Review Article

The Role of Adenosine A_{2A} Receptor, CYP450s, and PPARs in the Regulation of Vascular Tone

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Adenosine is an endogenous mediator involved in a myriad of physiologic functions, including vascular tone regulation. It is also implicated in some pathologic conditions. Four distinct receptor subtypes mediate the effects of adenosine, such as its role in the regulation of the vascular tone. Vascular tone regulation is a complex and continuous process which involves many mechanisms and mediators that are not fully disclosed. The vascular endothelium plays a pivotal role in regulating blood flow to and from all body organs. Also, the vascular endothelium is not merely a physical barrier; it is a complex tissue with numerous functions. Among adenosine receptors, A_{2A} receptor subtype (A_{2A}AR) stands out as the primary receptor responsible for the vasodilatory effects of adenosine. This review focuses on important effectors of the vascular endothelium, including adenosine, adenosine receptors, EETs (epoxyeicosatrienoic acids), HETEs (hydroxyeicosatetraenoic acids), PPARs (peroxisome proliferator-activated receptors), and K_{ATP} channels. Given the impact of vascular tone regulation in cardiovascular physiology and pathophysiology, better understanding of the mechanisms affecting it could have a significant potential for developing therapeutic agents for cardiovascular diseases.

1. Introduction

The vascular system of the human is known to be the major pathway for nutrition exchange among cells as well as organs (i.e., it connects the heart with organs and other tissues) [1]. Likewise, the blood vessels are the tree branches: they originate from the heart through the aorta and conduit arteries to fine capillaries. Blood runs in vessels by proper pressure, and therefore any change or deregulation of blood pressure will result in serious diseases [2]. For example, if blood pressure is low as in hypotension, it could result in organ failure (e.g., acute renal failure) [3]. On the other hand, if blood pressure is high as in hypertension, which is one of the main risk factors for cardiovascular disease, it results in serious diseases such as stroke and chronic renal failure [4]. Currently, hypertension affects over 25% of the human adult population globally [5]. Therefore, it is very important to have optimum blood pressure. Over the past decade, research has been focusing on investigating vascular tone regulation.

Physiologically, vascular tone is the contractile tension of a blood vessel relative to its maximum dilation. Vascular tone regulation is one of the complicated mechanisms in the human body. It involves multiple physiological mechanisms and mediators such as the atrial natriuretic peptide, eicosanoids, adrenal steroids, sodium and water excretion, neurologic control, kallikrein-kinin, and renomedullary endothelial systems [6]. The vascular wall is composed of three layers: intima, media, and adventitia [7]. In the intima layer, the vascular endothelium lines all vessels. The best description of the endothelium could be the printed circuit board (PCB), which is composed of wires, capacitors, and resistors that deliver a particular order by the programmer (i.e., it contains all necessary information for specific functions). Likewise, the vascular endothelium contains all important proteins, enzymes, and ion channels that serve in vascular tone regulation. However, the signaling pathways and their involvement in vascular tone are still not fully clear. The aim of this review is to summarize the previous work

of our lab for the past twelve years as well as touch on the related work from other laboratories in relation to vascular tone regulation. In particular, we will address the role of the endothelium, cytochrome P450s (CYPs), and peroxisome proliferator-activated receptors (PPARs) in vascular tone regulation employing disease models, genetic manipulation, and pharmacologic intervention.

2. The Role of the Endothelium in Vascular Tone Regulation

For a long time, the endothelium was considered an inert cellophane-like membrane, which coated the entire vascular tree, and its primary function was thought to only regulate the blood vessel wall permeability [8] or to be a mechanical barrier [6]. With the advancement of technology and research, the endothelium is currently viewed as a dynamic, heterogeneous, widely spread tissue that has numerous functions involving secretion, synthesis, metabolism, and immunology [8]. In a human adult, the endothelium consists of between 1 and 6×10^{13} cells, weighs about 1 kg, and covers a surface area of $3 m^2$. Also, the endothelium makes up the innermost layer, intima, of all vessels but has different structures and composition based on vessel type [9]. Considering the previous facts and figures, the endothelium is described by some as the largest organ [10, 11]. Since it is a disseminated tissue, it interacts with many systems and has an intricate role in "end organ disease of systems," which include neurologic, renal, hepatic, cardiac, dermatologic, immunologic, and vascular systems [12].

The role of the endothelium in the vasculature is not only merely to serving as a barrier between the circulation and surrounding tissue; it has a critical function in vascular tone regulation by producing some chemical substances (mediators) that affect vascular hemodynamics. These mediators can be divided into two types: (1) vasodilators, such as endothelium-derived relaxing factors (EDRFs), nitric oxide (NO), prostacyclin (PGI_2) [8], and endothelium-derived hyperpolarizing factors (EDHFs) [13–16], and (2) vasoconstrictors, which include endothelin-1, reactive oxygen species (ROS), platelet-activating factor (PAF) [8], and arachidonic acid (AA) cyclooxygenase-derived metabolites [17].

It is important to understand the functional complexity of the vascular endothelium; any chronic or acute disruption can cause endothelial dysfunction. This explains its involvement in many diseases such as cardiovascular disease, insulin resistance, obesity, and type 2 diabetes [18, 19]. A clear indicator of endothelial dysfunction is disabling the bioavailability of NO. Moreover, one or more of the following characteristics accompany endothelial dysfunction: reduction of endothelium-mediated vasorelaxation, enhanced cell layer permeability, increased production of reactive oxygen species (ROS), excessive oxidative stress, and overexpression of inflammatory genes [20–24].

The vascular endothelium plays a major role in mediating vascular tone. The cellular level, function, and pathogenesis of the vascular endothelium were explored deeply [8, 19, 25–28]; however, the exact signaling mechanism, enzymes, and substrates involved are not totally revealed. Therefore, we summarized the mechanisms and signaling pathways within the endothelium based on the work done at our lab and by others.

3. The Role of A_{2A} Adenosine Receptor in Vascular Tone Regulation

Adenosine receptors are involved in many signaling pathways and downstream effects [14, 15, 29–35]. For instance, they modulate and maintain many mechanisms such as cell growth, apoptosis, cell proliferation, angiogenesis, and immune response in addition to their involvement in diseases like asthma, Parkinson's disease, hypertension, and cancer [29, 36–38]. Moreover, adenosine receptors such as A_{2A} have significant effects in neurodegenerative diseases such as Alzheimer's disease [39], schizophrenia [40], and Huntington's disease [41]. However, A_1AR is implicated in pathological diseases such as Parkinson's disease and Alzheimer's disease [42]. Therefore, it is worthy to discuss the insightful mechanisms of adenosine and its receptors specifically in vascular tone regulation. The scope of this part is to discuss $A_{2A}AR$ and A_1AR in relation to CYPs, high salt, low salt, and soluble epoxide hydrolase (sEH) in vascular tone regulation. Both receptors belong to the G protein coupled receptors (GPCRs) family [43]. A_1AR and A_3AR bind to $G_{i/o}$ proteins family, whereas $A_{2A}AR$ and $A_{2B}AR$ bind with G_s protein. The distribution of $A_{2A}AR$ and A_1AR is not the same in the body [43, 44]. For instance, $A_{2A}AR$ is highly distributed in the spleen, thymus, leukocytes, blood platelets, heart, lung, blood vessels, and brain [43]. Particularly in the brain, $A_{2A}AR$ is highly expressed in the hippocampus [45], which is involved in Alzheimer's disease [46]. In addition, $A_{2A}AR$ is present in astrocytes, oligodendrocytes, microglia, and neurons [47, 48]. It is also reported that $A_{2A}AR$ is present in dendritic spines and postsynaptic regions of the basal ganglia [49]. However, with the high $A_{2A}AR$ expression in the brain, it has limited expression ("restrictive localization") in the striatum and olfactory bulb [50]. Moreover, $A_{2A}AR$ has various actions such as mediating vasodilation and aiding in new blood vessels build-up [51]. On the other hand, A_1AR is highly expressed in the CNS on neurons of the cortex, hippocampus, and cerebellum in the brain [50]. Adenosine A_1 receptor also exists in microglia [52], astrocytes [53], and oligodendrocytes [54]. In addition, A_1AR is found in other parts of the body such as adipose tissue, heart, and kidneys [44]. In the vascular system, A_1AR mediates the vasoconstrictive effect of adenosine in vascular beds [55, 56].

GPCRs, including A_1AR and $A_{2A}AR$, are known to form homo-, multi-, and heterodimers [57]. For example, if the same GPCRs bind together, they form a homodimer, whereas if different receptors bind together, they form a heterodimer [58]. If part of the heteromeric complex is activated, it will manipulate the receptor binding properties of the same complex [59]. Similarly, adenosine receptors are reported to form homodimers among each other and heterodimers with other GPCRs [58]. A good example of homodimer formation in ARs is A_1AR which has been reported in the intact tissue of the cortex in different species [60] and in Purkinje cells of the

TABLE 1: List of pharmacological drugs commonly used in vascular response investigation.

Class	Reagents	Reagents' full description
Nonspecific AR agonist	NECA	$5'$-N-Ethylcarboxamidoadenosine
Specific A_{2A} AR agonist	CGS 21680	2-p-(2-Carboxyethyl)phenethylamino-$5'$-N-ethylcarboxamidoadenosine hydrochloride hydrate
A_{2A} AR antagonist	ZM241385	4-(2-[7-Amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol
	SCH-58261	7-(2-Phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine
A_1 AR agonist	CCPA	2-Chloro-N6-cyclopentyladenosine
A_1 AR antagonist	DPCPX	8-Cyclopentyl-1,3-dipropylxanthine
eNOSi	L-NAME	N-Nitroarginine methyl ester
CYP-epoxygenases inhibitor	MS PPOH	N-(Methylsulfonyl)-2-(2-propynyloxy)-benzenehexanamide
CYP-hydroxylase	DDMS	Dibromododecenyl methylsulfimide
Cyclooxygenase inhibitor	Indomethacin	
sEH inhibitors	AUDA	12-(3-adamantan-1-yl-ureido)-dodecanoic acid
	t-AUCB	$trans$-4-[4-(3-Adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid
EET antagonist	14,15-EEZE	14,15-Epoxyeicosa-5(z)-enoic acid
20-HETE inhibitor	HET0016	N-Hydroxy-N′-(4-n-butyl-2-methylphenyl)formamidine
K_{ATP} channel blocker	Glibenclamide	
Mitochondrial-K_{ATP} channel blocker	5-HD	5-Hydroxydecanoate
PPARγ agonist	Rosiglitazone	
PPARα antagonist	GW 6471	N-((2S)-2-(((1Z)-1-Methyl-3-oxo-3-(4-(trifluoromethyl)phenyl)prop-1-enyl)amino)-3-(4-(2-(5-methyl-2-phenyl-1,3-oxazol-4-yl)ethoxy)phenyl)propyl)propanamide

cerebellum and hippocampal pyramidal neurons [61]. On the other hand, A_{2A} AR homodimers were reported in HeLa and HEK-293T cells cotransfected with different A_{2A} AR receptor structures [62]. Ciruela et al. illustrated heterodimerization of A_1 AR and A_{2A} AR on the cell surface of HEK-293T cells cotransfected with their cDNAs [63]. The activation of A_{2A} AR via CGS 21680 on these cells reduces the binding affinity of a selective radioligand to bind to A_1 AR [63]. Also, A_1 AR and A_{2A} AR formed heterodimers in rat astrocyte cultures [64]. The existence of A_1-A_{2A} AR heterodimers in the CNS was found to maintain neuronal excitability [58]. This finding is interesting because it indicates that ARs may affect each other's ability to respond to different ligands even in the case of what we label as selective ligands, as in the case of CGS 21680, which is a selective agonist of A_{2A} AR. More interestingly, it was found that GABA uptake was inhibited upon activating A_1 AR whereas GABA uptake was increased upon activating A_{2A} AR [64]. The data of adenosine receptor dimerization is mostly reported in the CNS; however, the AR oligomerization investigation in the vasculature is still lacking.

To study the mechanism of action of adenosine receptors, we utilized three major strategies: transgenic animal models, special diet, and pharmacological drugs. Genetically modified mice are utilized to discern the physiological importance of adenosine receptors [50]. Thus, most of the reported work

in our lab focused on A_{2A} AR-null, sEH-null, and eNOS-null mice. In the special feeding diet, high salt (HS) and normal salt (NS) intake were used as external factors to investigate how adenosine's effect on vascular response may change. Lastly, the pharmacological reagents were useful since they mimicked the actions and signaling pathway of adenosine. For example, CCPA is a selective A_1 AR agonist that can be used in humans, rats, and mice [43]. DPCPX, a xanthine analog, is a specific A_1 AR antagonist [65]. However, NECA, which was originally thought to be a selective A_2 AR agonist, is now considered nonselective for ARs after the development of the more selective A_{2A} AR agonist CGS 21680 [66] (more examples are available in Table 1). Taking into account the reports of dimerization of ARs, it would be prudent to rethink how we use and interpret the data from these pharmacologic agents. Combining the previous strategies provided a better understanding of A_{2A} AR's role and function in the vasculature.

Adenosine is a nucleoside generated through metabolic stress as well as high cellular activity [67]. Thus, it increases oxygen supply and decreases oxygen consumption [35]. It is mainly formed by the enzyme $5'$-nucleotidase CD73, which dephosphorylates adenosine $5'$-monophosphate (AMP) into adenosine that is distributed intracellularly and extracellularly. Intracellularly, adenosine is metabolized by adenosine kinase to AMP, whereas, extracellularly, it is degraded by

adenosine deaminase to inosine [68–70]. Since adenosine can vasodilate and hyperpolarize the vascular smooth muscle cells (VSMC), it is considered an EDHF [71]. There are four adenosine subfamily receptors in the vascular tissue that bind to adenosine, which are A_1AR, $A_{2A}AR$, $A_{2B}AR$, and A_3AR. These receptors are involved in vascular tone regulation via CYP enzymes family which will be discussed in more detail later. Most of the published data so far suggest that the activation of adenosine receptors $A_{2A}AR$ and $A_{2B}AR$ mediates vasodilation in various vessels [14, 15, 72–75], whereas the activation of A_1AR and A_3AR causes vasoconstriction [55, 56]. Moreover, both A_1AR and $A_{2A}AR$ are necessary for maintaining blood pressure (BP) [76].

The inception studies in our lab of adenosine receptors' role in vascular tone started by studying the vasodilation effect of activating $A_{2A}AR$ in $A_{2A}AR^{+/+}$ (wild type) and $A_{2A}AR^{-/-}$ (A_{2A} knockout) of mouse aorta. NECA (adenosine agonist) and CGS 21680 (a selective $A_{2A}AR$ agonist) caused increased vasodilation in $A_{2A}AR^{+/+}$ mouse aortae, whereas NECA caused vasoconstriction and CGS 21680 caused neither relaxation nor constriction in $A_{2A}AR^{-/-}$ mouse aortae (see Table 1 for all pharmacological reagents) [15]. These findings were important since they partly led to better understanding of the vasodilatory effect of $A_{2A}AR$ as evident in the lack of vasodilation when CGS 21680, which is a selective $A_{2A}AR$ agonist, was used in A_{2A} knockout mice [15]. However, the nonselective nature of NECA meant that, in the absence of A_{2A} receptors in $A_{2A}AR^{-/-}$ mice, this compound would activate the other adenosine receptors (A_1AR, $A_{2B}AR$, and A_3AR). The vasoconstrictive effect of A_1AR, along with the finding that the vasoconstrictive Cyp4a's expression is increased in $A_{2A}AR^{-/-}$ mice [15], could explain the NECA-mediated vasoconstriction in $A_{2A}AR^{-/-}$ mice [15]. Additionally, the data suggested that $A_{2A}AR$-mediated vasodilation is endothelium-dependant since NECA-induced vasodilation was observed only in aortic rings with intact endothelium, whereas endothelium-denuded rings responded by contraction [15]. In contrast, Arsyad and Dobson illustrated that adenosine-induced vasodilation in male Sprague-Dawley rats' thoracic aortic rings was partially endothelium-dependent [77]. This finding is in agreement with other reported studies in different vascular beds such as rat preglomerular microvessels [72, 78], porcine coronary artery wall [79], porcine retinal arterioles [80], rat pial arterioles [81], rat inferior mesenteric artery [82], and human coronary arterioles [83].

Other studies indicated that the activation of $A_{2A}AR$ leads to vascular relaxation via CYP-epoxygenase [15, 75, 84–88]. In these experiments, $A_{2A}AR^{+/+}$ and $A_{2A}AR^{-/-}$ mice aortae and pharmacological agents such as MS-PPOH (selective CYP-epoxygenase inhibitor), NECA (adenosine agonist), and CGS 21680 (a selective $A_{2A}AR$ agonist) were employed. The observed vasodilation induced by NECA and CGS 21680 was completely inhibited by MS-PPOH, which supports the findings we and others have reported in mouse aortae [15], rat preglomerular microvessels [72], and rat isolated perfused kidney [89]. Moreover, the activation of

$A_{2A}AR$ was associated with upregulation of Cyp2c29 (CYP-epoxygenase enzyme of mice species) in the endothelium-dependent relaxation. In comparison, in $A_{2A}AR^{-/-}$ mice, vasoconstriction via Cyp4a (an ω-hydroxylase enzyme) was observed [15]. This pathway was explored by using DDMS and HET-0016 (ω-hydroxylases inhibitors) (see Figure 1 and Table 1). Both inhibitors reversed vascular contraction, which suggested that the induced vasoconstriction in $A_{2A}AR^{-/-}$ mice was mediated by the Cyp4a enzyme [15]. Additionally, the endothelium layer was denuded to investigate its involvement in $A_{2A}AR$-induced vascular dilation. Vasoconstriction was observed in induced $A_{2A}AR$ aorta; that is, the CGS 21680 agonist effect on $A_{2A}AR$ was abolished in the denuded endothelium [15]. From the previous work and other studies, it is concluded that both CYP-epoxygenases and ω-hydroxylases were involved simultaneously with $A_{2A}AR$ in vascular tone regulation [15, 90].

A group of metabolites have been linked to the actions mediated by adenosine receptors; studies have demonstrated that the vasodilatory effects induced by adenosine through $A_{2A}AR$ and $A_{2B}AR$ are mediated by several physiological factors including NO [80, 91], EETs [92], and ATP-sensitive K^+ (K_{ATP}) channels [74, 80, 93, 94]. These studies also used pharmacological reagents, such as glibenclamide, for inhibiting the K_{ATP} channels as well as potassium channels openers (PCO) such as pinacidil and cromakalim. One of the major effects of the opening of K_{ATP} channels in vascular vessels is hyperpolarization of the smooth muscle resulting in vasorelaxation [95, 96]. Moreover, K_{ATP} channels were involved in the signaling pathways of adenosine-induced vasodilation as reported by others in coronary arterioles of miniature swine [97], human isolated small coronary arteries [98], rat retinal microvessels [99], porcine coronary artery [100], isolated rat pulmonary artery rings [101], and perfused hydronephrotic rat kidney [102]. Also, Arsyad and Dobson demonstrated that voltage-dependent K_v and K_{ATP} are opened in response to $A_{2A}AR$-induced vasodilation in response to adenosine activation [77]. Using PCO in both $A_{2A}AR^{-/-}$ and $A_{2A}AR^{+/+}$, vasorelaxation was significantly reduced in $A_{2A}AR^{-/-}$ compared to $A_{2A}AR^{+/+}$, which indicated a role of sarcolemmal K_{ATP} channels with $A_{2A}AR^{+/+}$ in vascular relaxation [95]. The PCO-induced effect was inhibited by glibenclamide, confirming the role of PCO in opening the K_{ATP} channels [95]. Therefore, the induced $A_{2A}AR$ effect through CGS 21680 was blocked by glibenclamide confirming the involvement of sarcolemmal K_{ATP} channels in the vasorelaxation process [95].

The involvement of $A_{2A}AR$ in vascular relaxation through sarcolemmal K_{ATP} channels was investigated. The principle of this study is based on utilizing PCO in $A_{2B}AR^{-/-}$ and $A_{2A/2B}AR^{-/-}$ mice aortae. The $A_{2B}AR^{-/-}$ had a comparable relaxation effect to the $A_{2B}AR^{+/+}$ (WT), whereas the $A_{2A/2B}AR^{-/-}$ had decreased relaxation compared to $A_{2B}AR^{-/-}$. Further blocking $A_{2A}AR$ with SCH 58261 ($A_{2A}AR$ antagonist) generated the same response as in the $A_{2A}AR^{-/-}$.
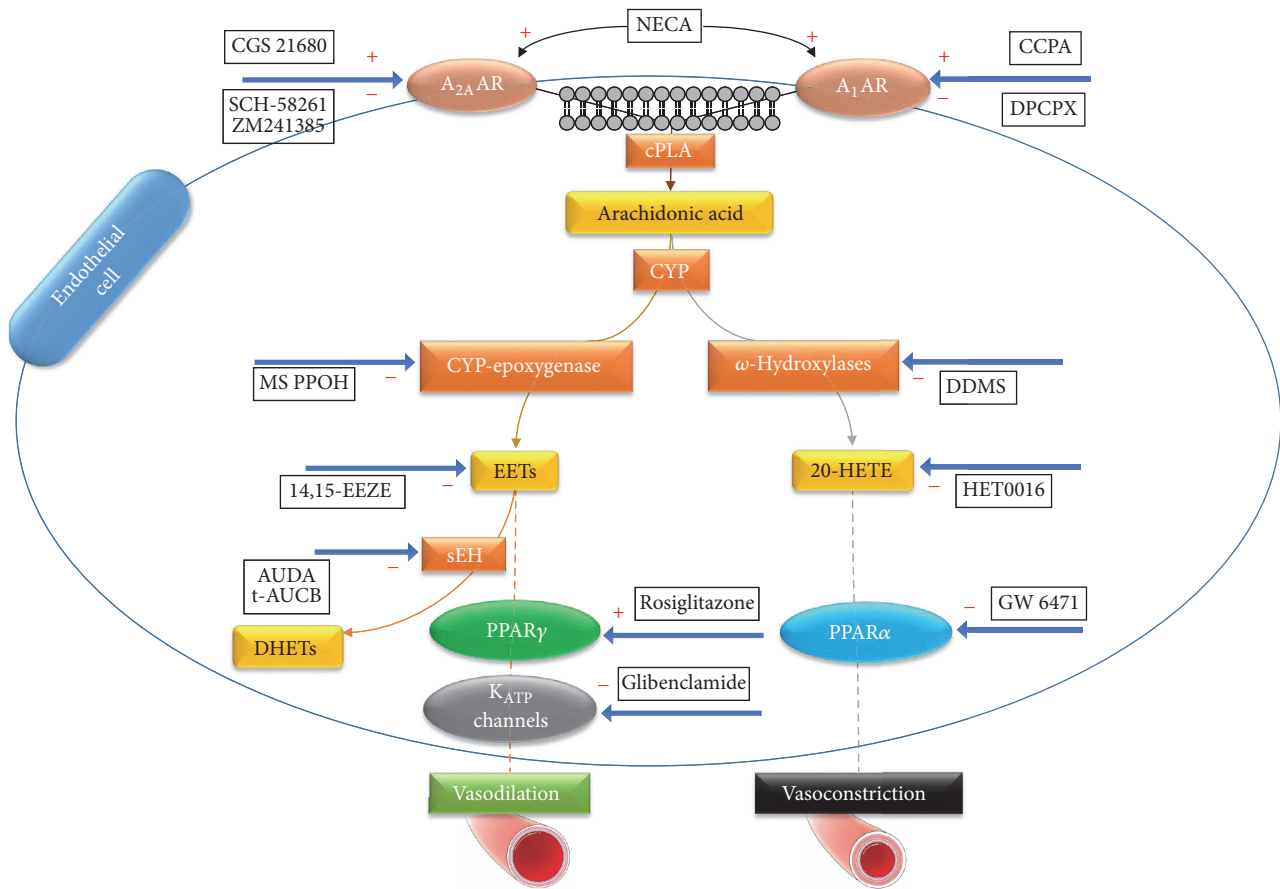
FIGURE 1: Illustration pathways of $A_{2A}AR$ and A_1AR induced in mice aorta. $A_{2A}AR$ induced demonstrates the involvement of CYP-epoxygenase which generates EETs from AA. EETs have substantial involvement in vascular relaxation and they undergo two pathways: (1) they are converted via sEH to DHETs which are inactive or less active metabolites and (2) they activate PPARγ which is involved in other signaling pathways in vascular relaxation. By contrast, A_1AR induced illustrates the involvement of ω-hydroxylases which utilize AA to form 20-HETE. Then, 20-HETE activates PPARα to produce vascular contraction. Different protein targets were probed using pharmacological agonists and antagonists to investigate the possible mechanism and signaling approaches (refer to Table 1 for drugs information). The solid line represents the reported pathways whereas the dashed line shows investigation still underway.

With the comparable effect observed from both $A_{2A}AR$ and $A_{2A}AR^{-/-}$, it is suggested that the potassium channels mediate the relaxation induced by $A_{2A}AR$.

It is reported by Ye et al. [92, 103] and Lu et al. [104] that EETs act as activators of K_{ATP} channels. Another study by Ponnoth et al. investigated the relationship between EETs and K_{ATP} channels [95]. By blocking the CYP-epoxygenases with MS-PPOH, the relaxation effect induced by pinacidil was greatly decreased. Other laboratories investigated the involvement of NO in $A_{2A}AR$-K_{ATP} pathway in pig coronary arterioles [74], porcine second-order retinal arterioles [80], and mouse aorta [95]. Their studies suggested that $A_{2A}AR$-mediated vasodilation was through NO (released by endothelium) and opening of K_{ATP} channels on smooth muscles [74]. A link in the signaling cascade between the K_{ATP} channels and adenosine-mediated vasorelaxation was reported in several vascular beds [97–102]. In our lab, the K_{ATP} channel openers pinacidil and cromakalim produced relaxation in both wild-type ($A_{2A}AR^{+/+}$) and $A_{2A}AR$ knockout

($A_{2A}AR^{-/-}$) mice but were significantly higher in the presence of $A_{2A}AR$ [95]. This finding confirmed that adenosine-mediated vasorelaxation is mediated by K_{ATP} channels [95]. Moreover, we demonstrated that NO played a role in the relaxation induced by pinacidil [95]. When L-NAME (NO synthase inhibitor) was used in wild type ($A_{2A}AR^{+/+}$) in the presence of pinacidil, the vascular relaxation was decreased to a level comparable to that in $A_{2A}AR^{-/-}$ mice [95]. Based on that, the signaling mechanism of adenosine at the $A_{2A}AR$ for aortic vascular relaxation involved CYP-epoxygenases through the following mediators arranged in order: EETs, nitric oxide (NO), and the opening of sarcolemmal K_{ATP} channels in the endothelium [95].

The involvement of 20-HETE with A_1AR was also investigated in vascular tone of mouse aorta [105]. Since 20-HETE is the active metabolite of AA through Cyp4a and is a potent vasoconstrictor [88], HET0016 was used to inhibit Cyp4a activity. As a result, the vascular contraction of induced A_1 was notably decreased in $A_{2A}AR^{+/+}$ and $A_{2A}AR^{-/-}$, suggesting that Cyp4a is downstream of A_1AR [105].

Other studies were conducted to reveal the involvement of CYP enzymes and signaling pathways in $A_{2A}AR^{+/+}$ and $A_{2A}AR^{-/-}$ vascular endothelium of mice aortae. First, in $A_{2A}AR^{-/-}$ mice, the data demonstrated vasoconstriction induced by Cyp4a through 20-HETE accompanied with upregulation of A_1AR, which mainly activates protein kinase C (PKC-α) and mitogen-activated protein kinase (MAPK) [105]. This effect on PKC is mediated by Gαi coupling [106]. The involvement of PKC-α and MAPK in induced A_1AR was also determined. In PKC-α, using Gö-6976, a selective PKC-α inhibitor, it primarily weakens the vasoconstriction response mediated by A_1AR in both $A_{2A}AR^{+/+}$ and $A_{2A}AR^{-/-}$ aortae [105]. However, the MAPK inhibitor, PD-98059, was used first to treat aortae, followed by CCPA to activate A_1AR. PD-98059 totally blocked A_1AR-induced vasoconstriction by CCPA suggesting the involvement of MAPK in vasoconstriction. Ponnoth et al. [105] and Ansari et al. [107] suggested that A_1AR-induced vascular contraction is through Cyp4a, which generates 20-HETE, and leads to the activation of PKC-α, which phosphorylates MAPK.

We mentioned earlier that the adenosine receptors, like other GPRCs, can interact with each other through dimerization. Ponnoth et al. reported that CCPA (a selective A_1 agonist) produced significantly higher contraction in $A_{2A}AR^{-/-}$ compared to wild-type ($A_{2A}AR^{+/+}$) mice [105]. It is also reported by the same authors [105] that the basal protein expression of A_1AR was higher in $A_{2A}AR^{-/-}$ mice compared to $A_{2A}AR^{+/+}$ mice [105]. This overexpression is due to lack of $A_{2A}AR$ inhibition effect over A_1AR [105]. The more enhanced contraction in $A_{2A}AR^{-/-}$ mice by an A_1AR agonist could be explained by the increased expression of A_1AR in this mouse genotype [105] and a possible lack of interaction, as suggested by the reported dimerization, between A_1AR and $A_{2A}AR$ due to the absence of the latter [58, 63, 64].

High salt (HS) intake is considered one of the risk factors for many diseases such as hypertension [108, 109]. HS also acts as an external factor to affect vascular tone, and therefore it is important to study its potential involvement with $A_{2A}AR$ and its downstream mechanism in vascular tone regulation, to which we turn our focus now. We previously reported a connection between adenosine receptors activation, particularly A_{2A}, and high dietary salt intake in mouse aorta [14, 33, 76] as done by others in isolated perfused rat kidney [89]. Therefore, the role of $A_{2A}AR$ in high salt diet was investigated [76]. Data showed upregulation of $A_{2A}AR$ expression in response to high salt intake [14, 76, 110] and subsequent increase in $A_{2A}AR$-induced vasorelaxation [14, 33, 72, 89, 110]. In contrast, A_1AR, which is involved in vasoconstriction, was downregulated in high salt-fed mice [14, 110]. Moreover, the produced adenosine levels were increased by HS diet ingestion in mice [111] and rats in other studies [33, 110, 112, 113].

Another study showed a relationship between activated $A_{2A}AR$ and HS diet [14, 16, 78]. The data suggested that HS in $A_{2A}AR^{+/+}$ improves $A_{2A}AR$-induced relaxation due to the enhanced CYP-epoxygenases activity, which produce more EETs. On the other hand, in $A_{2A}AR^{-/-}$, the HS caused vascular contraction due to the lower level of CYP-epoxygenases activity and enhanced level of A_1AR [76].

The use of NECA (a nonselective adenosine analog) and CGS 21680 (selective $A_{2A}AR$ agonist) resulted in enhanced aortic vasodilation in HS-fed versus NS-fed mice [14]. The close relationship of $A_{2A}AR$ either activated or induced via pharmacological drug and HS diet provided a better understanding of vascular modulation. Therefore, we investigated $A_{2A}AR^{+/+}$ and $A_{2A}R^{-/-}$ mice in HS and normal salt (NS) diet study [76]. In the presence of $A_{2A}AR$, data demonstrated that HS increases $A_{2A}AR$-induced vasodilation. On the other hand, in the knockout mice, HS increases contraction through increased levels of A_1AR [76]. The HS and NS diet studies confirm the involvement of $A_{2A}AR$ in vascular tone regulation. The $A_{2A}AR$ response to HS-fed mice is possibly adaptive since it showed upregulation of $A_{2A}AR$ and increased vasodilation of mice aortae [76] as previously demonstrated in preglomerular vessels of HS-fed mice [110].

Overall, the many experiments conducted by our lab and others have repeatedly confirmed the important role of adenosine, through its receptor subtypes, in vascular tone regulation. Also, they elucidated much of the downstream signaling pathways, which include CYP enzymes, EETs, ω-HETEs, PPARs, and K_{ATP} channels.

4. The Role of CYP-Epoxygenase in Vascular Tone Modulation

The investigated downstream mechanism starting from $A_{2A}AR$ and A_1AR until CYP enzymes was discussed above in detail. Now, we will continue the discussion of the other steps in the downstream cascade from CYPs until the hyperpolarization which ultimately leads to vascular smooth muscle relaxation. In this section, we will explore the involvement of CYP enzymes, sEH, PPARγ, and PPARα in vascular tone regulation (Figure 1) under normal and high salt conditions in the aorta and their roles in coronary reactive hyperemia (CRH) in response to ischemia.

$A_{2A}AR$-mediated vascular response involves CYP enzymes, which are related to cytochrome P450s (CYP450) enzyme family [90, 114]. CYP450 family are divided into two types, CYP-epoxygenases (CYP2C and CYP2J) and ω-hydroxylases (CYP4A and CYP4F), that are involved in maintaining vasculature tone [90, 114, 115]. Although both enzymes are from the same CYP450 family, they produce different metabolites from AA with widely different physiological functions. For instance, CYP-epoxygenases metabolize AA to generate epoxyeicosatrienoic acids (EETs), which have a vasodilatory effect [116]. On the other hand, the ω-hydroxylases convert AA to 20-hydroxyeicosatetraenoic acid (20-HETE), which is a vasoconstrictor [117]. Both groups of metabolites from AA are natriuretic agents.

EETs have four different regioisomers, 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET, and have many biological functions; for example, they are released from endothelial cells and act as EDHFs to produce vasodilation through hyperpolarization [118, 119]. Their relaxation of vascular

smooth muscle cells is believed to be through the activation of large conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) [118, 119]. Moreover, EETs have cardioprotective effects in response to ischemia/reperfusion injury (i.e., EETs reduced heart injury after ischemia) [120]. The vasodilatory effect of EETs is reported in different vascular beds such as the brain [121], preglomerular vasculature of the kidney [122], conduit arteries [14, 15, 32, 33], and intestines [116].

EETs are further metabolized to dihydroxyeicosatrienoic acids (DHETs), which are less active, through soluble epoxide hydrolase (sEH), the primary metabolic pathway for EETs [123]. DHETs were shown to be either inactive or less active than their parent compounds EETs on smooth muscle cells [124]. Other enzyme families involved in AA metabolism are cyclooxygenase (COX) and lipoxygenase (LOX), in which they generate prostanoids and midchain HETEs, respectively. Overall, the metabolites generated by CYPs enzymes from AA are known as oxylipins.

The link between $A_{2A}AR$ and CYP-epoxygenase was demonstrated earlier; most collected data so far demonstrated the relationship among $A_{2A}AR$, CYP-epoxygenase, sEH, and peroxisome proliferator-activated receptor gamma (PPARγ) in addition to the involvement of A_1AR and PPARα in $sEH^{+/+}$ (wild type) and $sEH^{-/-}$ (knockout) in regulating the vascular tone of mice aortae [32]. In $sEH^{-/-}$, the vascular relaxation induced by adenosine was driven by the upregulation of $A_{2A}AR$, CYP2J, and PPARγ and downregulation of A_1AR and PPARα [32]. However, in $sEH^{+/+}$, adenosine produced vasocontraction through PPARα [32]. Nayeem et al. illustrated that CGS 21680-induced relaxation in $sEH^{-/-}$ mice was induced by $A_{2A}AR$ activation, which resulted in CYP-epoxygenases generation of EETs. Moreover, CGS 21680-induced vasodilation was inhibited by the EET antagonist 14,15-EEZE in $sEH^{-/-}$ mice. This finding is in agreement with previous data in which the adenosine-induced vasodilation was blocked by 14,15-EEZE in $A_{2A}AR^{+/+}$ mouse aorta [14]. Likewise, Gauthier et al. confirmed the relationship between $A_{2A}AR$ and sEH in bovine coronary arteries [125]. Additionally, in $sEH^{-/-}$ mouse aorta, CYP2J5 protein level was upregulated compared to $sEH^{+/+}$, suggesting that the adenosine-induced vasodilation requires CYP-epoxygenases, such as CYP2J [32].

The sEH inhibitors were valuable in linking $A_{2A}AR$ with sEH enzyme in vascular tone regulation. Herein, blocking sEH, which converts EETs (vasodilator) to DHETE (inactive) by AUDA or t-AUCB, further enhanced the vascular relaxation induced by CGS 21680 [32]. The strong response was suggested to be due to an increase in EET level based on other published data [126]. Moreover, the use of EETs antagonist, 14,15-EEZE, blocked the vascular relaxation induced by CGS 21680, which confirmed that the enhanced vasodilation was due to the increased effect of EET [32].

We evaluated the effect of high salt intake and the role of CYP-epoxygenases in vascular tone regulation [76]. We mentioned above the significance of $A_{2A}AR$ and A_1AR in relation to HS intake in vascular modulation. The role of cyclooxygenase (COX) was investigated by using $A_{2A}AR^{+/+}$

and $A_{2A}AR^{-/-}$ mice aortae in vascular response. By treating HS-fed $A_{2A}AR^{+/+}$ with CGS 21680, in the presence or absence of a COX inhibitor and an eNOS inhibitor, the same vasodilation was produced. This can be explained by the notion that vascular dilation in response to CGS 21680 was COX- and NO-independent [76]. Moreover, the use of EETs antagonist inhibited CGS 21680-induced vasodilation. This illustration is in agreement with previous studies [14, 15, 72, 78]. Additionally, in HS-fed $A_{2A}AR^{+/+}$ mice, the level of CYP2c29, which generates EETs, was increased versus NS-fed mice [76]. In comparison with $A_{2A}AR^{-/-}$, the expression level of CYP2c29 was reduced in HS-fed versus NS diet $A_{2A}AR^{+/+}$. This observation suggests that CYP-epoxygenases, such as cyp2c29, have a substantial role in mediating vascular relaxation downstream of $A_{2A}AR$ in response to HS intake. In rat studies of HS-fed $A_{2A}AR^{-/-}$ [110], there was no increase of CYP-epoxygenase in response to HS, which supports this finding. Although the findings of Arsyad and Dobson supported our finding that COX was not involved in adenosine-induced vasodilation [77], they contrasted our finding that CGS 21680-induced vasodilation was NO-independent by demonstrating that the adenosine-induced vasodilation in thoracic aortic rings of male Sprague-Dawley rats was NO-dependent [77].

We sought to search for more evidence in our research on vascular tone regulation and moved a step forward from in vitro studies (vessel organ bath) to the ex vivo experiment Langendorff (isolated heart). This extension added more valuable understanding by bringing research data and hypothesis a step closer to translational, functional, and applied science. Herein, we evaluated the role of CYP-epoxygenases and sEH in coronary artery response to ischemia, or coronary reactive hyperemia (CRH), which is a protective mechanism to prevent potential damage to the heart due to ischemic insult [124]. When the heart is exposed to a brief ischemia, coronary blood flow immediately increases afterwards to compensate for the lack of blood supply and avoid any toxic metabolites build-up in the heart [127, 128]. We investigated the pharmacological inhibition on CYP-epoxygenases and sEH using two different genotypes of mice, sEH null ($sEH^{-/-}$) and wild type ($sEH^{+/+}$), using Langendorff (isolated heart) technique [124]. The inhibition of CYP-epoxygenases by MS-PPOH reduced CRH, whereas the inhibition of sEH by t-AUCB enhanced CRH in isolated mouse heart [124]. As mentioned previously, CYP-epoxygenases convert AA to EETs. It is reported that EETs were involved in insulin-induced augmentation of skeletal muscle perfusion. The study used MS-PPOH (CYP-epoxygenase inhibitor) to block EETs synthesis and confirmed EETs involvement in the augmentation of skeletal muscle perfusion [78]. Fleming reported that the majority of P450 enzymes are highly distributed in coronary arteries and small arterioles [119]. By blocking CYP-epoxygenases pathway, the synthesis of EETs was inhibited, and the protective CRH was attenuated as well [124]. On the other hand, by treating the heart with t-AUCB, CRH was enhanced versus

nontreated WT mice [124]. In treating $sEH^{-/-}$ mice compared with nontreated $sEH^{-/-}$ mice by t-AUCB, there were no significant differences, which emphasizes the selectivity of sEH inhibitor, t-AUCB, in CRH response to brief ischemia [124].

The vasodilatory and cardioprotective roles EETs are reiterated in a number of blood vessels. The release of EETs was also linked to $A_{2A}AR$ activation and was shown to be responsive to changes in EETs' generating enzymes (CYP-epoxygenases) and EETs' deactivating enzyme (sEH) [76]. These data further support the notion of finding clinical applications for these beneficial metabolites (EETs) through targeting different enzymes in their pathway.

5. The Role of PPARγ in $A_{2A}AR$-Induced Vascular Relaxation

PPARs are one of the downstream targets of EETs. Most of the epoxygenases effects are through PPARs stimulation; that is, PPARs activation produces similar effects to those of EETs and $A_{2A}AR$ [129]. For instance, the stimulation of PPARs induces vascular tone regulation, vascular cell proliferation, and cell relocation [130, 131]. Moreover, one of the functions of PPARγ in the endothelial cell as reported by Liu et al. [126] is an anti-inflammatory effect. In diabetic mice study, activation of PPARγ caused endothelium-dependent vasodilation, which was found in nondiabetic mice ($+db/+m$) [132]. Moreover, others suggested that PPARs have a role in adipogenesis, insulin sensitivity, and regulation of blood vessels tone [133]. In our $sEH^{+/+}$-$sEH^{-/-}$ mice study, the protein level of PPARγ was upregulated, and the proteins level of PPARα was downregulated in response to CGS 21680 in $sEH^{-/-}$ versus $sEH^{+/+}$ mouse aorta [76, 129]. Therefore, these data suggest that PPARγ is involved in CGS 21680-induced vasodilation in $sEH^{-/-}$, whereas the activation of PPARα causes vasocontraction [76, 129] (Figure 1). It was confirmed that the PPARα agonist (GW 7647) reduced the relaxation of CGS 21680 induced dose-dependently. Additionally, the vascular response induced by CGS 21680 was remarkably blocked by PPARγ antagonist in $sEH^{-/-}$ [32]. Overall, PPARγ in the vascular endothelial cell is activated by EETs to cause vascular relaxation. Since PPARγ is linked with EETs, which in turn are generated by enhanced CYP-epoxygenase activity via $A_{2A}AR$-induced vascular response, we reported the role of PPARγ in HS-fed $A_{2A}AR^{-/-}$ and $A_{2A}AR^{+/+}$ mice. In addition, we also reported the interplay between $A_{2A}AR$, sEH, EETs, PPARγ, and K_{ATP} in vascular response [129]. In this study, CGS 21680, T0070907, rosiglitazone, AUDA, and glibenclamide were utilized. The data demonstrated in HS-fed $A_{2A}AR^{+/+}$ that inhibiting PPARγ led to an increase in CGS 21680-induced vasodilation, which links $A_{2A}AR$ with PPARγ [129]. Moreover, PPARγ antagonist attenuated AUDA-induced vascular relaxation response, which confirms the link between EETs (through the sEH inhibitor AUDA) and PPARγ. The K_{ATP} channel blocker also attenuated the vasodilation-induced rosiglitazone; this establishes a connection between PPARγ and K_{ATP}. On the other hand, in HS-fed $A_{2A}AR^{-/-}$, the HS-induced vascular contraction was attenuated by the sEH inhibitor AUDA [129]. Overall, the vascular response to mediators, such as PPARγ agonist, PPARγ-antagonist, sEH inhibitors, and K_{ATP} channel blocker, was altered in response to HS feeding in both $A_{2A}AR^{-/-}$ and $A_{2A}AR^{+/+}$. These data indicated that these mediators (EETs, PPARγ, and K_{ATP} channels) are downstream of $A_{2A}AR$ [129].

In the isolated mouse heart, the data showed that the effect of CRH was decreased by PPARγ antagonist (T0070907), whereas PPARγ agonist (rosiglitazone) enhanced it [124]. Since the t-AUCB-enhanced CRH was reduced by the PPARγ antagonist, T0070907, in WT mouse heart, a link between EETs and PPARγ is more likely [124]. This is an indication that the modulation of CRH involves PPARγ receptors through CYP-epoxygenase-EET pathway [124]. Other studies also reported that PPARγ receptors are induced by EETs [129, 134–136]. Liu et al. suggested that activation of PPARγ increased retention of EETs in endothelial cells and enhanced the anti-inflammatory effect upon using a selective sEH inhibitor [136]. The previously reported data with our finding illustrated that PPARγ activation is downstream of CYP-epoxygenase-EET pathway to mediate CRH.

6. Conclusion

The vascular endothelium is a complex tissue with numerous functions and roles that potentially affect all body organs and systems. The main physiologic as well as pathologic role of the vascular endothelium is related to its regulation of blood flow, which is known as vascular tone regulation. The complicated process of vascular tone regulation involves receptors, enzymes, ion channels, and many mediators that have not been fully disclosed. Therefore, understanding the vascular tone modulation by different pharmacological reagents and polymorphic or allelic variant models is very significant. Particularly in mice aortae, activation of $A_{2A}AR$ triggers multiple steps to elicit a vascular response. $A_{2A}AR$ activation is associated with enhanced CYP-epoxygenases activity, which generate EETs from AA, and opening of sarcolemmal K_{ATP} channels. The reported data suggest that CYP-epoxygenases, EETs, PPARγ, and sarcolemmal K_{ATP} channels are involved in vascular tone regulation. By thoroughly understanding the mechanisms controlling vascular tone regulation, we will likely become closer to finding potential targets for pharmacologic intervention for treating pathologies affected by or involving the vascular endothelium.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

Acknowledgments

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