Chapter 14

Vascular Sympathetic Neurotransmission and Endothelial Dysfunction

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72442

Abstract

Endothelium is an important regulator of vascular tone via release of various endothelium-derived substances. Several studies have reported that endothelium may decrease the release of noradrenaline from vascular postganglionic sympathetic nerves and thus neurogenic vasoconstriction. Endothelium derived-mediators (adenosine and NO) can modify vascular sympathetic neurotransmission and are relevant for vascular homeostasis. This is a relevant issue in terms of vascular homeostasis and, any modification, may lead to a deregulation process and to pathologies. Focus on NO-mediated effects on vascular sympathetic transmission will be done, discriminating the effects ascribed to NO generated by NO synthases located in the different vascular layers. A comparison between mesenteric/tail arteries will also be explored, particularly the relevance of the transsynaptic modulation on noradrenaline release mediated by endothelial NO and adenosine in normotensive/hypertensive vascular tissues. Adenosinergic system, namely adenosine, nucleoside transporters and adenosine receptors, can be influenced by endothelium mediators, namely by NO, causing alterations on the way these players interact with each other. In conditions where endothelium is compromised, a deregulation occurs with an increase in vascular sympathetic neurotransmission (as a consequence of adenosinergic system dynamic alteration). In summary, the impact of endothelial dysfunction on vascular neurotransmission is debated with particular focus on adenosinergic and nitroxidergic system dynamics.

Keywords: endothelium, nitric oxide, adenosine, mesenteric artery, tail artery, sympathetic neurotransmission

1. Introduction

Endothelium has been described to present key roles in the vascular physiology: various endothelium-derived endogenous substances [1], namely contracting (endothelin, prostaglandin

F2a and thromboxane A2) and/or relaxing (prostaglandin I2 and nitric oxide, NO) factors [2, 3] can modulate blood vessel tone. These substances, known as endothelium-derived contracting factors (EDCF) or endothelium-derived relaxing factor (EDRF), can modify the vascular smooth muscle tone directly, acting on smooth muscle cells, or indirectly, by altering sympathetic transmission [4]. Nevertheless, when endothelium integrity and/or function is compromised, such regulation can be impaired. Indeed, evidence suggests that endothelial dysfunction (present an altered NO production and oxidative stress) may contribute to the pathogenesis of hypertension. As a consequence, an increase in peripheral vascular resistance occurs in conditions where endothelium is somehow injured. For example, endothelium dysfunction leads to the enhancement of contractile responses to vasoconstrictor agents [2, 5–8]. Nevertheless, in the literature, there are also innumerous other factors that can also influence endothelium function and, therefore, vascular responsiveness, such as tetrahydrobiopterin (BH4), sex hormones and gender, angiotensin, insulin, vascular endothelial growth factor, vitamin D, adiponectin, uric acid, lipids, oxygen-derived free radicals, aldosterone and epithelial sodium channels.

In this chapter, the impact of endothelial dysfunction on vascular neurotransmission is debated with particular focus on adenosinergic and nitroxidergic system dynamics.

2. Endothelium and vasodilation

The vascular wall is composed of layers that can be identified by their respective morphology and by the different functions exhibited by respective cells which, ultimately, are responsible for the vascular tone, influencing blood pressure. Arteries and veins have a similar structure presenting three layers: intima or endothelium, media or smooth muscle and adventitia.

The tunica intima is the inner and thinnest layer and surrounds the lumen. It is made up of endothelial cells lining the entire vasculature and includes circular elastic bands, the internal elastic lamina. The tunica media, also called muscle layer, is composed of vascular smooth muscle, which helps regulate the size of the lumen and externally present circular elastic bands, the external elastic lamina. This tunica differs between arteries and veins: arteries contain more smooth muscle than the tunica media of their counterpart, the veins, and this allows arteries to constrict and dilate to adjust the volume of blood needed by the tissues that they support. Additionally, the structure of arteries differs between large arteries and resistant arteries: in the first type, arteries present a media with large amount of elastic fibers disposed between smooth muscle cells and the thickness of the vascular wall is thinner than that exhibited by resistant arteries that often have multiple strands of smooth muscle layers. The external layer, adventitia layer is composed of connective tissue allowing the blood vessel to withstand forces acting on the vessel wall and of collagen fibers that anchor the vessel to surrounding tissues.

The endothelium can evoke effects, dilation or contraction of the underlying vascular smooth muscle, by releasing endothelium-derived relaxing factors (EDRF) such as NO or endothelium-derived contracting factors (EDCF) such as endothelin or prostanoids.

2.1. NO effects on vasodilation and endothelial dysfunction

NO is a well-known EDRF that induces vasodilation through the activation of soluble guanylyl cyclase in the vascular smooth muscle cells producing cyclic guanosine monophosphate (therefore, through the signaling pathway that can be represented as NO-cGMP/cGMP-dependent kinases).

It is well accepted that the benefits of NO released from endothelium are compromised in vascular diseases and aging since there is a reduced amount of NO. However, evidence also show that the production of NO can be upregulated, for example, by estrogens, exercise and dietary factors and downregulated by oxidative stress, smoking, pollution and oxidized low-density lipoproteins.

Moreover, when endothelium is dysfunctional, the vasodilation induced by endothelial mediators is impaired and it can even lead to vascular smooth muscle cells contraction. For instance, in aged subjects and in vascular diseases (essential hypertension and diabetes) when the production of NO is compromised, endothelium-dependent contractions are intensified.

NO is produced by three isoforms of NO synthase, presenting a more general distribution in the human body than that initially predicted: neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS). nNOS is constitutively expressed in central and peripheral nervous system contributing to regulation of blood pressure, smooth muscle dilation and vasodilation via peripheral nitrergic nerves. iNOS is expressed in several cell types and generates large amounts of NO, which is involved in the pathophysiology of inflammatory diseases, as regulatory effector molecule of the innate immune response and septic shock. eNOS is expressed mainly in endothelial cells and has several vasoprotective and anti-atherosclerotic effects as well as an important role in vascular tone and thus blood pressure regulation.

Many cardiovascular risk factors lead to oxidative stress, eNOS uncoupling and endothelial dysfunction in the vasculature. eNOS generates NO which results from the activity of two domains, the oxygenase domain that convert L-arginine to L-citrulline plus NO and the reductase domain that convert nitrites to NO [9].

As mentioned above, NO production from endothelium can be upregulated or downregulated by a number of factors of which vascular endothelial growth factor (VEGF) can upregulate eNOS. Interestingly, a chronic side effect of VEGF inhibitors is the occurrence of hypertension, suggesting a physiological role for VEGF in maintaining endothelial control of vasomotor tone [10–12]. In humans, in hypertension, VEGF inhibitors may cause increased production of endothelin-1 [13, 14] and reduced vascular response to acetylcholine [15, 16].

Acute and chronic increases in flow as well as the resulting augmentation in shear stress of the blood on the endothelial cells can be altered through Ca^{2+}-dependent and Ca^{2+}-independent pathways. It has been described that Ca^{2+}-independent pathway can increase both the expression and activity of eNOS and thus the release of NO [17]. The role played by the endothelial cells to protect against thrombin and other platelet products by increasing the activity of eNOS has been demonstrated both *in vitro* [18–26] and *in vivo* [27]. Serotonin and adenosine diphosphate are mediators released by aggregating platelets, which may activate eNOS and increase NO production. When endothelium is absent/dysfunctional, vasodilation is no longer observed,

and aggregating platelets induce contractions, because they release vasoconstrictors (thromboxane A2 and serotonin). When platelet aggregation occurs in a healthy artery (i.e, with an intact and physiologically active endothelium), serotonin (and ADP) release by the platelets as well as production of thrombin will increase NO release from endothelial cells. Thus, NO will be increased in the vicinity of smooth muscle cells inducing dilation, and consequently, increasing blood flow.

Another important factor influencing NO production relies on the presence of reactive oxygen/nitrogen species (ROS/RNS). Indeed, several enzymes from endothelium can produce superoxide anions such as nicotinamide adenine dinucleotide phosphate oxidase (NOX), xanthine oxidase (XO), cyclooxygenases (COX) and also eNOS but only when there is a deficient supply of substrate or of the cofactor BH4. Under pathophysiological conditions, superoxide anions scavenge NO resulting in the formation of peroxynitrite, reducing considerably the bioavailability of NO. Moreover, ROS can inactivate eNOS through S-glutathionylation. Taken together, these may explain why oxidative stress is often associated with endothelial dysfunction.

Moreover, intake of a number of natural products, such as flavonoids and other polyphenols, favors endothelium-dependent dilations and protects endothelium from dysfunction through increased production of NO. The protective effects of polyphenols against endothelial dysfunction involve increased production of NO in response to endothelium-derived vasodilators resulting from: facilitation of the effects of NO on the vascular smooth muscle cells, increased levels of BH4, calcium-independent phosphorylation of eNOS, antioxidant properties preventing the uncoupling of eNOS, activation of estrogen receptors and upregulation of AMP-activated protein kinase (AMPK) and of NAD(+)-dependent deacetylase (SIRT1) [28–31].

2.2. Influence of NO on another EDRF

Besides its direct role as a vasodilator, NO also modulates the release of other endothelium-derived mediators. Thus, in a number of larger arteries, endothelium-derived hyperpolarization (EDH)-mediated dilations become prominent only when the synthesis of NO is inhibited [32, 33]. Hence, EDH is able to take over, at least temporarily, in the case of 'classical' endothelial dysfunction associated with a loss of NO synthesis, demonstrating strong compensatory efficiency of EDH-mediated responses. Intriguingly, exogenous NO attenuates EDH-mediated responses in coronary arteries *in vitro* [34] and in coronary circulation *in vivo* [35, 36]. Moreover, NO has been shown to exert a negative feedback effect on endothelium-dependent dilation through cGMP-mediated desensitization in isolated coronary arteries [32]. Indeed, clinical studies show that chronic therapy with nitrate, used as a NO donor, in patients with ischemic heart disease does not yield a benefit on mortality [37, 38], confirming the importance of the physiological balance between NO and EDH. Moreover, the amount of NO formed in the endothelial cells controls the release of vasoconstrictor prostanoids [39, 40].

3. Endothelium and sympathetic neurotransmission

The sympathetic nervous system (SNS) is known to play a fundamental role in the short- and long-term regulation of different vascular functions. Vessels contain sympathetic nerves distributed between smooth muscle and adventitia layers [41]. Sympathetic nerve fibers are

enveloped in Schwann cells: most nerve fibers travel through individual channels in the Schwann cell, but small fibers are sometimes bundled together within a single channel [42]. The SNS signals to dilate or constrict the vessel, changing the lumen size, i.e., regulating vascular tone and, therefore, affecting blood pressure.

Nowadays, it is well established that SNS contributes to the modulation of vascular function and that this relationship is a key factor in the development of cardiovascular diseases. Several factors, such as the renin-angiotensin system, NO, ROS and endothelin, influence this modulation at central and peripheral level [43–45]. Moreover, endothelial function also seems to be regulated by SNS, mainly in the control of vascular tone. Additionally, endothelial dysfunction as well as increase in sympathetic activity has been associated to cardiovascular risk factors and disease. For example, in studies carried out in healthy subjects, an increase in sympathetic activity was associated with a decrease in endothelial function [46] Moreover, in humans, stiffness of large artery was also associated with an increased activity of SNS [47]. On the other hand, large artery stiffness can interfere with autonomic regulation by impairing carotid baroreflex sensitivity [48].

The influence of endothelium in noradrenaline release has also been previously demonstrated [49, 50]. This conclusion was obtained not only in arteries without endothelium but also in a model of endothelial dysfunction (i.e, essential hypertensive arteries), which is shown in **Table 1**. This type of information can be obtained from experiments where synapse events are mimicked allowing the evaluation of putative players able to alter neurotransmitter release from the nerves. Indeed, in such experiments, the use of selective pharmacological tools, such as agonists/antagonists of receptors or of activators/inhibitors of proteins or enzymes, can reveal their respective role in the neurotransmission dynamic. For instance, in experiments where rat vascular tissues, preincubated with [^{3}H]-noradrenaline, are electrically stimulated (5 Hz, 100 pulses, 1 ms, 50 mA), the release of ^{3}H is induced (which mimics a physiological depolarization) and can be measured by liquid scintillation spectrometry. In addition, by altering the receptors or proteins activated (with pharmacological tools), it is possible to evaluate the activity/role of a specific player in neurotransmitter release (please see previous articles from our group where the methodology is described in detail [49, 51, 52]). For example, in **Table 1**, data refer to tissues that were stimulated twice at 30-min interval: outflow (b_n) refers to the 5-min period immediately before each stimulation period. The electrically evoked tritium overflow (S_n) was calculated by subtracting the estimated basal outflow from total outflow observed during and in the 25-min period subsequent to S_1 and expressed as a percentage of the tissue ^{3}H content at the onset of stimulation. Two animal models have been used: spontaneously hypertensive rats (SHR), a well-established model of essential hypertension [53, 54], and the respective controls, the Wistar Kyoto (WKY) rats. Moreover, in WKY animals, some arteries were endothelium denuded. The influence of these conditions on the release of S_1 was evaluated, and the results are presented in **Table 1**.

The results in this table show that the outflow observed in the endothelium-denuded vascular tissue is lower than that obtained in intact tissue. Also, the S_2 values obtained in the endothelium-denuded arteries are altered, with values higher than those observed in intact tissues. These data reveal the importance of a healthy endothelium to the sympathetic neurotransmission homeostasis, once it seems to present a transsynaptic influence mediated by endothelium. In pathological conditions, this influence can be impaired augmenting the amount of noradrenaline release and causing vasoconstriction.

	Basal outflow (b_1) (fractional rate of outflow; min^{-1})	Evoked Overflow (S_1) (% of tissue tritium content)	S_2/S_1	n
Mesenteric artery				
WKY				
Endothelium intact	0.065 ± 0.004	0.202 ± 0.016	1.054 ± 0.038	12
Endothelium denuded	0.081 ± 0.002*	0.329 ± 0.036*	1.002 ± 0.026	12
SHR				
Endothelium intact	0.073 ± 0.003*	0.310 ± 0.041*	1.013 ± 0.031	10
Tail artery				
WKY				
Endothelium intact	0.084 ± 0.004	0.217 ± 0.012	0.932 ± 0.037	18
Endothelium denuded	0.069 ± 0.002*	0.317 ± 0.049*	0.929 ± 0.039	14
SHR				
Endothelium intact	0.063 ± 0.002*	0.259 ± 0.016*	1.034 ± 0.096	14

Tissue preparations of mesenteric and tail arteries from WKY and SHR animals were pre-incubated with [^{3}H]-noradrenaline for 40 min. After pre-incubation with [^{3}H]-noradrenaline, tissues were superfused with [^{3}H]-noradrenaline free medium containing desipramine (400 nM). Values presented are means ± SEM, and n denotes the number of tissue preparations. Significant differences from WKY intact arteries: *$P < 0.05$.

Table 1. Basal tritium outflow (b_1), electrically evoked tritium overflow (S_1) and S_2/S_1 ratios from normotensive (WKY) and hypertensive (SHR) vessels of the rat.

Several substances produced in endothelial cells, such as NO, adenosine, ROS and/or RNS (e.g. peroxides, superoxide, hydroxyl radical, and singlet oxygen) and prostaglandins can influence sympathetic transmission [55, 56]. Also, the activity of some enzymes, such as adenosine kinase, adenosine deaminase, NOX, XO and COX, can be altered leading to changes in the bioavailability of their respective products, influencing, indirectly, sympathetic neurotransmission.

3.1. NO and vascular neurotransmission

There is evidence demonstrating that NO can modulate sympathetic neurotransmission modifying vascular smooth muscle tone, in various vascular beds, such as in coronary [57, 58], mesenteric [50, 59, 60] and pulmonary arteries [61–63]. Indeed, and as illustrated in **Figure 1**, a NO donor, DEA-NONOATE (10 μM) altered noradrenaline release (measured as explained above, i.e., by determining the amount of ^{3}H overflow using liquid scintillation spectrometry) in differential mode depending on the vascular territory: an increase of noradrenaline release occurs in tail artery contrasting to mesenteric territory where noradrenaline release is reduced.

Another relevant data are related with NO source, i.e., the type of NOS that generates NO (**Figure 1**): in tail arteries, NO production is ascribed mainly to eNOS isoform, particularly to

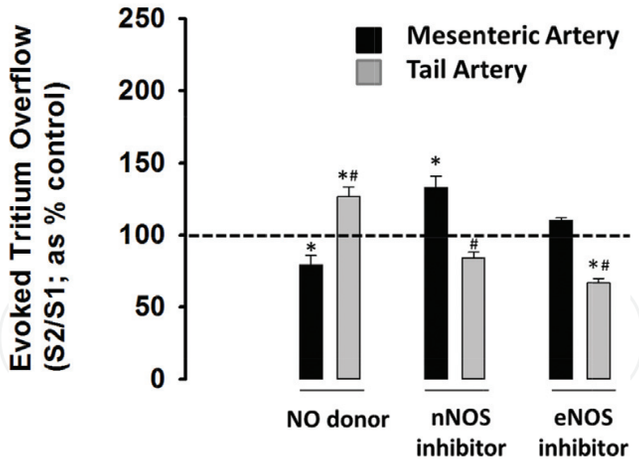

Figure 1. Influence of NOS inhibitors on vascular sympathetic transmission in mesenteric and tail rat arteries. Effect elicited by the NO donor, DEA-NONOate (10 μM), and the interaction exerted by the Nω-propyl-L-arginine hydrochloride, a specific neuronal NOS (nNOS) inhibitor (100 nM), and L-NIO dihydrochloride, a specific endothelial NOS (eNOS) inhibitor (500 nM), on the electrically evoked tritium overflow. Values are mean ± SEM from 5 to 12 artery segments. Significant differences from the appropriate control: $^{*}P < 0.05$ and from mesenteric artery: $^{\#}P < 0.05$ (ANOVA followed by post-hoc Holm-Sidak's multicomparisons t-test).

eNOS oxygenase domain with residual activity of the eNOS reductase domain [50], while in mesenteric arteries, nNOS, with both reductase and oxygenase domains being equally active, seems to be the most relevant isoform producing NO.

These differences in vascular neurotransmission elicited by NO can be explained by the activation of different pathways, leading to opposite outcomes. In resistant arteries, such as tail artery, the well-established NO-cGMP/cGMP-dependent kinases activating voltage-dependent-Ca^{2+} seem to be the predominant pathway [64], leading to vasoconstriction. However, in other vascular territories, such as the mesenteric artery, NO actions, in addition to the classically accepted activation of intracellular cGMP-dependent pathway [65], can also activate cGMP-independent pathways, namely by eliciting an energy decrease in mitochondria (i.e., ATP), particularly with an increase in ATP catabolism, with subsequent adenosine accumulation. Adenosine will then act on presynaptic A_1 receptors causing a reduction in cAMP formation and, consequently, of PKA. Therefore, a reduction of Ca^{2+} channels phosphorylation (by PKA) will occur reducing the intracellular amount of Ca^{2+}. Presynaptically, the amount of intracellular Ca^{2+} is critical for neurotransmission; therefore, lower amounts of calcium will cause a reduction of noradrenaline release and of the postsynaptic signal events triggered by noradrenaline, leading to vasodilation [66].

The location of enzyme isoforms is also relevant: nNOS in mesenteric arteries are located mostly in Schwann cells contrasting to tail arteries where their presence is very scarce (**Figure 2**).

3.2. Adenosine, endothelium and vascular neurotransmission

It is well established that adenosine can act as a physiological neuromodulator through activation of four types of adenosine receptors, A_1, A_{2A}, A_{2B} and A_3 in the vasculature [67]. These receptors present differential affinities for adenosine, with adenosine A_1 receptor requiring lower concentrations to get activated (KdA_1, 0.3–3 nM), followed by A_{2A} receptors with a Kd

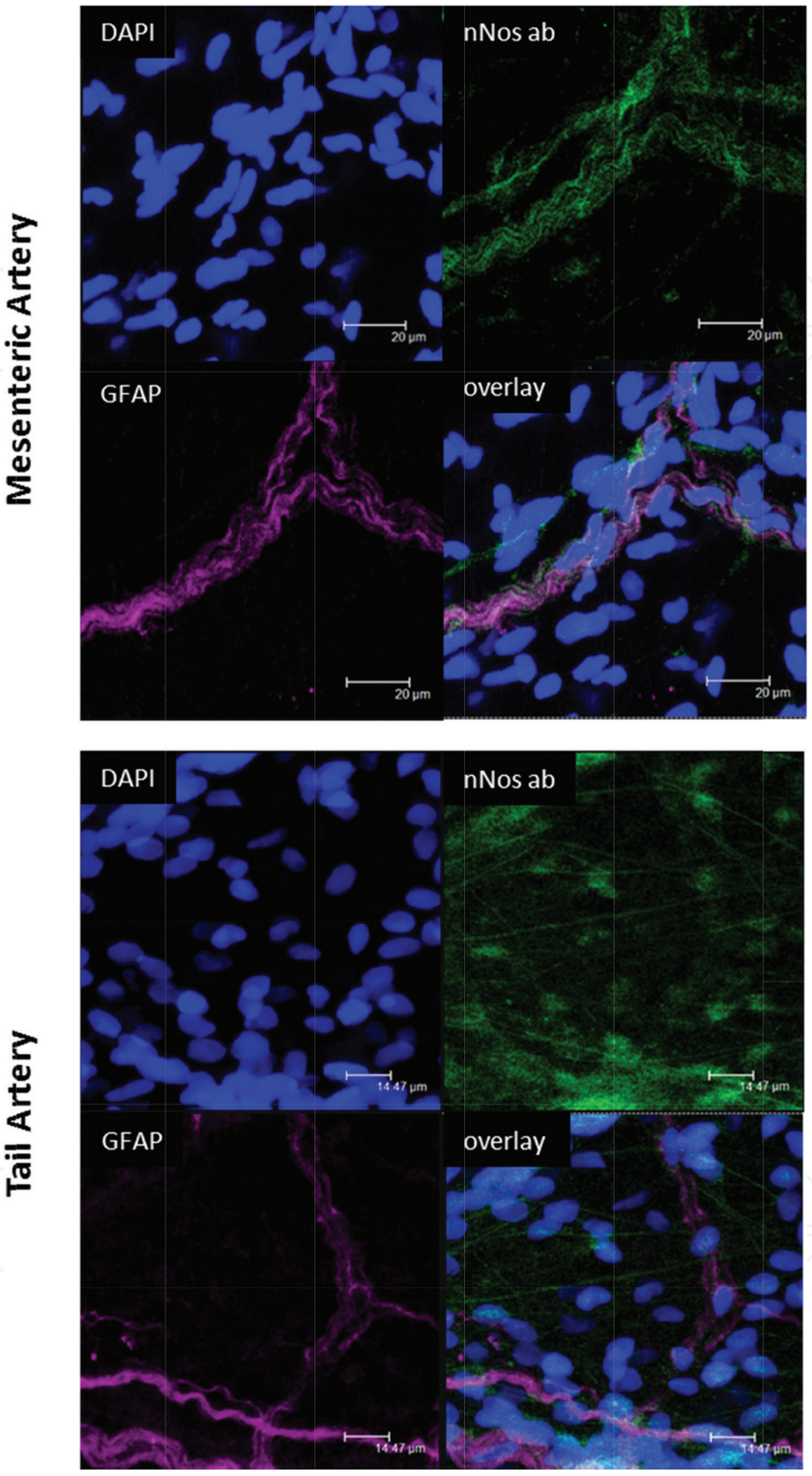

Figure 2. Adventitia mesenteric and tail rat arteries images. Images are representative reconstructions captured with a confocal microscope (Leica SP5 LSCM system fitted with an inverted microscope (x63 oil immersion lens). Stacks of 1-µm-thick serial optical images. Arteries were stained for nNOS (a primary mouse monoclonal anti-NOS1 and a species-specific secondary Alexa 488 antibody), GFAP (a primary rabbit anti-GFAP polyclonal antibody and a species-specific secondary Alexa 647 antibody) and DAPI (nuclear stain).

1–20 nM. Pharmacological studies have also revealed that A_{2B} and A_3 receptors are low affinity receptors for adenosine requiring concentrations higher than 1 μM, although these adenosine receptor subtypes present different Kd (A_3 subtype requires higher concentrations than A_{2B} receptors) [68].

Adenosine, a well-known nucleoside, results from the sequential catabolism of ATP, forming ADP, AMP and adenosine with this later step being mediated by 5′-nucleotidase. Adenosine can then be further converted in inosine by adenosine deaminase or, instead, can be reconverted to AMP by adenosine kinase. In addition to adenosine receptors and adenosine, adenosinergic system is also composed by nucleoside transporters (NTs), which are responsible for nucleoside transport into the cells and vice versa. Some of the NTs in particular some equilibrative nucleoside transporters (ENT)s have already been identified as capable of promoting adenosine transport in vasculature, namely the subtype ENT1 and ENT 4 [69, 70].

In vascular tissues as well as in some diseased states, such as hypertension, the bioavailability of adenosine varies [71], as presented in **Table 2**.

The amount of adenosine present in the vicinity of adenosine receptors depends on the adenosinergic system dynamics which, in turn, can be influenced by innumerous factors, such as NO, ROS, lipid peroxidation, endothelium dysfunction, etc., that can be altered in several pathological conditions, namely in hypertension, diabetes, aging and inflammation.

Another factor related with the relevance of vascular adenosine-mediated effects relies on adenosine receptor subtype distribution in the vasculature. All adenosine receptor subtypes have been identified not only in arteries, such as pulmonary [72], mesenteric [73–77], ear [73], aorta [78] and tail [51, 52, 79–81], but also in veins [75, 82]. In renal vessels, a role of adenosine receptors in sympathetic regulation was also demonstrated [83], conditioning the blood efflux

	Basal outflow (b_1) (pmol/mg of tissue)	**Evoked overflow (S_1) (pmol/mg of tissue)**	**n**
Mesenteric artery			
WKY	25.74 ± 2.57	26.47 ± 2.76	5
SHR	75.44 ± 4.22*	77.31 ± 5.47*	5
Tail artery			
WKY	45.64 ± 3.81	49.78 ± 5.29	5
SHR	64.81 ± 5.01*	67.82 ± 4.03*	5

Tissue preparations of mesenteric and tail arteries from WKY and SHR animals were superfused with Krebs-Henseleit. Tissues were stimulated twice at 30-min interval (S_1–S_2; 100 pulses, 5 Hz, 1 ms, 50 mA): b_1 refers to the 5-min period immediately before S_1. The superfusate was collected in 5-min period before and after stimulation, and each sample was heated at 80°C and derivatized using chloroacetaldehyde for 50 min at 70°C in a dry bath incubator. Identification of the ε-adenosine formed in this collected samples was confirmed by a gradient HPLC using a fluorescent detector at 230 nm excitation and 420 nm emission wavelengths. Values presented are means ± SEM of adenosine per mg of tissue, and n denotes the number of tissue preparations. Significant differences from WKY vessels: *$P < 0.05$.

Table 2. Basal (b_1) and electrically evoked (S_1) adenosine release from sympathetic nerve terminals from normotensive (WKY) and hypertensive (SHR) vessels of the rat.

in the afferent arteriole and, consequently, of renal filtration. In hypertensive arteries and veins, an impairment of the neuromodulation exerted by adenosine A_1 receptors [75–77, 82] was described, contrasting with a preserved adenosine A_{2A} receptor-mediated facilitation of noradrenaline release [75–77]. Note that a redistribution of adenosine A_1 receptors from sympathetic nerves to Schwann cells was reported in hypertensive state while adenosine A_{2A} receptors, in sympathetic nerves, were preserved [77]. Particular relevant information relies on the location of adenosine receptors on the vascular wall layers contributing to the understanding of the functional role ascribed to adenosine receptors.

In endothelium, the four adenosine receptor subtypes have been identified by functional and immunohistochemical assays, for instance in tail artery [80, 84] and aorta [84, 85]. The influence of endothelium in adenosine-mediated responses has been demonstrated, with endogenous adenosine inducing an inhibition on noradrenaline release, through activation of adenosine A_1 receptors, (**Figure 3**, effect of DPCPX, a selective A_1 receptor antagonist). Adenosine availability is a crucial factor (effect demonstrated by pentostatin and α,β-methylene ADP, which inhibit adenosine deaminase and ecto-5'nucleotidase, respectively), conditioning the type of adenosine receptor that is activated. In resistant arteries, this effect is impaired when endothelium is compromised (arteries denuded of endothelium or in essential hypertensive arteries) and, instead, a facilitatory effect mediated by adenosine A_{2A} receptors, revealed by a selective A_{2A} receptor antagonist, the SCH 58261, and by inhibition of adenosine kinase, revealed by an adenosine kinase inhibitor, 5′-iodotubercidin (ITU), and by ecto-5'nucleotidase inhibitor, α,β-methylene ADP, demonstrating the relevance of adenosinergic dynamics both in physiological and pathophysiological contexts, such as in hypertension [49]. The adenosinergic system dynamic is adjusted to the unfavorable conditions created by endothelium injury, with enzymes involved in adenosine formation, such as adenosine deaminase and 5′-nucleotidase operating to promote an increase in the adenosine amount available and favoring the activation of A_{2A} receptors. This occurs despite the efforts of nucleoside transporters to equilibrate the concentration of adenosine between the inner and outer space of cells. In mesenteric arteries, A_{2A} receptor effect is enough to counteract the existing inhibitory tonus mediated by adenosine A_1, but in resistant arteries, the facilitatory effect mediated by A_{2A} receptors (upon noradrenaline release) predominates.

3.3. Interplay between nitroxidergic and other pathways in neurotransmission

NO signaling events, in mesenteric arteries, cause an accumulation of adenosine (as previously described in Section 3.1). This condition may favor adenosine neuromodulation, namely by activation of adenosine A_1 receptors (which is revealed when blockade of A_1 receptors occurs in the presence of the NO donor, DEA-NONOATE; **Figure 4**) in the mesenteric artery. Activation of A_1 receptors leads to a reduction of noradrenaline release, and subsequently, the activation of α_1-adrenoceptors in vascular smooth muscle cells is reduced, leading to vasodilation. In the tail artery, such interplay does not occur, at least in the experimental conditions tested.

The interplay between nitroxidergic and adenosinergic pathways can occur in neurotransmission, with NO promoting the formation of enough amounts of adenosine capable of activating inhibitory A_1 receptors. However, this type of interplay is dependent on the type of vascular bed.

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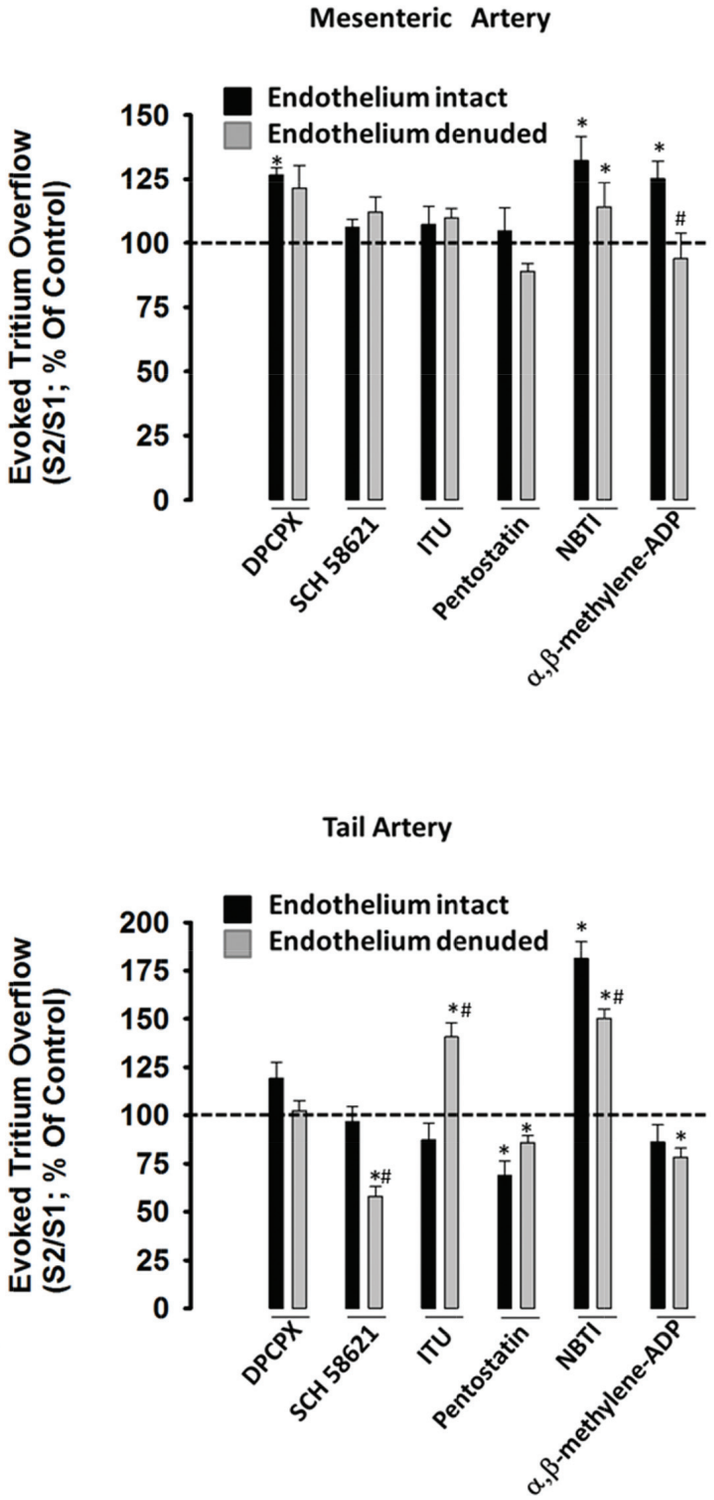

Figure 3. Influence of endogenous adenosine on vascular sympathetic transmission in mesenteric and tail arteries. Interaction with selective adenosine receptor antagonists, DPCPX (100 nM; A_1 subtype antagonist) and SCH 58261 (20 nM; A_{2A} subtype antagonist); adenosine kinase inhibitor, ITU (100 nM); adenosine deaminase inhibitor, Pentostatin (10 μM); a nucleoside transporter inhibitor, NBTI (5 μM) and an 5′-nucleotidase inhibitor, α,β-methylene-ADP (10 μM), on the electrically evoked tritium overflow. Values are mean ± SEM from 4 to 12 artery segments. Significant differences from the appropriate control: $^{*}P < 0.05$ and from intact arteries: $^{\#}P < 0.05$ (ANOVA followed by post-hoc Holm-Sidak's multicomparisons t-test).

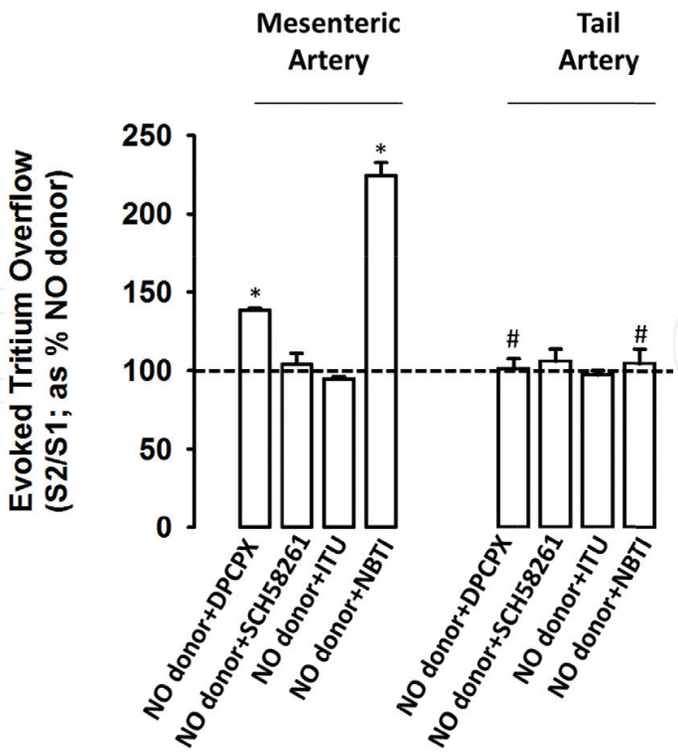

Figure 4. Influence of adenosine A_1 receptor antagonist (DPCPX, 100 nM) and adenosine A_{2A} receptor antagonist (SCH 58261, 20 nM), adenosine kinase inhibitor (ITU, 100 nM) and nucleoside transporter inhibitor (NBTI, 5 μM) in the effect elicited by a nitric oxide donor, DEA-NONOate (10 μM) on the electrically evoked tritium overflow, in mesenteric and tail arteries. Values are mean ± SEM from 4 to 12 artery segments. Significant differences from DEA-NONOate effect alone: $^{*}P < 0.05$ and from mesenteric artery: $^{\#}P < 0.05$ (ANOVA followed by post-hoc Holm-Sidak's multicomparisons t-test).

Furthermore, in the cardiovascular system, NO can also interplay with the adrenergic pathway. NO source is, most probably, endothelial since noradrenaline release in the presence of a β-adrenoceptor agonist, isoprenaline (300 nM), caused an increase of noradrenaline release (175.10 ± 13.8%, n = 11), but the increase observed was lower in endothelium-denuded arteries (129.92 ± 13.1%, n = 7). Therefore, these data support the possibility, previously raised by Balligand et al. [86] and by Conti et al. [87], that NO production can lead to an increase in noradrenaline release, as a consequence of adrenergic receptors activation, namely of facilitatory β-adrenoceptors.

4. Current and future developments

In addition to the direct effects exerted by several substances on smooth muscle cells, which can cause vasodilation or vasoconstriction, the evidence that endothelium-derived factors can also influence sympathetic neurotransmission that reinforces the importance of endothelium and of its putative role in pathologies. Indeed, vascular sympathetic neurotransmission and the interplay exerted by endothelium-derived substances are, therefore, relevant in the homeostasis of vascular tone. In pathophysiological conditions, especially when endothelium is injured, their impact on neurotransmission account, at least in part, for the occurring vasoconstriction.

NO has been viewed as a vasodilator substance since its direct effect on vascular smooth muscle cells causes dilation. However, NO can influence neurotransmission, and the interplay with adenosinergic and adrenergic pathways altering neurotransmission can, in some cases, cause an increase in noradrenaline release, which consequently will promote vasoconstriction. Therefore, the importance of NO is renewed as well as its ability to interplay with other signaling pathways involving sympathetic regulation such as the adrenergic and adenosinergic ones. Additional information and research on this field are, therefore, required to extend the knowledge on the insights of transsynaptic modulation of vascular neurotransmission. This is particularly important and can be useful to develop new therapeutic strategies, particularly in pathologies or clinical conditions, where the sympathetic system is hyperactivated.

Acknowledgements

This work received financial support from FCT, Fundação para a Ciência e Tecnologia) through project UID/QUI/50006/2013–POCI/01/0145/FEDER/007265 with financial support from FCT/MEC through national funds and co-financed by FEDER, under the Partnership Agreement PT2020. PhD grant [SFRH/BD//2009] supported by FCT. Authors would like to thank Professors Silvia M. Arribas and Maria Carmen González from Department of Physiology, School of Medicine, University Autónoma de Madrid, Spain, for their assistance in the use of the Confocal Microscopy.

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References

[1] Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature. 1980;**288**:373-376

[2] Moncada S, Palmer RM, Higgs EA. Nitric oxide: Physiology, pathophysiology, and pharmacology. Pharmacological Reviews. 1991;**43**:109-142

[3] Vanhoutte PM, Mombouli JV. Vascular endothelium: Vasoactive mediators. Progress in Cardiovascular Diseases. 1996;**39**:229-238

[4] Westcott EB, Segal SS. Perivascular innervation: A multiplicity of roles in vasomotor control and myoendothelial signaling. Microcirculation. 2013;**20**:217-238

[5] Stankevicius E, Kevelaitis E, Vainorius E, Simonsen U. Role of nitric oxide and other endothelium-derived factors. Medicina (Kaunas, Lithuania). 2003;**39**:333-341

[6] Torok J. Participation of nitric oxide in different models of experimental hypertension. Physiological Research. 2008;**57**:813-825

[7] Vanhoutte PM. Endothelial dysfunction: The first step toward coronary arteriosclerosis. Circulation Journal. 2009;**73**:595-601

[8] Vanhoutte PM, Shimokawa H, Tang EH, Feletou M. Endothelial dysfunction and vascular disease. Acta Physiologica (Oxford, England). 2009;**196**:193-222

[9] Gautier C, van Faassen E, Mikula I, Martasek P, Slama-Schwok A. Endothelial nitric oxide synthase reduces nitrite anions to NO under anoxia. Biochemical and Biophysical Research Communications. 2006;**341**:816-821

[10] Facemire CS, Nixon AB, Griffiths R, Hurwitz H, Coffman TM. Vascular endothelial growth factor receptor 2 controls blood pressure by regulating nitric oxide synthase expression. Hypertension. 2009;**54**:652-658

[11] Hou HH, Hammock BD, Su KH, Morisseau C, Kou YR, Imaoka S, Oguro A, Shyue SK, Zhao JF, Lee TS. N-terminal domain of soluble epoxide hydrolase negatively regulates the VEGF-mediated activation of endothelial nitric oxide synthase. Cardiovascular Research. 2012;**93**:120-129

[12] Skinner M, Philp K, Lengel D, Coverley L, Lamm Bergstrom E, Glaves P, Musgrove H, Prior H, Braddock M, Huby R, Curwen JO, Duffy P, Harmer AR. The contribution of VEGF signalling to fostamatinib-induced blood pressure elevation. British Journal of Pharmacology. 2014;**171**:2308-2320

[13] Kappers MH, de Beer VJ, Zhou Z, Danser AH, Sleijfer S, Duncker DJ, van den Meiracker AH, Merkus D. Sunitinib-induced systemic vasoconstriction in swine is endothelin mediated and does not involve nitric oxide or oxidative stress. Hypertension. 2012;**59**:151-157

[14] Lankhorst S, Kappers MH, van Esch JH, Danser AH, van den Meiracker AH. Hypertension during vascular endothelial growth factor inhibition: Focus on nitric oxide, endothelin-1, and oxidative stress. Antioxidants & Redox Signaling. 2014;**20**:135-145

[15] Thijs AM, van Herpen CM, Sweep FC, Geurts-Moespot A, Smits P, van der Graaf WT, Rongen GA. Role of endogenous vascular endothelial growth factor in endothelium-dependent vasodilation in humans. Hypertension. 2013;**61**:1060-1065

[16] Mayer EL, Dallabrida SM, Rupnick MA, Redline WM, Hannagan K, Ismail NS, Burstein HJ, Beckman JA. Contrary effects of the receptor tyrosine kinase inhibitor vandetanib on constitutive and flow-stimulated nitric oxide elaboration in humans. Hypertension. 2011;**58**:85-92

[17] Vanhoutte PM, Shimokawa H, Feletou M, Tang EH. Endothelial dysfunction and vascular disease - A 30th anniversary update. Acta Physiologica (Oxford, England). 2017;**219**:22-96

[18] De Mey JG, Claeys M, Vanhoutte PM. Endothelium-dependent inhibitory effects of acetylcholine, adenosine triphosphate, thrombin and arachidonic acid in the canine femoral artery. The Journal of Pharmacology and Experimental Therapeutics. 1982;**222**:166-173

[19] Cohen RA, Shepherd JT, Vanhoutte PM. Inhibitory role of the endothelium in the response of isolated coronary arteries to platelets. Science. 1983;**221**:273-274

[20] Cohen RA, Shepherd JT, Vanhoutte PM. Vasodilatation mediated by the coronary endothelium in response to aggregating platelets. Bibliotheca Cardiologica. 1984;**38**:35-42

[21] Houston DS, Shepherd JT, Vanhoutte PM. Adenine nucleotides, serotonin, and endothelium-dependent relaxations to platelets. The American Journal of Physiology. 1985;**248**:H389-H395

[22] Houston DS, Shepherd JT, Vanhoutte PM. Aggregating human platelets cause direct contraction and endothelium-dependent relaxation of isolated canine coronary arteries. Role of serotonin, thromboxane A2, and adenine nucleotides. The Journal of Clinical Investigation. 1986;**78**:539-544

[23] Shimokawa H, Kim P, Vanhoutte PM. Endothelium-dependent relaxation to aggregating platelets in isolated basilar arteries of control and hypercholesterolemic pigs. Circulation Research. 1988;**63**:604-612

[24] Derkach DN, Ihara E, Hirano K, Nishimura J, Takahashi S, Kanaide H. Thrombin causes endothelium-dependent biphasic regulation of vascular tone in the porcine renal interlobar artery. British Journal of Pharmacology. 2000;**131**:1635-1642

[25] Motley ED, Eguchi K, Patterson MM, Palmer PD, Suzuki H, Eguchi S. Mechanism of endothelial nitric oxide synthase phosphorylation and activation by thrombin. Hypertension. 2007;**49**:577-583

[26] Touyz RM. Regulation of endothelial nitric oxide synthase by thrombin. Hypertension. 2007;**49**:429-431

[27] Shimokawa H, Vanhoutte PM. Angiographic demonstration of hyperconstriction induced by serotonin and aggregating platelets in porcine coronary arteries with regenerated endothelium. Journal of the American College of Cardiology. 1991;**17**:1197-1202

[28] Bieganska-Hensoldt S, Rosolowska-Huszcz D. Polyphenols in preventing endothelial dysfunction. Postępy Higieny i Medycyny Doświadczalnej (Online). 2017;**71**:227-235

[29] Perez-Vizcaino F, Duarte J, Andriantsitohaina R. Endothelial function and cardiovascular disease: Effects of quercetin and wine polyphenols. Free Radical Research. 2006;**40**:1054-1065

[30] Perez-Vizcaino F, Duarte J. Flavonols and cardiovascular disease. Molecular Aspects of Medicine. 2010;**31**:478-494

[31] Yamagata K, Tagami M, Yamori Y. Dietary polyphenols regulate endothelial function and prevent cardiovascular disease. Nutrition. 2015;**31**:28-37

[32] Olmos L, Mombouli JV, Illiano S, Vanhoutte PM. cGMP mediates the desensitization to bradykinin in isolated canine coronary arteries. The American Journal of Physiology. 1995;**268**:H865-H870

[33] Ozkor MA, Quyyumi AA. Endothelium-derived hyperpolarizing factor and vascular function. Cardiology Research and Practice. 2011;**2011**:156146

[34] Bauersachs J, Popp R, Hecker M, Sauer E, Fleming I, Busse R. Nitric oxide attenuates the release of endothelium-derived hyperpolarizing factor. Circulation. 1996;**94**:3341-3347

[35] Nishikawa Y, Stepp DW, Chilian WM. Nitric oxide exerts feedback inhibition on EDHF-induced coronary arteriolar dilation in vivo. American Journal of Physiology. Heart and Circulatory Physiology. 2000;**279**:H459-H465

[36] Shimokawa H, Godo S. Diverse functions of endothelial NO synthases system: NO and EDH. Journal of Cardiovascular Pharmacology. 2016;**67**:361-366

[37] Kojima S, Matsui K, Sakamoto T, Ishihara M, Kimura K, Miyazaki S, Yamagishi M, Tei C, Hiraoka H, Sonoda M, Tsuchihashi K, Shimoyama N, Honda T, Ogata Y, Ogawa H, Japanese Acute I. Coronary syndrome study, long-term nitrate therapy after acute myocardial infarction does not improve or aggravate prognosis. Circulation Journal. 2007;**71**:301-307

[38] Ambrosio G, Del Pinto M, Tritto I, Agnelli G, Bentivoglio M, Zuchi C, Anderson FA, Gore JM, Lopez-Sendon J, Wyman A, Kennelly BM, Fox KA, Investigators G. Chronic nitrate therapy is associated with different presentation and evolution of acute coronary syndromes: Insights from 52,693 patients in the global registry of acute coronary events. European Heart Journal. 2010;**31**:430-438

[39] Banerjee D, Mazumder S, Sinha AK. The role of inhibition of nitric oxide synthesis in the aggregation of platelets due to the stimulated production of thromboxane A2. Blood Coagulation & Fibrinolysis. 2014;**25**:585-591

[40] Sandoo A, van Zanten JJ, Metsios GS, Carroll D, Kitas GD. The endothelium and its role in regulating vascular tone. Open Cardiovascular Medicine Journal. 2010;**4**:302-312

[41] Birch DJ, Turmaine M, Boulos PB, Burnstock G. Sympathetic innervation of human mesenteric artery and vein. Journal of Vascular Research. 2008;**45**:323-332

[42] Saladin KS. Anatomy and Physiology. 6th ed. New YorK: McGraw-Hill; 2011

[43] Zucker IH, Liu JL. Angiotensin II–nitric oxide interactions in the control of sympathetic outflow in heart failure. Heart Failure Reviews. 2000;**5**:27-43

[44] Bruno RM, Ghiadoni L, Seravalle G, Dell'oro R, Taddei S, Grassi G. Sympathetic regulation of vascular function in health and disease. Frontiers in Physiology. 2012;**3**:284

[45] Forstermann U, Sessa WC. Nitric oxide synthases: regulation and function. Eur Heart J. 2012;**33**:829-837 837a-837d

[46] Amiya E, Watanabe M, Komuro I. The relationship between vascular function and the autonomic nervous system. Annals of Vascular Diseases. 2014;**7**:109-119

[47] Parati G, Salvi P. Arterial stiffness and the sympathetic nervous system, in: ME Safar, MF O'Rourke, ED Frohlich (Ed.) Blood Pressure and Arterial Wall Mechanics in Cardiovascular Diseases, Springer, London; 2014

[48] Cao Q, Zhang J, Xu G. Hemodynamic changes and baroreflex sensitivity associated with carotid endarterectomy and carotid artery stenting. Interventional Neurology. 2015;**3**:13-21

[49] Sousa JB, Fresco P, Diniz C. Endothelial dysfunction impairs vascular neurotransmission in tail arteries. Neurochemistry International. 2015;**80**:7-13

[50] Sousa JB, Vieira-Rocha MS, Arribas SM, Gonzalez MC, Fresco P, Diniz C. Endothelial and neuronal nitric oxide activate distinct pathways on sympathetic neurotransmission in rat tail and mesenteric arteries. PLoS One. 2015;**10**:e0129224

[51] Fresco P, Diniz C, Goncalves J. Facilitation of noradrenaline release by activation of adenosine a(2A) receptors triggers both phospholipase C and adenylate cyclase pathways in rat tail artery. Cardiovascular Research. 2004;**63**:739-746

[52] Fresco P, Oliveira JM, Kunc F, Soares AS, Rocha-Pereira C, Goncalves J, Diniz C. A2A adenosine-receptor-mediated facilitation of noradrenaline release in rat tail artery involves protein kinase C activation and betagamma subunits formed after alpha2-adrenoceptor activation. Neurochemistry International. 2007;**51**:47-56

[53] Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. Japanese Circulation Journal. 1963;**27**:282-293

[54] Trippodo NC, Frohlich ED. Similarities of genetic (spontaneous) hypertension. Man and rat. Circulation Research. 1981;**48**:309-319

[55] Reid JJ, Lieu AT, Rand MJ. Interactions between endothelin-1 and other chronotropic agents in rat isolated atria. European Journal of Pharmacology. 1991;**194**:173-181

[56] Wiklund NP, Wiklund CU, Cederqvist B, Ohlen A, Hedqvist P, Gustafsson LE. Endothelin modulation of neuroeffector transmission in smooth muscle. Journal of Cardiovascular Pharmacology. 1991;**17**(Suppl 7):S335-S339

[57] Addicks K, Bloch W, Feelisch M. Nitric oxide modulates sympathetic neurotransmission at the prejunctional level. Microscopy Research and Technique. 1994;**29**:161-168

[58] Schwarz P, Diem R, Dun NJ, Forstermann U. Endogenous and exogenous nitric oxide inhibits norepinephrine release from rat heart sympathetic nerves. Circulation Research. 1995;**77**:841-848

[59] Boerman EM, Segal SS. Depressed perivascular sensory innervation of mouse mesenteric arteries with advanced age. The Journal of Physiology. 2016;**594**:2323-2338

[60] Koyama T, Hatanaka Y, Jin X, Yokomizo A, Fujiwara H, Goda M, Hobara N, Zamami Y, Kitamura Y, Kawasaki H. Altered function of nitrergic nerves inhibiting sympathetic neurotransmission in mesenteric vascular beds of renovascular hypertensive rats. Hypertension Research. 2010;**33**:485-491

[61] Yu M, McAndrew RP, Al-Saghir R, Maier KG, Medhora M, Roman RJ, Jacobs ER. Nitric oxide contributes to 20-HETE-induced relaxation of pulmonary arteries. J Appl Physiol (1985). 2002;**93**:1391-1399

[62] Brassai A, Mako K, Domjanschitz L, Sperlagh B. Lack of prejunctional modulation of noradrenaline release by endogenous nitric oxide in guinea pig pulmonary artery. Neurochemistry International. 2002;**41**:279-283

[63] Vaz-da-Silva MJ, Guimaraes S, Moura D. Adenosine and the endothelium-dependent modulation of 3H-noradrenaline release in the canine pulmonary artery. Naunyn-Schmiedeberg's Archives of Pharmacology. 1995;**352**:640-645

[64] Chen C, Schofield GG. Nitric oxide donors enhanced Ca2+ currents and blocked noradrenaline-induced Ca2+ current inhibition in rat sympathetic neurons. The Journal of Physiology. 1995;**482**(Pt 3):521-531

[65] Martire M, Altobelli D, Cannizzaro C, Preziosi P. Effects of nitric oxide donors on basal and K+−evoked release of [3H]noradrenaline from rat cerebral cortex synaptosomes. European Journal of Pharmacology. 1998;**350**:345-351

[66] Lu Y, Chung HJ, Li Y, Rosenberg PA. NMDA receptor-mediated extracellular adenosine accumulation in rat forebrain neurons in culture is associated with inhibition of adenosine kinase. The European Journal of Neuroscience. 2003;**17**:1213-1222

[67] Ralevic V, Dunn WR. Purinergic transmission in blood vessels. Autonomic Neuroscience. 2015;**191**:48-66

[68] Dunwiddie TV, Masino SA. The role and regulation of adenosine in the central nervous system. Annual Review of Neuroscience. 2001;**24**:31-55

[69] Baldwin SA, Beal PR, Yao SY, King AE, Cass CE, Young JD. The equilibrative nucleoside transporter family, SLC29. Pflügers Archiv. 2004;**447**:735-743

[70] Loffler M, Morote-Garcia JC, Eltzschig SA, Coe IR, Eltzschig HK. Physiological roles of vascular nucleoside transporters. Arteriosclerosis, Thrombosis, and Vascular Biology. 2007;**27**:1004-1013

[71] Sousa JB, Diniz C. The Adenosinergic system as a therapeutic target in the vasculature: New ligands and challenges. Molecules. 2017;**22**

[72] Wiklund NP, Cederqvist B, Gustafsson LE. Adenosine enhancement of adrenergic neuroeffector transmission in guinea-pig pulmonary artery. British Journal of Pharmacology. 1989;**96**:425-433

[73] Zhang GL, Miyahara H, Suzuki H. Inhibitory actions of adenosine differ between ear and mesenteric arteries in the rabbit. Pflügers Archiv. 1989;**415**:56-62

[74] Talaia C, Morato M, Quintas C, Goncalves J, Queiroz G. Functional crosstalk of prejunctional receptors on the modulation of noradrenaline release in mesenteric vessels: A differential study of artery and vein. European Journal of Pharmacology. 2011;**652**:33-39

[75] Rocha-Pereira C, Sousa JB, Vieira-Rocha MS, Fresco P, Goncalves J, Diniz C. Differential inhibition of noradrenaline release mediated by inhibitory a(1)-adenosine receptors in the mesenteric vein and artery from normotensive and hypertensive rats. Neurochemistry International. 2013;**62**:399-405

[76] Rocha-Pereira C, Arribas SM, Fresco P, Gonzalez MC, Goncalves J, Diniz C. Impaired inhibitory function of presynaptic A1-adenosine receptors in SHR mesenteric arteries. Journal of Pharmacological Sciences. 2013;**122**:59-70

[77] Sousa JB, Vieira-Rocha MS, Sa C, Ferreirinha F, Correia-de-Sa P, Fresco P, Diniz C. Lack of endogenous adenosine tonus on sympathetic neurotransmission in spontaneously hypertensive rat mesenteric artery. PLoS One. 2014;**9**:e105540

[78] Stoggall SM, Shaw JS. The coexistence of adenosine A1 and A2 receptors in guinea-pig aorta. European Journal of Pharmacology. 1990;**190**:329-335

[79] Goncalves J, Queiroz G. Purinoceptor modulation of noradrenaline release in rat tail artery: Tonic modulation mediated by inhibitory P2Y- and facilitatory A2A-purinoceptors. British Journal of Pharmacology. 1996;**117**:156-160

[80] Diniz C, Fresco P, Leal S, Goncalves J. Adenosine receptors involved in modulation of noradrenaline release in isolated rat tail artery. European Journal of Pharmacology. 2004;**504**:17-25

[81] Fresco P, Diniz C, Queiroz G, Goncalves J. Release inhibitory receptors activation favours the A2A-adenosine receptor-mediated facilitation of noradrenaline release in isolated rat tail artery. British Journal of Pharmacology. 2002;**136**:230-236

[82] Sangsiri S, Dong H, Swain GM, Galligan JJ, Xu H. Impaired function of prejunctional adenosine A1 receptors expressed by perivascular sympathetic nerves in DOCA-salt hypertensive rats. The Journal of Pharmacology and Experimental Therapeutics. 2013;**345**:32-40

[83] Jackson EK, Cheng D, Tofovic SP, Mi Z. Endogenous adenosine contributes to renal sympathetic neurotransmission via postjunctional A1 receptor-mediated coincident signaling. American Journal of Physiology. Renal Physiology. 2012;**302**:F466-F476

[84] Leal S, Sa C, Goncalves J, Fresco P, Diniz C. Immunohistochemical characterization of adenosine receptors in rat aorta and tail arteries. Microscopy Research and Technique. 2008;**71**:703-709

[85] Ansari HR, Nadeem A, Tilley SL, Mustafa SJ. Involvement of COX-1 in A3 adenosine receptor-mediated contraction through endothelium in mice aorta. American Journal of Physiology. Heart and Circulatory Physiology. 2007;**293**:H3448-H3455

[86] Balligand JL, Kelly RA, Marsden PA, Smith TW, Michel T. Control of cardiac muscle cell function by an endogenous nitric oxide signaling system. Proceedings of the National Academy of Sciences of the United States of America. 1993;**90**:347-351

[87] Conti V, Russomanno G, Corbi G, Izzo V, Vecchione C, Filippelli A. Adrenoreceptors and nitric oxide in the cardiovascular system. Frontiers in Physiology. 2013;**4**:321

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