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REVIEW

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Cycling matters: Sex hormone regulation of vascular potassium channels

Samuel N Baldwin[a], Thomas A Jepps[a], and Iain A Greenwood[b]

[a]Vascular Biology Group, Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark; [b]Vascular Biology Research Centre, Institute of Molecular and Clinical Sciences, St George's University of London, London, UK

ABSTRACT

Sex hormones and the reproductive cycle (estrus in rodents and menstrual in humans) have a known impact on arterial function. In spite of this, sex hormones and the estrus/menstrual cycle are often neglected experimental factors in vascular basic preclinical scientific research. Recent research by our own laboratory indicates that cyclical changes in serum concentrations of sex - hormones across the rat estrus cycle, primarily estradiol, have significant consequences for the subcellular trafficking and function of K_V7. Vascular potassium channels, including K_V7, are essential components of vascular reactivity. Our study represents a small part of a growing field of literature aimed at determining the role of sex hormones in regulating arterial ion channel function. This review covers key findings describing the current understanding of sex hormone regulation of vascular potassium channels, with a focus on K_V7 channels. Further, we highlight areas of research where the estrus cycle should be considered in future studies to determine the consequences of physiological oscillations in concentrations of sex hormones on vascular potassium channel function.

Introduction

Cyclical changes in sex hormones driven by the human or rodent reproductive cycle coincide with changes in vascular function [1]. To date, research regarding the impact of sex hormones on vascular function has focused on sex-hormone-mediated changes in the production of endothelial-derived relaxing factors such as nitric oxide (NO), prostaglandin I_2 (PGI_2), or endothelial-derived hyperpolarization (EDH) [2–5]. These pathways depend on the activation of vascular smooth muscle cell (VSMC) potassium (K^+) channels to elicit their effect. For example, large conductance calcium-activated potassium channels (BK_{Ca}), voltage-gated potassium channels (K_V), and inwardly rectifying potassium channels are activated in response to NO [6,7], PGI_2 [8–12], and EDH [13,14], respectively. Yet, comparably little is known about the influence of sex hormones on vascular smooth muscle K^+ channels. Recently, Baldwin *et al.* 2022 and 2023 showed the functional impact of arterial KCNQ-encoded voltage-dependent potassium channels (termed Kv7s) changed considerably across the

rat estrus cycle [12,15]. These works are in the vanguard of research identifying estrus-cycle-dependent oscillations in smooth muscle functionality outside of the sex-dependent differences in arterial responsiveness conventionally reported [16]. Alterations in smooth muscle ion channel activity driven by sex hormone oscillations have potential implications in disease manifestations and pharmacological treatments, highlighting why estrus "cycling" matters. This review provides an overview of this nascent and emerging field of research and draws comparisons with known work in other cell types. It focuses primarily on Kv7 channels, but also includes aspects on how sex and sex hormones regulate other K^+ channels.

The estrus cycle

Sex hormones, principally estrogens, progesterone, and androgens, fluctuate throughout the course of the estrus (rodent) and menstrual (human) cycles. A direct comparison between rodent and human cycles is difficult as the rodent estrus cycle last

only 4–5 days and is split into four stages (proestrus, estrus, metestrus and diestrus); whereas the human cycle lasts roughly 28 days and has three phases (follicular, ovulation and luteal), followed by menses. Deducing the impact of sex hormones on vascular reactivity throughout the menstrual cycle is challenging, not least because of additional genetic and environmental factors that will also drive significant changes in human vascular reactivity. Thus, there is a paucity of human data describing arterial function changes throughout the menstrual cycle. As such, this review will focus primarily on the rodent estrus cycle, which is described comprehensively by Nilsson *et al.* (2015) [17]. A brief overview of each hormone and their vascular receptors will be given here.

Estrogen

The bioactive form of estrogen, 17-β estradiol (E2), is synthesized in the theca and granulosa cells of the ovaries, the adrenal glands and locally within the vasculature by aromatase enzymes. E2 produces both genomic and fast acting non-genomic vascular effects through nuclear- and membrane-bound estrogen receptors (ERs). ERα/ERβ, which are canonically considered cytoplasmic nuclear receptors, dimerize to form homo/hetromers upon binding E2, which translocate to the nucleus. Active nuclear ERs bind estrogen response elements within the genome and regulate transcription [18,19]. E2 also exerts rapid non-genomic effects that derive from membrane bound ERα/β [20], which evoke a myriad of intracellular signaling cascades attributed to mitogen activated protein kinase (MAPK)/extracellular signal- regulated kinases (ERK), serine and threonine kinases, phosphoinositide 3-kinase (PI3K), and cAMP [21–24]. ERα/ERβ are ubiquitously expressed across the vasculature [19,25]. A G protein-coupled estrogen receptor (GPER1), designated GPR30 initially, is also expressed in the membrane of the sarcoplasmic reticulum, the golgi apparatus and the plasma membrane of VSMCs and endothelial cells (ECs) [19]. GPER1 couples to a complex array of signaling cascades via $G_{\alpha s,i,q/ll}$ and is further complicated via receptor cross talk with the mineralocorticoid and epidermal growth factor receptors [26,27].

Progesterone

Progesterone is a steroidal hormone produced within the adrenal cortex, gonads and the corpus luteum of the pregnant rat. Its receptors have been found within both ECs and VSMCs of rats, mice and humans [28]. Compared to E2, the literature on progesterone within the vasculature is sparse, though it can be broken down into nuclear and non-nuclear signaling. Progesterone binds two distinct cytoplasmic nuclear receptors termed progesterone receptor (PR) A/B, which are derived from a single gene. Upon binding progesterone, the receptors dimerize, forming homo/heteromers, which translocate to the nucleus and bind progesterone response elements that subsequently regulate gene transcription [29,30]. Moreover, a novel class of membrane-bound progesterone receptor (mPR) has been discovered and includes three subtypes, α, β and γ. The activation of mPR is associated with $G_{\alpha i}$ and subsequent decrease in cAMP, as well as the activation of MAPKs, ERK1/2, and c-Jun N-terminal kinase (JNK1/2) [31–33].

Testosterone

Testosterone, and its more potent analogue di-hydro testosterone, are produced within the gonads and adrenal glands of both sexes. Like E2 and progesterone, testosterone activates both nuclear- and membrane-bound androgen receptors (ARs). Classic ARs include two isoforms, A/B, which are cytosolic nuclear transcription factors that dimerize and translocate to the nucleus where they bind androgen response elements, mediating a change in gene transcription [24]. Recently, a novel class of testosterone-activated G protein-coupled receptor was discovered [34,35], in addition to membrane-associated nuclear ARs. Upon binding testosterone, activated membrane-associated nuclear, and membrane-bound G protein-coupled ARs undergo a conformational change in shape, and can mediated rapid non-genomic effects associated with the activation of a protein kinase C (PKC), protein kinase A (PKA),

MAPK, cAMP, ERK, and protein kinase G (PKG) [24,34,35]. Testosterone can also be converted to E2 locally within the vasculature by aromatase activity, which complicates delineation between vascular testosterone and E2 signaling.

K+ channels

K+ channels are a fundamental factor in the generation of VSMC resting membrane potential, which is roughly −70 to −55 mV [36]. As K+ channels open, the cell becomes permeable to K+ ions, which move down their electrochemical gradient, exiting the cell, thus moving VSMC membrane potential (V_m) toward the equilibrium potential for K+ (E_k). As E_k within VSMC is roughly −90 mV, the cell membrane becomes hyperpolarized. Despite the known role for K+ channels in VSMC reactivity within the male, their role within the female vasculature and how their activity is affected by hormonal fluctuations is poorly understood. K+ channels are principally comprised of homo or heterotetrameric configurations of α-subunits that exist within the membrane in associated with β-auxiliary subunits. β-auxiliary subunits regulate the biophysical, physiological and pharmacological properties of the channel. Both the α- and β-subunits are candidate targets for sex hormone regulation of ion channel function, and will be discussed below. See Table 1 for a summary of the effect of sex hormones on vascular and non-vascular K+ channels.

K$_V$7 channels

K$_V$7 channels are a K$_V$ channel subfamily encoded for by the genes KCNQ1–5, which give rise to distinct α-subunit proteins, termed K$_V$7.1–7.5, respectively. K$_V$7 channels activate slowly upon depolarization with a relatively negative threshold compared to other K$_V$ channels (~-40 mV) and exhibit little inactivation. Every human or rodent vascular bed studied expresses K$_V$7 channel transcripts, with Kcnq1, Kcnq4, and Kcnq5 being the principally expressed genes [92,93]. There is comparably little contribution from Kcnq3 and rarely expression of Kcnq2. K$_V$7 channel stoichiometry varies between systems. Vascular K$_V$7.1 is commonly thought of as exclusively homotetrameric

[94], although Oliveras et al. (2014) demonstrated a K$_V$7.1/7.5 heteromer within coronary artery VSMCs from rats [95]. Vascular K$_V$7.4 readily forms heterotetrameric channels with Kv7.5, but are also found as homotetrameric channels, whereas K$_V$7.5 exists predominantly with K$_V$7.4 in native VSMCs [96,97]. Within rat [98] and murine [99] mesenteric VSMCs, K$_V$7.4 and Kv7.4/7.5 channels exist in close proximity with the KCNE4 β-auxiliary subunit protein, which positively regulates channel trafficking and biophysical/pharmacological properties [99].

K$_V$7 channels regulate arterial smooth muscle contractility and are a key component of receptor-mediated vasodilations in arteries from male animals. Application of the pan-K$_V$7 channel blockers, linopirdine or XE991, depolarizes rat V_m [100,101] and mediates a rise in baseline tension in both human and rodent arteries [92,102–105]. In contrast, K$_V$7.1-specific blockers like HMR-1556 or chromanol 293B do not effect baseline tension in rat aorta, mesenteric nor intrapulmonary arteries [106]. Functional vascular K$_V$7 channel activity is further evidenced by relaxation of pre-contracted arterial tone in response to several K$_V$7.2–7.5 channel activators (e.g. retigabine, acrylamide S-1, ML213) across a number of rodent arteries [93,105,107,108]. Similarly, activators of K$_V$7.1, including (R)-L3, Mefenamic acid and ML277 [106,108], are effective and reversible relaxants of contracted arteries. These data suggest that K$_V$7.4/K$_V$7.5 are regulators of resting arterial smooth muscle V_m due to their low activation threshold and that their activity hyperpolarizes the membrane potential reducing the open probability of voltage-gated calcium channels, thereby negating Ca^{2+} influx. Whereas K$_V$7.1 channels do not contribute to resting V_m, but are functionally expressed.

Pan-K$_V$7 blockers and molecular knockdown of K$_V$7.4/K$_V$7.5 channels also impair receptor-mediated vasorelaxation generated by β-adrenoreceptor, adenosine, calcitonin-gene related peptide, and prostacyclin (IP) receptor agonists (cAMP-linked [12,96,104,109,110]), as well as atrial natriuretic peptide and nitric oxide-mediated responses (cGMP-linked [111–113]) in many arteries from male rats. Interestingly, β-adrenoreceptor mediated relaxation differentially

Table 1. The effect of sex hormones on vascular and non-vascular K$^+$ channels. *= hormone mediator speculated.

Ion channel	Hormone	Upregulated			Downregulated			No effect		
		Subtype	Model	Ref	Subtype	Model	Ref	Subtype	Model	Ref
K_V7	Estrogen				$K_V7.1$	Xenopus Oocytes HT29cl.19A Rat: Distal colic crypt cells	[37] [38] [38–40]			
					$K_V7.1$: KCNE1	Mouse/Guinea pig: Ventricular myocytes	[41]			
					$K_V7.1$: KCNE3	Chinese Hamster Ovarian cells	[40]			
					$K_V7.4$	Rat mesenteric/ renal VSMC	[12,15]			
	Progesterone	$K_V7.1$/ $K_V7.5$/ Kcne1	Murine uterus*	[42]				$K_V7.2$–5	Rat mesenteric artery	[12]
	Testosterone	$K_V7.1$ KCNE4	Rat cardiac myocyte Mouse cardiac myocyte	[43] [44]						
K_V1	Estrogen				$K_V1.5$	Murine ventricular myocytes	[41,45]			
	Progesterone	$K_V1.5$	H9C2 cells	[46]	$K_V1.1$–6	Murine ventricular myocytes	[46]			
	Testosterone							$K_V1.5$	Murine ventricular myocytes	[47,48]
								$K_V1.5$	Rat aorta	[49]
K_V2	Estrogen				$K_V2.1$	Human osteoblast-like MG63 cells Cultured rat hippocampal neurons Guinea pig heat	[50] [51] [41]			
					$K_V2.1$/ 2.2	Murine B-pancreatic cells	[52]			
					$K_V2.2$	Cultured rat cerebellar granule HEK-293 cells	[53] [53]			
	Progesterone	$K_V2.1$	H9C2 cells	[46]				$K_V2.1$	HEK293 cells	[54]
$K_V11.1$	Estrogen	$K_V11.1$	Murine ventricular myocytes Human ECG HEK293B	[55] [57] [57]	$K_V11.1$	Guinea pig heat HEK293B Computational	[41,56] [56,58] [59]			
	Progesterone	$K_V11.1$	Guinea pig ventricular myocytes Computational	[60] [60]	$K_V11.1$	Rat neonatal cardiac myocytes Murine uterus*	[61] [62]			
					$K_V11.1$: KCNE2	Human uterus*	[63]			
	Testosterone	$K_V11.1$	HEK293B Rabbit ventricular myocytes	[64] [65]						

(Continued)

Table 1. (Continued).

Ion channel	Hormone	Channel function/expression								
		Upregulated			Downregulated			No effect		
		Subtype	Model	Ref	Subtype	Model	Ref	Subtype	Model	Ref
BK_{Ca}	Estrogen	BK_{Ca}1.1: K_{Ca}β1	Rat cerebral arteries	[66]	BK_{Ca}1.1	HUV-EC-C	[67]			
		BK_{Ca}1.1	Human coronary arteries	[68,69]						
			Porcine coronary arteries	[70]						
			Cultured human coronary/aortic VSMCs	[71]						
			Ovine uterus arteries	[72–74]						
	Progesterone				BK_{Ca}1.1	Xenopus oocytes	[75]	BK_{Ca}1.1	Ovine uterus arteries	[72]
	Testosterone	BK_{Ca}1.1	Porcine coronary arteries	[76,77]						
			Rat mesenteric arteries	[78]						
K_{ATP}	Estrogen	K_{ATP}: SUR1, mKATP	Murine myocardium	[79]	K_{ATP}	Murine β-pancreatic cells	[80–82]	K_{ATP}	Human coronary artery	[83]
		K_{ATP}: SUR2A	H9c2	[84]						
		K_{ATP}: SUR1	Rat brain cortices	[85]						
		K_{ATP}	Murine gonadotrophin releasing neurons	[86,87]						
			Human myocardium	[83]						
	Testosterone	K_{ATP}	Canine coronary artery	[88]				K_{ATP}	Rat aorta	[89]
			Rat aorta	[90]						
			Human corporal artery	[91]						

couples to K_V7 in a vascular bed specific manner, via PKA in renal arteries, and a novel alternate signaling cascade mediated by exchange protein activated by cAMP (EPAC) in mesenteric arteries [109]. $K_V7.1$-specific blockers do not affect an array of receptor-mediated vasorelaxations, although Baldwin et al. (2022) revealed that relaxations of rat mesenteric artery induced by activation of IP receptors, either with iloprost or MRE-269, were attenuated by HMR1556 to the same degree as the pan-Kv7 blocker linopirdine.

A growing body of literature indicates sexual dimorphisms in vascular K_V7 physiology. Abbott and Jepps (2015) observed that mesenteric arteries from male *Kcne4-/-* knockout mice showed an increased sensitivity to α-1 adrenoreceptor agonist methoxamine and decreased sensitivity to $K_V7.2$–7.5 activator ML213, which was not observed in females [98]. Further, Berg (2018) observed that vascular K_V7 channel function was preserved in female hypertensive animals when compared to

males [114]. As above, Baldwin *et al.* (2022) demonstrated a novel role for $K_V7.1$ as the downstream target of IP receptor evoked relaxation in mesenteric arteries from both male and female rats [12]. However, the sensitivity of IP receptor-mediated relaxation to inhibition of $K_V7.1$ by pre-incubation of HMR-1556 $(10\,\mu mol\text{-}L^{-1})$ was absent in arteries from animals harvested during proestrus or estrus stages of the estrus cycle [12], stages associated with an initial spike in serum concentration of E2 [17]. This correlated with a significant decrease in sensitivity to IP receptor-mediated relaxation when compared to mesenteric arteries from female animals harvested from dies-trus/met-estrus, where HMR-1556 still had an effect. These findings were the first demonstration of an estrus cycle-sensitive regulation of arterial K_V7 function.

Subsequently, Baldwin *et al.* (2023) focused on responsiveness of several K_V7 channel activators across the estrus cycle of the rat. These

experiments revealed that during cycle stages associated with high E2, proestrus/estrus, $K_V7.2$–5 channel activator-mediated relaxations were markedly impaired when compared to diestrus/metestrus, where serum E2 was low [15]. Similarly, thromboxane A2 (TXA2) receptor-mediated vasoconstriction was enhanced and β adrenoceptor-mediated relaxation was impaired during proestrus/diestrus when compared to diestrus/metestrus [15]. The sensitivity of these responses to K_V7 inhibition during proestrus/estrus was also diminished [15]. An impairment of vascular function during proestrus/estrus was associated with a translocation of $K_V7.4$ from the plasma membrane. Both K_V7 function and protein membrane abundance were impaired by application of exogenous E2 and the GPER1 agonist, G1, in arteries from females form low serum E2 stages of the estrus cycle, diestrus/metestrus, but not proestrus/metestrus. These *ex vivo* manipulations were prevented by prior application of the GPER1 antagonist, G36. The findings of Baldwin et al. (2023) were the first to identify a cyclical estrous-regulation of an ion channel in the vasculature and the functional consequences. This work reinforces earlier studies [115–117], indicating that $K_V7.4$ channel abundance in the plasma membrane is labile and at the mercy of the arterial environment.

Whilst the impact of sex hormones on vascular Kv7 channels is a nascent field of research, work in other cell types also indicates an acute estrogenic inhibition of K_V7 function and expression. For example, supraphysiological concentrations of E2 ($1\,\mu mol\text{-}L^{-1}$) inhibit $K_V7.1$:KCNE1 channel derived currents in an heterologous expression system [37] and the E2 receptor antagonist, tamoxifen, increased $K_V7.1$ function and expression in murine and guinea pig ventricular myocytes [41], suggesting a tonic estrogenic suppression of the channel. E2-induced inhibition of $K_V7.1$ derived currents in rat distal colic crypt cells occurs in a PKC-δ-dependent manner, independent of the classical estrogen receptors [39], and basolateral K_V currents responsible for Cl^- secretion are inhibited by E2 via uncoupling of $K_V7.1$:KCNE3 [40]. Moreover, in HT29cl.19A cells, E2 abrogated $K_V7.1$ membrane expression through PKC-δ-dependent channel endocytosis into early endosomes, which is then recycled to the membrane [38]. Taken as a collective, the aforementioned studies provide strong evidence of estrogenic K_V7:KCNE channel regulation in a complex process of channel recycling. Within renal arteries from female rats, VSMC KCNE4 membrane abundance did not fluctuate across the estrus cycle [15]. Consequently, the role of KCNE4 in E2 regulation of vascular $K_V7.4$ remains to be determined, though a process of E2-mediated vascular $K_V7.4$ recycling similar to that described in Rapetti-Mauss *et al.* (2013) is plausible, in light of the rapid recovery of vascular $K_V7.2$–5 function between estrus cycle stages [15].

In contrast to E2, short-term supplemental progesterone incubation on ex vivo mesenteric arteries from female rats had no effect on the vasorelaxation elicited by $K_V7.2$–7.5 channel activators across the estrus cycle [15]. These data cannot conclusively rule out a role for progesterone on K_V7 channel function or expression, as K_V7 channel modulator sensitivity and membrane abundance of $K_V7.4$ was enhanced during metestrus/diestrus, where serum progesterone was highest [15]. Similarly, in mouse myometrial tissue, the total copy number for *Kcnq1*, *Kcnq5* and *Kcne1* increases during metestrus [42]. Future experiments with antiprogestogens, and long-term incubation of arteries (+24hrs) are required to determine the impact of progesterone on vascular K_V7 channel function.

Within male rodents, testosterone increases the relative expression of *Kcnq1* in cardiomyocytes [43]. This has been postulated to underpin testosterone mediated QT interval shortening [43]. The impact of testosterone signaling on vascular K_V7 channels has yet to be investigated, however, testosterone upregulates *Kcne4* [44] expression in ventricular myocytes from male mice. *Kcne4* transcript is more abundantly expressed in mesenteric arteries from male mice when compared to females, but Kv7.4 protein expression is lower; however, the responses to ML213 were the same [98]. These data potentially suggest that females have more $K_V7.4$ channels but the testosterone-mediated upregulation of *Kcne4* increases the channel function in the males. Though this remains to be determined.

K_V7 channels are being considered as a novel therapeutic target in the treatment of hypertension

and other diseases [118]. Though the gross impact of "estrus cycling" vascular K_V7 channel function on blood pressure and health has yet to be established, current understanding indicates that the pharmacology required to target the channel conventionally, through activators for example, would change dramatically across the menstrual cycle, thereby excluding these channels as convenient targets in pre-menopausal women.

K_V1 channels

Several studies have found transcript and protein expression of different K_V1 channel subtypes across various vascular beds from multiple animal models, with Kv1.5 predominating [119–126]. $K_V1.5$ channels are implicated in regulating myogenic tone [124,125] and receptor-mediated contraction [127] and relaxation [128–132]. Interestingly, $K_V1.3$ has been implicated in the phenotypic switch to contractile from proliferative VSMCs via PLCγ, independent of K^+ influx [133,134], prospectively through their c-terminal domain [135].

Within murine mesenteric arteries, no difference in the abundance of $K_V1.5$ was detected between males and females [136], however whole cell K_V currents were greater in mesenteric arteries from female mice [136] and rats [66]. Though estrus cycle regulation of vascular K_V1 channels has yet to be investigated, Saito *et al.* (2009) observed diminished $K_V1.5$ currents and protein in murine ventricular myocytes harvested from females in estrus when compared to di-estrus [45]. Further, supplemental E2 inhibited ventricular $K_V1.5$ currents and protein expression in ovariectomized mice [45] and tamoxifen increased the relative abundance of $K_V1.5$ [41]. Some evidence indicates that levels of progesterone seen during pregnancy can impair $K_V1.3/1.5$ transcript expression in native cardiac myocytes and heterologous expression systems [46]. Within the vasculature, testosterone deprivation impairs K_V current and $K_V1.5$ expression in aortic VSMCs [49]. Similarly, within ventricular myocytes, castration diminished and testosterone replacement enhanced $K_V1.5$

expression and function [47,48]. Evidence therefore exists for estrus cycle dependent regulation of K_V1 channels [45], with E2 impairing and testosterone promoting K_V1 function, though this remains to be investigated within the vasculature.

K_V2 channels

Several studies have identified *KCNB*-encoded $K_V2.1$ and $K_V2.2$ in whole lysates of arteries [137,138]. Of note, $K_V2.2$ is minimally expressed compared to $K_V2.1$ in rat middle cerebral artery [139] and aorta [140]. As $K_V2.1$ and $K_V2.2$ form heterotetramers [141], the effects of $K_V2.2$ can be difficult to delineate. Consequently, the current literature has focused predominantly on $K_V2.1$ by comparison to $K_V2.2$. $K_V2.1$ contributes to K_V currents in rat pulmonary [142], mesenteric [143] and aortic [144,145] VSMCs. Functionally, $K_V2.1$ regulates resting V_m in rat middle cerebral artery and opposes myogenic tone in a pressure-sensitive process [146] in heteromultimeric configurations of $K_V2.1/K_V9.3$ [139]. $K_V2.1$ is also upregulated in response atrial natriuretic peptide- and NO-dependent signaling within the rat aorta [140].

The only study to consider sex-differences in vascular K_V2 channels to date found that mesenteric VSMCs from female C57BL/6J mice expressed greater $K_V2.1$ protein and current when compared to males [136]. Paradoxically, these findings were observed in conjunction with enhanced $Ca_V1.2$ open probability, greater pressure mediated $[Ca^{2+}]_i$ increase and pressure induced tone within arteries from female mice [136]. To explain these contrasting findings, O'Dwyer *et al.* (2020) argue for a novel role for non-conductive $K_V2.1$ channels in regulating VSMC plasmalemmal $Ca_V1.2$ channel clustering and function [136]. Thus, in their model, a greater ratio of $K_V2.1:Ca_V1.2$ in arteries from female animals leads to enhanced Ca^{2+} influx and constriction [136].

Though the estrus cycle was not considered in the previous study, some evidence exists for the regulation of K_V2 by sex hormones. Supplemental E2 diminishes both native $K_V2.1$

currents in osteocytes, in a process speculated to be non-genomic [50], and over-expressed $K_V2.1$ in cultured hippocampal neurons [51]. Further, ER inhibition with tamoxifen increases $K_V2.1$ expression in guinea pig heart [41], ER_β signaling impairs $K_V2.1/2.2$ currents in β-pancreatic cells [52] and GPER1 activation inhibits $K_V2.2$ expression in a PKC-sensitive signaling cascade [53]. Though in murine ventricular myocytes, no estrus cycle-sensitive change in $K_V2.1$ current or protein expression was observed, which calls into question whether Kv2 channels are regulated by estrous/menstrual cycle-dependent changes in E2 levels [45]. The role of progesterone in regulating K_V2 is even less clear. Progesterone (1 μmol-L^{-1}) diminished $K_V2.1$ expression in murine cardiac myocytes via mPR, which reportedly underpins prolonged QT intervals and action potential duration [46]. Though progesterone (10 μmol-L^{-1}) had no effect on $K_V2.1$-encoded currents in HEK 293 cells [54]. The reason for this discrepancy is currently unclear, however, a serum concentration of 1 μmol-L^{-1} progesterone is not achieved during pregnancy [147]. As such, estrus cycle regulation of vascular K_V2 channels remains speculative; however, sex differences in vascular K_V2 expression and function is indicated and merits further investigation.

$K_V11.1$ channels

Isoforms of *KCNH2* encode $K_V11.1$, commonly known as *ether-a-go-go* (ERG)-related channels, including ERG1a and ERG1b, contribute to the fast-component of the delayed rectifying current (QT interval) of the cardiac action potential [148,149]. Within the vasculature, currents sensitive to the ERG1 blocker, E-4031, were demonstrated in murine portal vein [150,151], and K_V11 protein and transcript were identified within isolated murine aortic, carotid and femoral arterial smooth muscle cells [152], though no ERG currents were detectable. Modulation of $K_V11.1$ did however alter VSMC proliferation [152]. Additionally, $K_V11.1$ inhibitor dofetilide had no functional effect in quiescent vessels, including rat aorta, mesenteric and intralobar arteries [153]. The current consensus is that $K_V11.1$ functionally contributes to repolarization kinetics of spontaneously contractile smooth muscle, including portal vein [62,151,152], esophageal [154], jejunum [155], myometrial [62], and bladder [156], as well as playing a role in VSMC proliferation.

No papers to date have investigated the role of $K_V11.1$ within the vasculature of the female; however, a host of literature has investigated the role of sex hormones in regulating cardiac ERG channels. Ablation of E2 production by aromatase knockout in a mouse model impaired QT interval sensitivity to E-4031 [55]. Furthermore, E2 drives $K_V11.1$ forward trafficking in cardiomyocytes by enhanced interaction with HSP90, thereby increasing ventricular repolarization [57]. However, the E2 receptor inhibitor tamoxifen increased $K_V11.1$ protein expression [41] and physiological concentrations of E2 reduce $K_V11.1$ currents in guinea pig hearts [56] and HEK cells [58]. Additionally, mutagenesis [56], pharmacological [58], and computational [59] studies indicate that E2 binds to the $K_V11.1$ pore in a manner similar to $K_V11.1$ inhibitor dofetilide [59].

In contrast to E2, the role of progesterone and testosterone derivatives in regulating $K_V11.1$ is clearer. Physiological levels of progesterone seen during the follicular phase upregulate cardiac $K_V11.1$ currents in guinea pig ventricular myocytes [60]. Though progesterone, as seen during pregnancy, impairs $K_V11.1$ forward trafficking in rat neonatal cardiac myocytes [61]. Similarly, the considerable impact of $K_V11.1$ blockers on myometrial contractility is lost in pregnant mice and humans as they progress through pregnancy [62,63]. This effect is associated with an increase in KCNE2 proteins as labor develops [62,63], reinforcing the crucial role for auxiliary subunit proteins in K^+ channel regulation and as the site of hormone based regulation. Similarly to progesterone, testosterone improves native $K_V11.1$ function in HEK cells [64] and rabbit ventricular myocytes in a non-genomic post-transcriptional mechanism which enhances $K_V11.1$ current density and voltage sensitivity [65].

These findings implicate a cyclical regulation of E2 suppression and progesterone promotion of $K_V11.1$ function across the estrus cycle, either through channel block or through auxiliary

subunit regulation, respectively. As no ERG currents have been demonstrated in $K_V11.1$ expressing quiescent systemic vascular beds [152], the functional consequence for sex hormones in mediating sex-differences in $K_V11.1$ regulation may be slight. Though in light of the role of ERG in mediating VSMC proliferation, there is a potential role for androgenic regulation of $K_V11.1$ in sex-differences in vessel size [157], angiogenesis [158], vascularization [159], and angiogenesis [160].

BK$_{Ca}$ channels

KCNMA1 encodes for the pore-forming α subunit $K_{Ca}1.1$, which in turn gives rise to homotetrameric channels within the membrane termed BK_{Ca}. Unique amongst K_{Ca}, BK_{Ca} are both Ca^{2+} and voltage activated [161–163]. Within the vasculature, BK_{Ca} expression is predominantly restricted to VSMCs, though some expression may be present within the endothelium [164,165]. Vascular BK_{Ca} channels primarily couple with the $K_{Ca}\beta1$ auxiliary subunit, which improves $[Ca^{2+}]_i$ and voltage sensitivity when compared to $K_{Ca}1.1$ alone [166–168]. In a vascular bed specific manner, inhibitors of BK_{Ca} channels increase basal and pressure-induced tone [11,169–173]. These descriptions provide only a brief insight into the role of BK_{Ca} channels within the vasculature, for more detail see [174].

BK_{Ca} channels have been identified in male and female human mesenteric [175] and coronary [176] arteries, though data was not separated dependent on sex. Functional BK_{Ca} channels have also been demonstrated in omental and myometrial arteries from women [177,178]. Within rodents, BK_{Ca}-derived currents are greater within middle cerebral artery VSMCs isolated from adult female rats when compared to age-matched males, though this phenotype dissipates with age, due to a decrease in $K_{Ca}\beta1$ expression [66]. Though the estrus cycle was not factored into this study, ovariectomy diminished BK_{Ca} channel expression and enhanced pressure-mediated increases in vessel tone [66] and BK_{Ca} expression and vessel function was ameliorated by supplemental E2 dietary pellets [66].

Mechanistic insight into E2-mediated changes in BK_{Ca} function indicate that E2 (0.02–5 μmol-L^{-1})-GPER1 signaling upregulated vascular BK_{Ca} currents in human [68,69] and porcine [70] coronary artery VSMCs, via PKC. Further, ER_α-derived PI3K signaling increased $BK_{Ca}1.1$ transcript expression in cultured human coronary and aortic VSMCs [71] and E2 (0.3 nmol-L^{-1}) treatment increased BK_{Ca} currents in ovine uterine arteries via $K_{Ca}\beta1$ modulation [72–74]. The importance of the discrepancies in the receptor responsible for E2-mediated increases in vascular BK_{Ca} activity is debatable, as E2 has similar affinities for both $ER_{\alpha/\beta}$ and GPER1 [179]. These data strongly support an upregulation of BK_{Ca} channels in VSMCs by E2, although it should be noted that outside VSMCs, including in cultured human endothelial cells [67], BK_{Ca} channel expression and function is negatively regulated by E2 [180].

Investigations regarding the effect of progesterone on vascular BK_{Ca} are sparse. In heterologous expression systems, progesterone (10 μmol-L^{-1}) inhibits BK_{Ca} derived currents [75], in a process speculated to be due to an increase in intracellular pH, and a decrease in cytosolic Ca^{2+}/cAMP. Though progesterone (0.1 μmol-L^{-1}) has no effect on native BK_{Ca} currents in uterine artery VSMCs from sheep [72].

Conversely, testosterone can enhance BK_{Ca}-mediated relaxations through activation of the NO-PKG cascade in porcine coronary [76,77] and rat mesenteric arteries [78]. As both serum testosterone and E2 are raised significantly during proestrus [17], current literature would indicate an increase in vascular responsiveness during proestrus. In contrast to this hypothesis, we observed no change in relaxation response to the BK_{Ca} channel activator NS11021 in mesenteric nor renal arteries harvested from female animals across the estrus cycle [15]. The reason for this discrepancy remains to be determined and requires further investigation.

K$_{ATP}$ channels

ATP-sensitive K^+ channels (K_{ATP}) are a member of the inwardly rectifying K^+ channel (K_{IR}) subfamily and are derived from $K_{IR}6.x$ proteins. K_{ATP} however express poor inward rectification by

comparison to other K_{IR} channels [181,182]. Expression of $K_{IR}6.x$ alone does not form functional K^+ channels, but requires the co-expression of sulphonylurea receptors (SURs) [183–187], including SUR1, SUR2A and SUR2B [188]. K_{ATP} are now recognized as intracellular sensors that convey changes in metabolic states to electrical signals across the membrane. K_{ATP} inhibitors contract coronary [189,190] and renal arteries [191] in normotensive animals, implicating K_{ATP} in the regulation of basal arterial tone. K_{ATP} is the target of myriad receptor-mediated signaling cascades, for more detail, see [174].

Functional K_{ATP} channels are an essential components of human chorionic plate, umbilical and myometrial artery reactivity [178,192–195]. K_{ATP} channels have been demonstrated in middle cerebral, meningeal, superficial temporal and cutaneous arteries within male and female humans [196–200]. Reports of sexual dimorphisms in vascular K_{ATP} function are contradicting. For example, there are no apparent sex-dependent differences in sensitivity to K_{ATP} channel modulators in rodent cerebral [201,202] nor mesenteric [203] arteries from normotensive rats, though a later study found impaired K_{ATP} activator mediated relaxation within arteries from male hypertensive rats when compared to females [203]. Similarly, inhibition of K_{ATP} had greater impact on skeletal muscle tissue oxygenation in female rats when compared to males [204], reportedly as a result of increased VSMC K_{ATP} activity in female rats [204].

As regards to the role of sex hormones in regulating K_{ATP}, E2 protects against ischemic reperfusion injury within myocardium [79] and neurons [85] by enhanced mitochondrial-K_{ATP} (mKATP) and $K_{IR}6.2/SUR1$ function, respectively. mKATP channels when activated prevent apoptosis, enhance respiration and reduce matrix Ca^{2+} accumulation [205]. E2-mediated upregulation of K_{ATP} function is reported as both a genomic and non-genomic process, for example, E2 upregulates SUR expression within myocardial and neuronal cells [79,84,85] and also gives rise to a PKA-PKC-mediated increases in K_{ATP} activity in neuronal cells [86,87]. Similarly within humans, following angioplasty, E2 protects against myocardial ischemia by increasing myocardial K_{ATP} activity and through enhanced coronary artery vasodilation [83], however, increased coronary artery vasodilation in response to E2 was insensitive to K_{ATP} inhibition [83]. In contrast to neuronal and myocardial cells, E2 rapidly inhibited K_{ATP} function within murine β-pancreatic cells via ERβ [80,81] in a cGMP-PKG-sensitive signaling cascade [82]. Consequently, the role of E2 in regulating vascular K_{ATP} expression or activity remains speculative, and may be either positive or negative.

A comprehensive study on the effect of progesterone on K_{ATP} is yet to be undertaken. Within our own study, we observed an estrus cycle-dependent shift in sensitivity to relaxations elicited by a K_{ATP} activator within mesenteric, but not renal, arteries from female rats within proestrus/estrus, when compared to arteries from female rats within diestrus/metestrus [15]. When comparing serum sex hormone concentration between these groups we observed raised E2 in the former and raised progesterone in the later [15]. Consequently, it is unclear if mesenteric vascular K_{ATP} channel function is impaired by E2 or enhanced by progesterone [15]; however, pinacidil sensitivity was comparable between arteries from females of a low-serum estradiol stage of the estrus cycle and arteries from male animals (data not published), indicative of E2 inhibition of vascular K_{ATP} function. Further, the cause of the vascular bed specific estrus cycle regulation of K_{ATP} channel function was also unclear.

Finally, conflicting reports indicate testosterone as either a K_{ATP}-dependent or K_{ATP}-independent vasodilator [206]. For example, testosterone mediates K_{ATP} sensitive vasorelaxation in canine coronary [88], rat aortic [90] and human corporal [91] arteries, either via release of endothelial derived relaxant factors [88,90] or via direct interaction with the channel [91]. Conversely, the K_{ATP} inhibitor Glibenclamide had no effect on testosterone-mediated relaxation in rat aorta [89]. The reason for the discrepancy between Honda *et al.* (1999) and Ding and Stallone (2001), may be accounted for by differences in rat models. Both studies employ similar concentrations of both testosterone and Glibenclamide yielding conflicting results, warranting further investigation.

Conclusion

This brief review demonstrates a range of extra-vascular and vascular studies, which detail sexual dimorphisms in ion channel function. These differences are further complicated by genomic and non-genomic sex hormone regulation of ion channels. However, fluctuations in sex hormone levels across the estrus cycle is rarely taken into consideration and studies commonly favor supraphysiological concentrations of sex hormones to investigate their role. Our lack of understanding of female biology from a basic preclinical science perspective has culminated in a greater prevalence in adverse drug reactions within women when compared with age-matched men [207]. This review highlights the need to revisit basic principles of ion channels within the vasculature of the female, with keen consideration of the estrus cycle.

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Data availability statement

Data sharing is not applicable to this article.

References

[1] Pabbidi MR, Kuppusamy M, Didion SP, et al. Sex differences in the vascular function and related mechanisms: role of 17β-estradiol. Am J Physiol Circ Physiol. 2018 Sep 7;315(6):H1499–518. InternetAvailable from. doi:10.1152/ajpheart.00194.2018.

[2] Niţă AR, Knock GA, Heads RJ. Signalling mechanisms in the cardiovascular protective effects of estrogen: with a focus on rapid/membrane signalling. Curr Res Physiol. 2021;4:103–118.

[3] Willemars MMA, Nabben M, Verdonschot JAJ, et al. Evaluation of the interaction of sex hormones and cardiovascular function and health. Curr Heart Fail Rep. 2022 Aug;19(4):200–212.

[4] Sobrino A, Oviedo PJ, Novella S, et al. Estradiol selectively stimulates endothelial prostacyclin production through estrogen receptor-{alpha}. J Mol Endocrinol. 2010 Apr;44(4):237–246.

[5] Leung SWS, Vanhoutte PM. Endothelium-dependent hyperpolarization: age, gender and blood pressure, do they matter? Acta Physiol (Oxf). 2017 Jan;219(1):108–123.

[6] Fukao M, Mason HS, Britton FC, et al. Cyclic GMP-dependent protein kinase activates cloned BKCa channels expressed in mammalian cells by direct phosphorylation at serine 1072. J Biol Chem. 1999 Apr;274(16):10927–10935.

[7] Tanaka Y, Koike K, Toro L. MaxiK channel roles in blood vessel relaxations induced by endothelium-derived relaxing factors and their molecular mechanisms. J Smooth Muscle Res. 2004;40(4,5):125–153.

[8] Dumas M, Dumas JP, Rochette L, et al. Role of potassium channels and nitric oxide in the effects of iloprost and prostaglandin E1 on hypoxic vasoconstriction in the isolated perfused lung of the rat. Br J Pharmacol. 1997;120(3):405–410.

[9] Lombard JH, Liu Y, Fredricks KT, et al. Electrical and mechanical responses of rat middle cerebral arteries to reduced PO2 and prostacyclin. Am J Physiol - Hear Circ Physiol. 1999;276(2):H509–516.

[10] Schubert R, Serebryakov VN, Engel H, et al. Iloprost activates K(Ca) channels of vascular smooth muscle cells: role of Camp-dependent protein kinase. Am J Physiol - Cell Physiol. 1996;271(4):C1203–1211.

[11] Schubert R, Serebryakov VN, Mewes H, et al. Iloprost dilates rat small arteries: role of K(ATP)- and K(Ca)-channel activation by Camp-dependent protein kinase. Am J Physiol - Hear Circ Physiol. 1997;272(3):H1147–1156.

[12] Baldwin SN, Forrester EA, McEwan L, et al. Sexual dimorphism in prostacyclin-mimetic responses within rat mesenteric arteries: a novel role for K(V) 7.1 in shaping IP-receptor mediated relaxation. Br J Pharmacol. 2022;179(7):1338–1352. DOI:10.1111/bph.15722

[13] Goto K, Rummery NM, Grayson TH, et al. Attenuation of conducted vasodilatation in rat mesenteric arteries during hypertension: role of inwardly rectifying potassium channels. J Physiol. 2004;561(Pt 1):215–231. InternetAvailable from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1665331&tool=pmcentrez&rendertype=abstract

[14] Sonkusare SK, Dalsgaard T, Bonev AD, et al. Inward rectifier potassium (Kir2.1) channels as end-stage boosters of endothelium-dependent vasodilators. J Physiol. 2016;594(12):3271–3285.

[15] Baldwin SN, Forrester EA, Homer NZM, et al. Marked oestrus cycle-dependent regulation of rat arterial KV7.4 channels driven by GPER1. Br J Pharmacol. 2023;180(2):17–193. DOI:10.1111/bph.15947

[16] Jaimes L, Vinet R, Knox M, et al. A review of the actions of endogenous and exogenous vasoactive substances during the estrous cycle and pregnancy in rats. Anim An Open Access J From MDPI. 2019 May;9(6):288.

[17] Nilsson ME, Vandenput L, Tivesten Å, et al. Measurement of a comprehensive sex steroid profile in rodent serum by high-sensitive gas chromatography-tandem mass spectrometry. Endocrinology. 2015 Jul;156(7):2492–2502.

[18] Matsuda KI, Ochiai I, Nishi M, et al. Colocalization and ligand-dependent discrete distribution of the estrogen receptor (ER) α and ERβ. Mol Endocrinol. 2002;16 (10):2215–2230.

[19] Caroccia B, Seccia TM, Barton M, et al. Estrogen signaling in the adrenal cortex: implications for blood pressure sex differences. Hypertension. 2016;68 (4):840–848.

[20] Razandi M, Pedram A, Greene GL, et al. Cell membrane and nuclear estrogen receptors (ERs) originate from a single transcript: studies of ERα and ERβ expressed in Chinese hamster ovary cells. Mol Endocrinol. 1999;13(2):307–319.

[21] Ronda AC, Buitrago C, Boland R. Role of estrogen receptors, PKC and Src in ERK2 and p38 MAPK signaling triggered by 17β-estradiol in skeletal muscle cells. J Steroid Biochem Mol Biol. 2010 Oct;122 (4):287–294.

[22] Meyer MR, Haas E, Prossnitz ER, et al. Non-genomic regulation of vascular cell function and growth by estrogen. Mol Cell Endocrinol. 2009 Sep;308(1–2):9–16.

[23] Traupe T, Stettler CD, Li H, et al. Distinct roles of estrogen receptors α and β mediating acute vasodilation of epicardial coronary arteries. Hypertension. 2007;49(6):1364–1370. DOI:10.1161/HYPERTENSIONAHA.106.081554

[24] Connelly PJ, Casey H, Montezano AC, et al. Sex steroids receptors, hypertension, and vascular ageing. J Hum Hypertens. 2021 Jul;36(2):120–125.

[25] Haas E, Meyer MR, Schurr U, et al. Differential effects of 17beta-estradiol on function and expression of estrogen receptor alpha, estrogen receptor beta, and GPR30 in arteries and veins of patients with atherosclerosis. Hypertens (Dallas, Tex 1979). 2007 Jun;49(6):1358–1363.

[26] DeLeon C, Wang DQH, CK A. G protein-coupled estrogen receptor, GPER1, offers a novel target for the treatment of digestive diseases. Front Endocrinol. 2020;11:578536.

[27] Gohar EY. G protein-coupled estrogen receptor 1 as a novel regulator of blood pressure. Am J Physiol - Ren Physiol. 2020;319(4):F612–7.

[28] Goletiani NV, Keith DR, Gorsky SJ. Progesterone: review of safety for clinical studies. Exp Clin Psychopharmacol. 2007 Oct;15(5):427–444.

[29] Conneely OM, Mulac-Jericevic B, DeMayo F, et al. Reproductive functions of progesterone receptors. Recent Prog Horm Res. 2002;57:339–355.

[30] Leonhardt SA, Boonyaratanakornkit V, Edwards DP. Progesterone receptor transcription and non-transcription signaling mechanisms. Steroids. 2003 Nov;68(10–13):761–770.

[31] Zhu Y, Bond J, Thomas P. Identification, classification, and partial characterization of genes in humans and other vertebrates homologous to a fish membrane progestin receptor. Proc Natl Acad Sci. 2003;100 (5):2237–2242.

[32] Karteris E, Zervou S, Pang Y, et al. Progesterone signaling in human myometrium through two novel membrane G protein-coupled receptors: potential role in functional progesterone withdrawal at term. Mol Endocrinol. 2006;20(7):1519–1534. DOI:10.1210/me. 2005-0243

[33] dos Santos RL, da Silva FB, Ribeiro RFJ, et al. Sex hormones in the cardiovascular system. Horm Mol Biol Clin Investig. 2014 May;18(2):89–103.

[34] Shihan M, Bulldan A, Scheiner-Bobis G. Non-classical testosterone signaling is mediated by a G-protein-coupled receptor interacting with Gnα11. Biochim Biophys Acta. 2014 Jun;1843(6):1172–1181.

[35] Lorigo M, Mariana M, Lemos MC, et al. Vascular mechanisms of testosterone: the non-genomic point of view. J Steroid Biochem Mol Biol. 2020;196:105496. InternetAvailable from. https://www.sciencedirect.com/science/article/pii/S0960076019302493

[36] Hirst GD, Edwards FR. Sympathetic neuroeffector transmission in arteries and arterioles. Physiol Rev. 1989 Apr;69(2):546–604.

[37] Waldegger S, Lang U, Herzer T, et al. Inhibition of minK protein induced K+ channels in Xenopus oocytes by estrogens. Pflugers Arch Eur J Physiol. 1996 Dec;354(6):698–702.

[38] Rapetti-Mauss R, O'Mahony F, Sepulveda FV, et al. Oestrogen promotes KCNQ1 potassium channel endocytosis and postendocytic trafficking in colonic epithelium. J Physiol. 2013;591(11):2813–2831.

[39] O'Mahony F, Alzamora R, Betts V, et al. Female gender-specific inhibition of KCNQ1 channels and chloride secretion by 17β-estradiol in rat distal colonic crypts. J Biol Chem. 2007;282(34):24563–24573. DOI:10.1074/jbc.M611682200

[40] Alzamora R, O'Mahony F, Bustos V, et al. Sexual dimorphism and oestrogen regulation of KCNE3 expression modulates the functional properties of KCNQ1 K+channels. J Physiol. 2011;589 (21):5091–5107. DOI:10.1113/jphysiol.2011.215772

[41] El Gebeily G, Fiset C. Upregulation of ventricular potassium channels by chronic tamoxifen treatment. Cardiovasc Res. 2011;90(1):68–76.

[42] McCallum LA, Greenwood IA, Tribe RM. Expression and function of Kv7 channels in murine myometrium throughout oestrous cycle. Pflügers Archiv - European J Physiol. 2009;457(5):1111–1120.

[43] Masuda K, Takanari H, Morishima M, et al. Testosterone-mediated upregulation of delayed rectifier potassium channel in cardiomyocytes causes

abbreviation of QT intervals in rats. J Physiol Sci. 2018;68(6):759–767. DOI:10.1007/s12576-017-0590-4

[44] Crump SM, Hu Z, Kant R, et al. Kcne4 deletion sex- and age-specifically impairs cardiac repolarization in mice. FASEB J Off Publ Fed Am Soc Exp Biol. 2016 Jan;30(1):360–369.

[45] Saito T, Ciobotaru A, Bopassa JC, et al. Estrogen contributes to gender differences in mouse ventricular repolarization. Circ Res. 2009;105(4):343–352.

[46] Liang S, Sun YS, Li L, et al. Progesterone changes the pregnancy-induced adaptation of cardiomyocyte Kv2.1 Channels via MicroRNA-29b. Cardiovasc Ther. 2022;2022:1–19.

[47] Liu J, Tsang S, Wong TM. Testosterone is required for delayed cardioprotection and enhanced heat shock protein 70 expression induced by preconditioning. Endocrinology. 2006 Oct;147(10):4569–4577.

[48] Brouillette J, Rivard K, Lizotte E, et al. Sex and strain differences in adult mouse cardiac repolarization: importance of androgens. Cardiovasc Res. 2005 Jan;65 (1):148–157.

[49] Zhou P, Fu L, Pan Z, et al. Testosterone deprivation by castration impairs expression of voltage-dependent potassium channels in rat aorta. Eur J Pharmacol. 2008 Sep;593(1–3):87–91.

[50] Li X, Zheng S, Dong X, et al. 17β-Estradiol inhibits outward voltage-gated k+ currents in human osteoblast-like MG63 cells. J Membr Biol. 2013;246(1):39–45.

[51] Zhang W, Jin HW, Zhang HX, et al. [Effect of 17beta-estradiol on Kv2.1 current and delayed rectifier potassium current in cultured rat hippocampal neurons]. Yao Xue Xue Bao. 2004 Sep;39(9):686–690.

[52] Martinez-Pinna J, Marroqui L, Hmadcha A, et al. Oestrogen receptor β mediates the actions of bisphe-nol-A on ion channel expression in mouse pancreatic beta cells. Diabetologia. 2019;62(9):1667–1680. DOI:10. 1007/s00125-019-4925-y

[53] Dong WH, Chen JC, He YL, et al. Resveratrol inhibits Kv2.2 currents through the estrogen receptor GPR30-mediated PKC pathway. Am J Physiol - Cell Physiol. 2013;305(5):547–557.

[54] Liu X, Fu Y, Yang H, et al. Potential independent action of sigma receptor ligands through inhibition of the Kv2.1 channel. Oncotarget. 2017Aug; 8 (35):59345–59358. InternetAvailable from https://eur opepmc.org/articles/PMC5601737

[55] Kurokawa J, Sasano T, Kodama M, et al. Aromatase knockout mice reveal an impact of estrogen on drug-induced alternation of murine electrocardiography parameters. J Toxicol Sci. 2015;40(3):339–348. DOI:10.2131/jts.40.339

[56] Kurokawa J, Tamagawa M, Harada N, et al. Acute effects of oestrogen on the guinea pig and human IKr channels and drug-induced prolongation of cardiac repolarization. J Physiol. 2008;586(12):2961–2973. DOI:10.1113/jphysiol.2007.150367

[57] Anneken L, Baumann S, Vigneault P, et al. Estradiol regulates human QT-interval: acceleration of cardiac repolarization by enhanced KCNH2 membrane trafficking. Eur Heart J. 2016 Feb;37(7):640–650.

[58] Ando F, Kuruma A, Kawano S. Synergic effects of β-estradiol and erythromycin on hERG currents. J Membr Biol. 2011 May;241(1):31–38.

[59] Yang PC, Perissinotti LL, López-Redondo F, et al. A multiscale computational modelling approach predicts mechanisms of female sex risk in the setting of arousal-induced arrhythmias. J Physiol. 2017;595 (14):4695–4723. DOI:10.1113/JP273142

[60] Nakamura H, Kurokawa J, Bai CX, et al. Progesterone regulates cardiac repolarization through a nongenomic pathway: an in vitro patch-clamp and computational modeling study. Circulation. 2007 Dec;116 (25):2913–2922.

[61] Wu ZY, Yu DJ, Soong TW, et al. Progesterone impairs human ether-a-go-go-related gene (HERG) trafficking by disruption of intracellular cholesterol homeostasis. InternetAvailable from J Biol Chem. 2011;286 (25):22186–22194. doi: 10.1074/jbc.M110.198853

[62] Greenwood IA, Yeung SY, Tribe RM, et al. Loss of functional K+ channels encoded by ether-à-go-go-related genes in mouse myometrium prior to labour onset. J Physiol. 2009 May;587(Pt 10):2313–2326.

[63] Parkington HC, Stevenson J, Tonta MA, et al. Diminished hERG K+ channel activity facilitates strong human labour contractions but is dysregulated in obese women. Nat Commun. 2014 Jun;5:4108.

[64] Wu ZY, Chen K, Haendler B, et al. Stimulation of N-terminal truncated isoform of androgen receptor stabilizes human ether-á-go-go-related gene-encoded potassium channel protein via activation of extracellular signal regulated kinase 1/2. Endocrinology. 2008 Oct;149(10):5061–5069.

[65] Liu XK, Katchman A, Whitfield BH, et al. In vivo androgen treatment shortens the QT interval and increases the densities of inward and delayed rectifier potassium currents in orchiectomized male rabbits. Cardiovasc Res. 2003;57(1):28–36. DOI:10.1016/ S0008-6363(02)00673-9

[66] Reed JT, Pareek T, Sriramula S, et al. Aging influences cerebrovascular myogenic reactivity and BK channel function in a sex-specific manner. Cardiovasc Res. 2020 Jun;116(7):1372–1385.

[67] Chiang HT, Wu SN. Inhibition of large-conductance calcium-activated potassium channel by 2-methoxyestradiol in cultured vascular endothelial (HUV-EC-C) cells. J Membr Biol. 2001 Aug;182 (3):203–212.

[68] White RE, Han G, Maunz M, et al. Endothelium-independent effect of estrogen on Ca2±activated K+ channels in human coronary artery smooth muscle cells. Cardiovasc Res. 2002;53(3):650–661. DOI:10. 1016/S0008-6363(01)00428-X

[69] White RE, Darkow DJ, Lang JL. Estrogen relaxes coronary arteries by opening BKCa channels through a Cgmp-dependent mechanism. Circ Res. 1995 Nov;77 (5):936–942.

[70] Yu X, Ma H, Barman SA, et al. Activation of G protein-coupled estrogen receptor induces endothelium-independent relaxation of coronary artery smooth muscle. Am J Physiol Endocrinol Metab. 2011 Nov;301(5):E882–8.

[71] Danesh SM, Kundu P, Lu R, et al. Distinct transcriptional regulation of human large conductance voltage- and calcium-activated K+ channel gene (hSlo1) by activated estrogen receptor alpha and c-Src tyrosine kinase. J Biol Chem. 2011 Sep;286(36):31064–31071.

[72] Hu XQ, Xiao D, Zhu R, et al. Pregnancy upregulates large-conductance Ca 2±activated K + channel activity and attenuates myogenic tone in uterine arteries. Hypertension. 2011;58(6):1132–1139. DOI:10.1161/HYPERTENSIONAHA.111.179952

[73] Nagar D, Liu XT, Rosenfeld CR. Estrogen regulates β1-subunit expression in Ca 2±activated K+ channels in arteries from reproductive tissues. Am J Physiol - Hear Circ Physiol. 2005;289(4 58–4):1417–1427.

[74] Hu XQ, Song R, Romero M, et al. Pregnancy increases Ca(2+) sparks/spontaneous transient outward currents and reduces uterine arterial myogenic tone. Hypertens (Dallas, Tex 1979). 2019 Mar;73(3):691–702.

[75] Wong CM, Tsang SY, Yao X, et al. Differential effects of estrogen and progesterone on potassium channels expressed in Xenopus oocytes. Steroids. 2008;73 (3):272–279.

[76] Deenadayalu VP, White RE, Stallone JN, et al. Testosterone relaxes coronary arteries by opening the large-conductance, calcium-activated potassium channel. Am J Physiol - Hear Circ Physiol. 2001;281 (4 50–4):1720–1727.

[77] Deenadayalu V, Puttabyatappa Y, Liu AT, et al. Testosterone-induced relaxation of coronary arteries: activation of Bk ca channels via the Cgmp-dependent protein kinase. Am J Physiol - Hear Circ Physiol. 2012;302(1):115–123.

[78] Tep-Areenan P, Kendall DA, Randall MD. Testosterone-induced vasorelaxation in the rat mesenteric arterial bed is mediated predominantly via potassium channels. Br J Pharmacol. 2002;135(3):735–740.

[79] Gao J, Xu D, Sabat G, et al. Disrupting KATP channels diminishes the estrogen-mediated protection in female mutant mice during ischemia-reperfusion. Clin Proteomics. 2014;11(1):1–15.

[80] Nadal A, Rovira JM, Laribi O, et al. Rapid insulinotropic effect of 17beta-estradiol via a plasma membrane receptor. FASEB J Off Publ Fed Am Soc Exp Biol. 1998 Oct;12(13):1341–1348.

[81] Soriano S, Ropero AB, Alonso-Magdalena P, et al. Rapid regulation of K(ATP) channel activity by 17 {beta}-estradiol in pancreatic {beta}-cells involves the estrogen receptor {beta} and the atrial natriuretic peptide receptor. Mol Endocrinol. 2009 Dec;23 (12):1973–1982.

[82] Ropero AB, Fuentes E, Rovira JM, et al. Non-genomic actions of 17β-oestradiol in mouse pancreatic β-cells are mediated by a Cgmp-dependent protein kinase. Journal of Physiology. 1999;521(2):397–407.

[83] Lee TM, Chou TF, Tsai CH. Differential role of K ATP channels activated by conjugated estrogens in the regulation of myocardial and coronary protective effects. Circulation. 2003;107(1):49–54.

[84] Ranki HJ, Budas GR, RM C, et al. 17β-Estradiol regulates expression of KATPchannels in heart-derived H9c2 cells. J Am Coll Cardiol. 2002 Jul 17;40 (2):367–374. InternetAvailable from. doi:10.1016/S0735-1097(02)01947-2.

[85] Zhang D, Xia H, Xu L, et al. Neuroprotective effects of 17β-estradiol associate with KATP in rat brain. Neuroreport. 2012 Nov;23(16):952–957.

[86] Zhang C, Bosch MA, Levine JE, et al. Gonadotropin-releasing hormone neurons express K(ATP) channels that are regulated by estrogen and responsive to glucose and metabolic inhibition. J Neurosci Off J Soc Neurosci. 2007 Sep;27(38):10153–10164.

[87] Zhang C, Kelly MJ, Rønnekleiv OK. 17 β-estradiol rapidly increases ATP-sensitive potassium channel activity in gonadotropin-releasing hormone neurons [corrected] via a protein kinase signaling pathway. Endocrinology. 2010 Sep;151(9):4477–4484.

[88] Chou TM, Sudhir K, Hutchison SJ, et al. Testosterone induces dilation of canine coronary conductance and resistance arteries in vivo. Circulation. 1996 Nov;94 (10):2614–2619.

[89] Ding AQ, Stallone JN. Testosterone-induced relaxation of rat aorta is androgen structure specific and involves K+ channel activation. J Appl Physiol. 2001 Dec;91 (6):2742–2750.

[90] Honda H, Unemoto T, Kogo H. Different mechanisms for testosterone-induced relaxation of aorta between normotensive and spontaneously hypertensive rats. Hypertens (Dallas, Tex 1979). 1999 Dec;34 (6):1232–1236.

[91] Han DH, Chae MR, Jung JH, et al. Effect of testosterone on potassium channel opening in human corporal smooth muscle cells. J Sex Med. 2008 Apr;5 (4):822–832.

[92] Ng FL, Davis AJ, Jepps TA, et al. Expression and function of the K + channel KCNQ genes in human arteries. Br J Pharmacol. 2011;162(1):42–53. DOI:10.1111/j.1476-5381.2010.01027.x

[93] Ohya S, Sergeant GP, Greenwood IA, et al. Molecular variants of KCNQ channels expressed in murine portal vein myocytes: a role in delayed rectifier current. Circ Res. 2003;92(9):1016–1023.

[94] Schwake M, Jentsch TJ, Friedrich T. A carboxy-terminal domain determines the subunit specificity of KCNQ K+ channel assembly. EMBO Rep. 2003;4(1):76–81.

[95] Oliveras A, Roura-Ferrer M, Solé L, et al. Functional assembly of Kv7.1/Kv7.5 channels with emerging properties on vascular muscle physiology. Arterioscler Thromb Vasc Biol. 2014;34(7):1522–1530. DOI:10.1161/ATVBAHA.114.303801

[96] Chadha PS, Jepps TA, Carr G, et al. Contribution of Kv7.4/Kv7.5 heteromers to intrinsic and calcitonin gene-related peptide-induced cerebral reactivity. Arterioscler Thromb Vasc Biol. 2014;34(4):887–893. DOI:10.1161/ATVBAHA.114.303405

[97] Brueggemann LI, Haick JM, Cribbs LL, et al. Differential activation of vascular smooth muscle Kv7.4, Kv7.5, and Kv7.4/7.5 channels by ML213 and ICA-069673. Mol Pharmacol. 2014;86(3):330–341.

[98] Abbott GW, Jepps TA. Kcne4 deletion sex-dependently alters vascular reactivity. J Vasc Res. 2016;53(3–4):138–148.

[99] Jepps TA, Carr G, Lundegaard PR, et al. Fundamental role for the KCNE4 ancillary subunit in Kv7.4 regulation of arterial tone. J Physiol. 2015;593(24):5325–5340.

[100] Mackie AR, Brueggemann LI, Henderson KK, et al. Vascular KCNQ potassium channels as novel targets for the control of mesenteric artery constriction by vasopressin, based on studies in single cells, pressurized arteries, and in vivo measurements of mesenteric vascular resistance. J Pharmacol Exp Ther. 2008;325 (2):475–483. DOI:10.1124/jpet.107.135764

[101] Mani BK, Brueggemann LI, Cribbs LL, et al. Activation of vascular KCNQ (Kv7) potassium channels reverses spasmogen-induced constrictor responses in rat basilar artery. Br J Pharmacol. 2011 Sep;164(2):237–249.

[102] Zhong XZ, Harhun MI, Olesen SP, et al. Participation of KCNQ (Kv7) potassium channels in myogenic control of cerebral arterial diameter. J Physiol. 2010;588 (17):3277–3293. DOI:10.1113/jphysiol.2010.192823

[103] Lee S, Yang Y, Tanner MA, et al. Heterogeneity in Kv7 channel function in the cerebral and coronary circulation. Microcirculation. 2015;22(2):109–121.

[104] Chadha PS, Zunke F, Zhu HL, et al. Reduced KCNQ4-encoded voltage-dependent potassium channel activity underlies impaired β-adrenoceptor-mediated relaxation of renal arteries in hypertension. Hypertension. 2012;59(4):877–884. DOI:10.1161/HYPERTENSIONAHA.111.187427

[105] Yeung SYM, Pucovský V, Moffatt JD, et al. Molecular expression and pharmacological identification of a role for Kv7 channels in murine vascular reactivity. Br J Pharmacol. 2007;151(6):758–770. DOI:10.1038/sj.bjp.0707284

[106] Chadha PS, Zunke F, Davis AJ, et al. Pharmacological dissection of Kv7.1 channels in systemic and pulmonary arteries. Br J Pharmacol. 2012;166 (4):1377–1387. DOI:10.1111/j.1476-5381.2012.01863.x

[107] Jepps TA, Bentzen BH, Stott JB, et al. Vasorelaxant effects of novel Kv7.4 channel enhancers ML213 and NS15370. Br J Pharmacol. 2014;171(19):4413–4424. DOI:10.1111/bph.12805

[108] Baldwin SN, Sandow SL, Mondéjar-Parreño G, et al. KV7 channel expression and function within rat mesenteric endothelial cells. Front Physiol. 2020;11 (December):1–16.

[109] Stott JB, Barrese V, Greenwood IA. Kv7 channel activation underpins EPAC-Dependent relaxations of rat arteries. Arterioscler Thromb Vasc Biol. 2016;36 (12):2404–2411.

[110] van der Horst J, Rognant S, Abbott GW, et al. Dynein regulates Kv7.4 channel trafficking from the cell membrane. J Gen Physiol. 2021 Mar;153(3). doi: 10.1085/jgp.202012760.

[111] Stott JB, Barrese V, Jepps TA, et al. Contribution of Kv7 channels to natriuretic peptide mediated vasodilation in normal and hypertensive rats. Hypertension. 2015;65(3):676–682.

[112] Jepps TA, Olesen SP, Greenwood IA, et al. Molecular and functional characterization of Kv7 channels in penile arteries and corpus cavernosum of healthy and metabolic syndrome rats. Br J Pharmacol. 2016;173 (9):1478–1490.

[113] Mondéjar-Parreño G, Moral-Sanz J, Barreira B, et al. Activation of Kv7 channels as a novel mechanism for NO/NO/Cgmp-induced pulmonary vasodilation. Br J Pharmacol. 2019. doi:10.1111/bph.14662.

[114] Berg T. Kv7(kcnq)-K±Channels influence total peripheral resistance in female but not male rats, and hamper catecholamine release in hypertensive rats of both sexes. Front Physiol. 2018;9. DOI:10.3389/fphys.2018.00117

[115] Jepps TA, Chadha PS, Davis AJ, et al. Downregulation of Kv7.4 channel activity in primary and secondary hypertension. Circulation. 2011;124(5):602–611. DOI:10.1161/CIRCULATIONAHA.111.032136

[116] Carr G, Barrese V, Stott JB, et al. MicroRNA-153 targeting of KCNQ4 contributes to vascular dysfunction in hypertension. Cardiovasc Res. 2016;112 (2):581–589. DOI:10.1093/cvr/cvw177

[117] Barrese V, Stott JB, Figueiredo HB, et al. Angiotensin II Promotes K V 7.4 channels degradation through reduced interaction with HSP90 (Heat Shock Protein 90)Novelty and significance. Hypertension. 2018;71 (6):1091–1100. DOI:10.1161/HYPERTENSIONAHA.118.11116

[118] Barrese V, Stott JB, Greenwood IA. KCNQ-Encoded potassium channels as therapeutic targets. Annu Rev Pharmacol Toxicol. 2018;58(1):625–648.

[119] Cheong A, Dedman AM, Beech DJ. Expression and function of native potassium channel [K(v)alpha1]

subunits in terminal arterioles of rabbit. J Physiol. 2001 Aug;534(Pt 3):691–700.

[120] Cheong A, Dedman AM, Xu SZ, et al. K(v)alpha1 channels in murine arterioles: differential cellular expression and regulation of diameter. Am J Physiol Heart Circ Physiol. 2001 Sep;281(3): H1057–65.

[121] Kerr PM, Clément-Chomienne O, Thorneloe KS, et al. Heteromultimeric Kv1.2-Kv1.5 channels underlie 4-aminopyridine-sensitive delayed rectifier K(+) current of rabbit vascular myocytes. Circ Res. 2001;89(11):1038–1044. DOI:10.1161/hh2301.100803

[122] Fergus DJ, Martens JR, England SK. Kv channel subunits that contribute to voltage-gated K+ current in renal vascular smooth muscle. Pflugers Arch. 2003 Mar;445(6):697–704.

[123] Cox RH, Fromme S. Functional expression profile of voltage-Gated K(+) channel subunits in rat small mesenteric arteries. Cell Biochem Biophys. 2016 Jun;74(2):263–276.

[124] Chen TT, Luykenaar KD, Walsh EJ, et al. Key role of Kv1 channels in vasoregulation. Circ Res. 2006 Jul;99(1):53–60.

[125] Plane F, Johnson R, Kerr P, et al. Heteromultimeric Kv1 channels contribute to myogenic control of arterial diameter. Circ Res. 2005 Feb;96(2):216–224.

[126] Thorneloe KS, Chen TT, Kerr PM, et al. Molecular composition of 4-aminopyridine-sensitive voltage-gated K+ channels of vascular smooth muscle. Circ Res. 2001;89(11):1030–1037. DOI:10.1161/hh2301.100817

[127] Cogolludo A, Moreno L, Lodi F, et al. Serotonin inhibits voltage-gated K+ currents in pulmonary artery smooth muscle cells: role of 5-HT2A receptors, caveolin-1, and KV1.5 channel internalization. Circ Res. 2006 Apr;98(7):931–938.

[128] Kwak YG, Hu N, Wei J, et al. Protein kinase a phosphorylation alters Kvbeta1.3 subunit-mediated inactivation of the Kv1.5 potassium channel. J Biol Chem. 1999 May;274(20):13928–13932.

[129] Aiello EA, Walsh MP, Cole WC. Phosphorylation by protein kinase a enhances delayed rectifier K+ current in rabbit vascular smooth muscle cells. Am J Physiol. 1995 Feb;268(2 Pt 2):H926–34.

[130] Aiello EA, Malcolm AT, Walsh MP, et al. Beta-adrenoceptor activation and PKA regulate delayed rectifier K+ channels of vascular smooth muscle cells. Am J Physiol. 1998 Aug;275(2):H448–59.

[131] Johnson RP, El-Yazbi AF, Hughes MF, et al. Identification and functional characterization of protein kinase A-catalyzed phosphorylation of potassium channel Kv1.2 at serine 449. J Biol Chem. 2009 Jun;284(24):16562–16574.

[132] Jackson WF. K V channels and the regulation of vascular smooth muscle tone. Microcirculation. 2018 Jan;25(1):e12421.

[133] Cidad P, Jiménez-Pérez L, García-Arribas D, et al. Kv1.3 channels can modulate cell proliferation during phenotypic switch by an ion-flux independent mechanism. Arterioscler Thromb Vasc Biol. 2012 May;32(5):1299–1307.

[134] Cidad P, Miguel-Velado E, Ruiz-McDavitt C, et al. Kv1.3 channels modulate human vascular smooth muscle cells proliferation independently of mTOR signaling pathway. Pflugers Arch. 2015 Aug;467(8):1711–1722.

[135] Jiménez-Pérez L, Cidad P, Álvarez-Miguel I, et al. Molecular Determinants of Kv1.3 potassium channels-induced proliferation. J Biol Chem. 2016 Feb;291(7):3569–3580.

[136] O'Dwyer SC, Palacio S, Matsumoto C, et al. Kv2.1 channels play opposing roles in regulating membrane potential, Ca2+ channel function, and myogenic tone in arterial smooth muscle. Proc Natl Acad Sci U S A. 2020;117(7):3858–3866. DOI:10.1073/pnas.1917879117

[137] Xu C, Lu Y, Tang G, et al. Expression of voltage-dependent K(+) channel genes in mesenteric artery smooth muscle cells. Am J Physiol. 1999 Nov;277(5):G1055–63.

[138] Schmalz F, Kinsella J, Koh SD, et al. Molecular identification of a component of delayed rectifier current in gastrointestinal smooth muscles. Am J Physiol Liver Physiol. 1998;274(5):G901–11. DOI:10.1152/ajpgi.1998.274.5.G901

[139] Zhong XZ, Abd-Elrahman KS, Liao CH, et al. Stromatoxin-sensitive, heteromultimeric Kv2.1/Kv9.3 channels contribute to myogenic control of cerebral arterial diameter. J Physiol. 2010 Nov;588(Pt 22):4519–4537.

[140] Tanaka Y, Tang G, Takizawa K, et al. Kv channels contribute to nitric oxide- and atrial natriuretic peptide-induced relaxation of a rat conduit artery. J Pharmacol Exp Ther. 2006 Apr;317(1):341–354.

[141] Blaine JT, Ribera AB. Heteromultimeric potassium channels formed by members of the Kv2 subfamily. J Neurosci. 1998 Dec;18(23):9585–9593.

[142] Smirnov SV, Beck R, Tammaro P, et al. Electrophysiologically distinct smooth muscle cell subtypes in rat conduit and resistance pulmonary arteries. J Physiol. 2002 Feb;538(Pt 3):867–878.

[143] Lu Y, Hanna ST, Tang G, et al. Contributions of Kv1.2, Kv1.5 and Kv2.1 subunits to the native delayed rectifier K(+) current in rat mesenteric artery smooth muscle cells. Life Sci. 2002 Aug;71(12):1465–1473.

[144] Tammaro P, Smith AL, Hutchings SR, et al. Pharmacological evidence for a key role of voltage-gated K+ channels in the function of rat aortic smooth muscle cells. Br J Pharmacol. 2004 Sep;143(2):303–317.

[145] Belevych AE, Beck R, Tammaro LC, et al. Developmental changes in the functional characteristics and expression of voltage-gated K+channel

currents in rat aortic myocytes. Cardiovasc Res. 2002;54(1):152–161.

[146] Amberg GC, Santana LF. Kv2 channels oppose myogenic constriction of rat cerebral arteries. Am J Physiol Cell Physiol. 2006 Aug;291(2):C348–56.

[147] Ku CW, Allen JCJ, Lek SM, et al. Serum progesterone distribution in normal pregnancies compared to pregnancies complicated by threatened miscarriage from 5 to 13 weeks gestation: a prospective cohort study. Bmc Pregnancy Childbirth. 2018 Sep;18(1):360.

[148] Lees-Miller JP, Kondo C, Wang L, et al. Electrophysiological characterization of an alternatively processed ERG K+ channel in mouse and human hearts. Circ Res. 1997 Nov;81(5):719–726.

[149] Larsen AP, Olesen SP, Grunnet M, et al. Characterization of hErg1a and hErg1b potassium channels-a possible role for hErg1b in the I (Kr) current. Pflugers Arch. 2008 Sep;456(6):1137–1148.

[150] Ohya S, Horowitz B, Greenwood IA. Functional and molecular identification of ERG channels in murine portal vein myocytes. Am J Physiol Cell Physiol. 2002 Sep;283(3):C866–77.

[151] Yeung SYM, Greenwood IA. Pharmacological and biophysical isolation of K+ currents encoded by ether-à-go-go-related genes in murine hepatic portal vein smooth muscle cells. Am J Physiol Cell Physiol. 2007 Jan;292 (1):C468–76.

[152] Barrese V, Cidad P, Yeung SY, et al. Proliferative Role of Kv11 Channels in Murine Arteries. Front Physiol. 2017;8:500.

[153] Doggrell SA, Nand V. Effects of dofetilide on cardiovascular tissues from normo- and hypertensive rats. J Pharm Pharmacol. 2002 May;54(5):707–715.

[154] Akbarali HI, Thatte H, He XD, et al. Role of HERG-like K(+) currents in opossum esophageal circular smooth muscle. Am J Physiol. 1999 Dec;277(6): C1284–90.

[155] Farrelly AM, Ro S, Callaghan BP, et al. Expression and function of KCNH2 (HERG) in the human jejunum. Am J Physiol Gastrointest Liver Physiol. 2003 Jun;284 (6):G883–95.

[156] Barrese V, Wehbe Z, Linden A, et al. Key role for Kv11.1 (ether-a-go-go related gene) channels in rat bladder contractility. Physiol Rep. 2023 Feb;11(3):e15583.

[157] Hiteshi AK, Li D, Gao Y, et al. Gender differences in coronary artery diameter are not related to body habitus or left ventricular mass. Clin Cardiol. 2014 Oct;37 (10):605–609.

[158] Sodhi EU, Philpott HT, Carter MM, et al. Sex-differences and associations between complement activation and synovial vascularization in patients with late-stage knee osteoarthritis. Front Immunol. 2022;13:890094.

[159] Irani YD, Pulford E, Mortimer L, et al. Sex differences in corneal neovascularization in response to superficial corneal cautery in the rat. PLoS ONE. 2019;14(9):1–17. DOI:10.1371/journal.pone.0221566

[160] Fairweather D. Sex differences in inflammation during atherosclerosis. Clin Med Insights Cardiol. 2014;8 (Suppl 3):49–59.

[161] Marty A. Ca-dependent K channels with large unitary conductance in chromaffin cell membranes. Nature. 1981;291(5815):497–500. doi:10.1038/291497a0. InternetAvailable from.

[162] Golowasch J, Kirkwood A, Miller C. Allosteric effects of Mg2+ on the gating of Ca2±activated K+ channels from mammalian skeletal muscle. J Exp Biol. 1986 Sep;124:5–13.

[163] Zhang J, Yan J. Regulation of BK channels by auxiliary γ subunits. Front Physiol. 2014;5:401.

[164] Benham CD, Bolton TB, Lang RJ, et al. Calcium-activated potassium channels in single smooth muscle cells of rabbit jejunum and guinea-pig mesenteric artery. J Physiol. 1986 Feb;371:45–67.

[165] Sandow SL, Grayson TH. Limits of isolation and culture: intact vascular endothelium and BKCa. Am J Physiol Heart Circ Physiol. 2009 Jul;297(1):H1–7.

[166] McCobb DP, Fowler NL, Featherstone T, et al. A human calcium-activated potassium channel gene expressed in vascular smooth muscle. Am J Physiol. 1995 Sep;269(3 Pt 2):H767–77.

[167] McManus OB, Helms LM, Pallanck L, et al. Functional role of the beta subunit of high conductance calcium-activated potassium channels. Neuron. 1995 Mar;14(3):645–650.

[168] Meera P, Wallner M, Jiang Z, et al. A calcium switch for the functional coupling between alpha (hslo) and beta subunits (Kv,ca beta) of maxi K channels. FEBS Lett. 1996 Mar;382(1–2):84–88.

[169] Berczi V, Stekiel WJ, Contney SJ, et al. Pressure-induced activation of membrane K+ current in rat saphenous artery. Hypertension. 1992 Jun;19(6 Pt 2):725–729. Hypertens (Dallas, Tex 1979). doi:10.1161/01.HYP.19.6.725.

[170] Howitt L, Sandow SL, Grayson TH, et al. Differential effects of diet-induced obesity on BKCa {beta}1--subunit expression and function in rat skeletal muscle arterioles and small cerebral arteries. Am J Physiol Heart Circ Physiol. 2011 Jul;301(1):H29–40.

[171] Jackson-Weaver O, Paredes DA, Gonzalez Bosc LV, et al. Intermittent hypoxia in rats increases myogenic tone through loss of hydrogen sulfide activation of large-conductance Ca(2+)-activated potassium channels. Circ Res. 2011 Jun;108(12):1439–1447.

[172] Jackson WF, Huebner JM, Rusch NJ. Enzymatic isolation and characterization of single vascular smooth muscle cells from cremasteric arterioles. Microcirculation. 1997 Mar;4(1):35–50.

[173] Jackson WF. Potassium channels and regulation of the microcirculation. Microcirculation. 1998;5(2–3):85–90.

[174] Tykocki NR, Boerman EM, Jackson WF. Smooth muscle ion channels and regulation of vascular tone in resistance arteries and arterioles. Compr Physiol. 2017;7(2):485–581. DOI:10.1002/cphy.c160011

[175] Yang Y, Li PY, Cheng J, et al. Function of BKCa channels is reduced in human vascular smooth muscle cells from han Chinese patients with hypertension. Hypertension. 2013;61(2):519–525. DOI:10.1161/HYPERTENSIONAHA.111.00211

[176] Lu T, Chai Q, Jiao G, et al. Downregulation of BK channel function and protein expression in coronary arteriolar smooth muscle cells of type 2 diabetic patients. Cardiovasc Res. 2019 Jan;115(1):145–153.

[177] Worton SA, Pritchard HAT, Greenwood SL, et al. Kynurenine relaxes arteries of normotensive women and those with preeclampsia. Circ Res. 2021 May;128 (11):1679–1693.

[178] Fallahi S, Houck JA, Euser AG, et al. High altitude differentially modulates potassium channel-evoked vasodilatation in pregnant human myometrial arteries. J Physiol. 2022 Dec;600(24):5353–5364.

[179] Bologa CG, Revankar CM, Young SM, et al. Virtual and biomolecular screening converge on a selective agonist for GPR30. Nat Chem Biol. 2006 Apr;2 (4):207–212.

[180] Kow LM, Pfaff DW. Rapid estrogen actions on ion channels: a survey in search for mechanisms. Steroids. 2016 Jul;111:46–53.

[181] Taglialatela M, Ficker E, Wible BA, et al. C-terminus determinants for Mg2+ and polyamine block of the inward rectifier K+ channel IRK1. Embo J. 1995 Nov;14(22):5532–5541.

[182] Lu Z, MacKinnon R. Electrostatic tuning of Mg2+ affinity in an inward-rectifier K+ channel. Nature. 1994 Sep;371(6494):243–246.

[183] Inagaki N, Tsuura Y, Namba N, et al. Cloning and functional characterization of a novel ATP-sensitive potassium channel ubiquitously expressed in rat tissues, including pancreatic islets, pituitary, skeletal muscle, and heart (*). J Biol Chem. 1995;270 (11):5691–5694. DOI:10.1074/jbc.270.11.5691

[184] Aguilar-Bryan L, Nichols CG, Wechsler SW, et al. Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. Science. 1995;268(5209):423–426. DOI:10.1126/science.7716547

[185] Chutkow WA, Simon MC, Le Beau MM, et al. Cloning, tissue expression, and chromosomal localization of SUR2, the putative drug-binding subunit of cardiac, skeletal muscle, and vascular KATP channels. Diabetes. 1996 Oct;45(10):1439–1445.

[186] Yamada M, Isomoto S, Matsumoto S, et al. Sulphonylurea receptor 2B and Kir6.1 form a sulphonylurea-sensitive but ATP-insensitive K+ channel. J Physiol. 1997 Mar;499(Pt 3(Pt 3):715–720.

[187] Isomoto S, Kondo C, Yamada M, et al. A novel sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K+ channel. J Biol Chem. 1996 Oct;271(40):24321–24324.

[188] Aguilar-Bryan L, Clement JP 4th, Gonzalez G, et al. Toward understanding the assembly and structure of KATP channels. Physiol Rev. 1998 Jan;78(1):227–245.

[189] Duncker DJ, van Zon NS, Pavek TJ, et al. Endogenous adenosine mediates coronary vasodilation during exercise after K(ATP)+ channel blockade. J Clin Invest. 1995 Jan;95(1):285–295.

[190] Farouque HMO, Worthley SG, Meredith IT. Effect of ATP-sensitive potassium channel inhibition on coronary metabolic vasodilation in humans. Arterioscler Thromb Vasc Biol. 2004 May;24(5):905–910.

[191] Colburn TD, Holdsworth CT, Craig JC, et al. ATP-sensitive K(+) channel inhibition in rats decreases kidney and skeletal muscle blood flow without increasing sympathetic nerve discharge. Respir Physiol Neurobiol. 2020 Jul;278:103444.

[192] Wareing M, Bai X, Seghier F, et al. Expression and function of potassium channels in the human placental vasculature. Am J Physiol Regul Integr Comp Physiol. 2006;291(2):0–9. DOI:10.1152/ajpregu.00040.2006

[193] Jewsbury S, Baker PN, Wareing M. Relaxation of human placental arteries and veins by ATP-sensitive potassium channel openers. Eur J Clin Invest. 2007 Jan;37(1):65–72.

[194] Yin B, Zhang Y, Wei X, et al. The expression of ATP-sensitive potassium channels in human umbilical arteries with severe pre-eclampsia. Sci Rep. 2021;11 (1):1–8. InternetAvailable from. doi:10.1038/s41598-021-87146-6.

[195] Li H, Shin SE, Seo MS, et al. Alterations of ATP-sensitive K(+) channels in human umbilical arterial smooth muscle during gestational diabetes mellitus. Pflugers Arch. 2018 Sep;470(9):1325–1333.

[196] Al-Karagholi MAM, Ghanizada H, Nielsen CAW, et al. Cerebrovascular effects of glibenclamide investigated using high-resolution magnetic resonance imaging in healthy volunteers. J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab. 2021 Jun;41(6):1328–1337.

[197] Fujii N, McGarr GW, Kenny GP, et al. NO-mediated activation of KATP channels contributes to cutaneous thermal hyperemia in young adults. Am J Physiol Regul Integr Comp Physiol. 2020;318(2):R390–8. DOI:10.1152/ajpregu.00176.2019

[198] Al-Karagholi MAM, Ghanizada H, Hansen JM, et al. Levcromakalim, an adenosine triphosphate-sensitive potassium channel opener, dilates extracerebral but not cerebral arteries. Headache. 2019;59(9):1468–1480. DOI:10.1111/head.13634

[199] Fujii N, Louie JC, McNeely BD, et al. Mechanisms of nicotine-induced cutaneous vasodilation and sweating in young adults: roles for KCa, KATP, and KV channels, nitric oxide, and prostanoids. Appl Physiol Nutr Metab. 2017;42(5):470–478.

[200] Protić D, Beleslin-Čokić B, Spremović-RaCrossed d signenović S, et al. The different effects of resveratrol and naringenin on isolated human umbilical vein: the role of ATP-sensitive K+ channels. Phyther Res. 2014;28(9):1412–1418. DOI:10.1002/ptr.5145

[201] Chrissobolis S, Sobey CG. Influence of Gender on K ±Induced Cerebral Vasodilatation. Stroke. 2004;35 (3):747–752.

[202] Saha PS, Knecht TM, Arrick DM, et al. Prenatal exposure to alcohol impairs responses of cerebral arterioles to activation of potassium channels: role of oxidative stress. Alcohol Clin Exp Res. 2022 May;47(1):1–8.

[203] Small HY, McNeilly S, Mary S, et al. Resistin mediates sex-dependent effects of perivascular adipose tissue on vascular function in the shrsp. Sci Rep. 2019 May;9 (1):6897.

[204] Colburn TD, Weber RE, Schulze KM, et al. Sexual dimorphism in vascular ATP-sensitive K(+) channel function supporting interstitial PO2 via convective and/or diffusive O(2) transport. J Physiol. 2021 Jul;599(13):3279–3293.

[205] Wang Y, Haider HK, Ahmad N, et al. Mechanisms by which K(ATP) channel openers produce acute and delayed cardioprotection. Vasc Pharmacol. 2005;42(5–6):253–264.

[206] Jones RD, Pugh PJ, Jones TH, et al. The vasodilatory action of testosterone: a potassium-channel opening or a calcium antagonistic action? Br J Pharmacol. 2003;138(5):733–744.

[207] Zopf Y, Rabe C, Neubert A, et al. Women encounter ADRs more often than do men. Eur J Clin Pharmacol. 2008 Oct;64(10):999–1004.