to 2-arachidonoylglycerol (2-AG) in human mesenteric arteries. *Pharm. Res.* **2014**, *81*, 74–82. [CrossRef] [PubMed]
176. Bondarenko, A.I.; Panasiuk, O.; Drachuk, K.; Montecucco, F.; Brandt, K.J.; Mach, F. The quest for endothelial atypical cannabinoid receptor: BK_{Ca} channels act as cellular sensors for cannabinoids in in vitro and in situ endothelial cells. *Vasc. Pharm.* **2018**, *102*, 44–55. [CrossRef]
177. Brandes, R.P.; Schmitz-Winnenthal, F.H.; Feletou, M.; Godecke, A.; Huang, P.L.; Vanhoutte, P.M.; Fleming, I.; Busse, R. An endothelium-derived hyperpolarizing factor distinct from NO and prostacyclin is a major endothelium-dependent vasodilator in resistance vessels of wild-type and endothelial NO synthase knockout mice. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 9747–9752. [CrossRef] [PubMed]
178. Limbu, R.; Cottrell, G.S.; McNeish, A.J. Characterisation of the vasodilation effects of DHA and EPA, n-3 PUFAs (fish oils), in rat aorta and mesenteric resistance arteries. *PLoS ONE* **2018**, *13*, e0192484. [CrossRef]
179. Idris Khodja, N.; Chataigneau, T.; Auger, C.; Schini-Kerth, V.B. Grape-derived polyphenols improve aging-related endothelial dysfunction in rat mesenteric artery: Role of oxidative stress and the angiotensin system. *PLoS ONE* **2012**, *7*, e32039.
180. Han, J.; Xu, H.H.; Chen, X.L.; Hu, H.R.; Hu, K.M.; Chen, Z.W.; He, G.W. Total Flavone of Rhododendron Improves Cerebral Ischemia Injury by Activating Vascular TRPV4 to Induce Endothelium-Derived Hyperpolarizing Factor-Mediated Responses. *Evid.-Based Complement. Altern. Med.* **2018**, *2018*, 8919867. [CrossRef]
181. Ruamyod, K.; Watanapa, W.B.; Shayakul, C. Testosterone rapidly increases Ca^{2+}-activated K^+ currents causing hyperpolarization in human coronary artery endothelial cells. *J. Steroid Biochem. Mol. Biol.* **2017**, *168*, 118–126. [CrossRef]
182. Mazzuca, M.Q.; Mata, K.M.; Li, W.; Rangan, S.S.; Khalil, R.A. Estrogen receptor subtypes mediate distinct microvascular dilation and reduction in $[Ca^{2+}]_i$ in mesenteric microvessels of female rat. *J. Pharm. Exp.* **2015**, *352*, 291–304. [CrossRef]
183. Aird, W.C. Spatial and temporal dynamics of the endothelium. *J. Thromb. Haemost.* **2005**, *3*, 1392–1406.
184. Fishman, A.P. Endothelium: A distributed organ of diverse capabilities. *Ann. N. Y. Acad. Sci.* **1982**, *401*, 1–8. [CrossRef] [PubMed]

185. Hielscher, D.; Kaebisch, C.; Braun, B.J.V.; Gray, K.; Tobiasch, E. Stem Cell Sources and Graft Material for Vascular Tissue Engineering. *Stem Cell Rev.* **2018**, *14*, 642–667. [CrossRef] [PubMed]
186. Mauricio, M.D.; Guerra-Ojeda, S.; Marchio, P.; Valles, S.L.; Aldasoro, M.; Escribano-Lopez, I.; Herance, J.R.; Rocha, M.; Vila, J.M.; Victor, V.M. Nanoparticles in Medicine: A Focus on Vascular Oxidative Stress. *Oxid. Med. Cell. Longev.* **2018**, *2018*, 6231482. [CrossRef]
187. Phan, D.T.; Bender, R.H.F.; Andrejecsk, J.W.; Sobrino, A.; Hachey, S.J.; George, S.C.; Hughes, C.C. Blood-brain barrier-on-a-chip: MicroPhysiol.ogical systems that capture the complexity of the blood-central nervous system interface. *Exp. Biol. Med. (Maywood)* **2017**, *242*, 1669–1678. [CrossRef] [PubMed]

© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).