REVIEW

Purinergic regulation of pulmonary vascular tone

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Abstract

Purinergic signaling is a crucial determinant in the regulation of pulmonary vascular physiology and presents a promising avenue for addressing lung diseases. This intricate signaling system encompasses two primary receptor classes: P1 and P2 receptors. P1 receptors selectively bind adenosine, while P2 receptors exhibit an affinity for ATP, ADP, UTP, and UDP. Functionally, P1 receptors are associated with vasodilation, while P2 receptors mediate vasoconstriction, particularly in basally relaxed vessels, through modulation of intracellular Ca^{2+} levels. The P2X subtype receptors facilitate extracellular Ca^{2+} influx, while the P2Y subtype receptors are linked to endoplasmic reticulum Ca^{2+} release. Notably, the primary receptor responsible for ATP-induced vasoconstriction is P2X1, with α,β-meATP and UDP being identified as potent vasoconstrictor agonists. Interestingly, ATP has been shown to induce endothelium-dependent vasodilation in pre-constricted vessels, associated with nitric oxide (NO) release. In the context of P1 receptors, adenosine stimulation of pulmonary vessels has been unequivocally demonstrated to induce vasodilation, with a clear dependency on the A_{2B} receptor, as evidenced in studies involving guinea pigs and rats. Importantly, evidence strongly suggests that this vasodilation occurs independently of endothelium-mediated mechanisms. Furthermore, studies have revealed variations in the expression of purinergic receptors across different vessel sizes, with reports indicating notably higher expression of $P2Y_1$, $P2Y_2$, and $P2Y_4$ receptors in small pulmonary arteries. While the existing evidence in this area is still emerging, it underscores the urgent need for a comprehensive examination of the specific characteristics of purinergic signaling in the regulation of pulmonary vascular tone, particularly focusing on the disparities observed across different intrapulmonary vessel sizes. Consequently, this review aims to meticulously explore the current evidence regarding the role of purinergic signaling in pulmonary vascular tone regulation, with a specific emphasis on the variations observed in intrapulmonary vessel sizes. This endeavor is critical, as purinergic signaling holds substantial promise in the modulation of vascular tone and in the proactive prevention and treatment of pulmonary vascular diseases.

Keywords Pulmonary circulation · Pulmonary vascular tone · Purinergic agonists · Purinergic receptors · Purinergic signaling

Introduction

To ensure adequate blood perfusion of the alveoli, dynamic and regulated pulmonary vascular tone is necessary and a ubiquitous signaling pathway can be identified in humans and other species: the purinergic signaling pathway. Recent investigations using endogenous and exogenous purinergic receptor agonists and antagonists have expanded the knowledge related to this field. In addition, there is growing evidence concerning the role of purinergic signaling in vascular pathophysiology of lung diseases, such as pulmonary arterial hypertension and chronic obstructive pulmonary disease. This review will address the characteristics of purinergic

signaling and its role in pulmonary vascular dynamics, highlighting the differences between different sizes and types of pulmonary vessels [1–6].

Purinergic signaling pathways

Receptors and ligands

Purinergic receptors (PRs) can be divided into two groups; P1 receptors, which bind adenosine and P2 receptors, which bind nucleotides, such as ATP (adenosine 5′-triphosphate) and ADP (adenosine 5′-diphosphate) [7]. P1 receptors, also known as adenosine (A) receptors, are G protein-coupled receptors [8]. A_1 and A_3 receptors are coupled to G_i protein,

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whereas A_{2A} and A_{2B} receptors are coupled to G_s and G_q proteins, respectively [8]. On the other hand, P2 receptors are divided into P2X and P2Y receptors [8]. P2X receptors are non-selective, monovalent, and divalent cation channels with a preference for Na^+, K^+, and Ca^{2+} [9]. They are activated by ATP and consist of 7 subtypes: P2X1, P2X2, P2X3, P2X4, P2X5, P2X6, and P2X7 receptors [9]. They form trimers and have similarities in their aminoacidic structure ranging from 41 to 55.4% in humans [9]. P2Y receptors are G protein-coupled receptors and there are eight subtypes: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ receptors [10]. P2Y receptors have affinity not just for purine nucleotides but also for pyrimidine (uracil) nucleotides, such as UTP (uridine triphosphate) and UDP (uridine diphosphate) [11, 12].

Ligand metabolism

Regarding purinergic ligands and their effects on the pulmonary circulation, it is important to consider that they are subject to extracellular metabolism because this will determine which receptor and pathway will be activated. Studies have shown that this is conducted by nucleotidase enzymes (ectonucleotidases) in the extracellular medium, changing the available circulating ligands to PRs at the cell membrane. Ectonucleotidases are a family of oligomerizable glycosylated proteins with two transmembrane domains, and they can be grouped into two: ecto-nucleoside triphosphate diphosphohydrolase (e-NTPDase) and ecto-5′-nucleotidase [13]. Conversion of ATP to ADP and ADP to adenosine 5′-monophosphate (AMP) by e-NTPDase has been demonstrated, while adenosine production from AMP by ecto-5′-nucleotidase has been described [14, 15]. For uracil nucleotides (UTP, UDP, UMP) the metabolic pathway is similar although with different effectiveness [13, 16]. Four of the eight existing e-NTPDs have been located on the cell surface with an extracellular catalytic site: e-NTPDase1, e-NTPDase2, e-NTPDase3, and e-NTPDase8 [13, 16]. All of them require Ca^{2+} and Mg^{2+} in the millimolar concentration range to function [13, 16] and to hydrolyze nucleoside triphosphate, including ATP and UTP. In vessels, e-NTPD1 has been described as the main e-NTPD [13, 16], and e-NTPD2 has also been associated with the vascular system, where both e-NTPDases participate in hemostatic regulation [16]. In the lung, ecto-5′-nucleotidase has been observed in capillary endothelium and erythrocytes. Additionally, increased expression of e-NTPDase and ecto-5–nucleotidase mRNA has been observed in human bronchial epithelial cell lines following exposure to cyclic mechanical stress, and increased e-NTPDase1/ecto-5′-nucleotidase mRNA expression and protein levels in the lungs of mechanical ventilated mice have also been observed, where e-NTPDase1/

ecto-5′-nucleotidases were located in the epithelium and pulmonary endothelium [17]. However, there is still little evidence about the distribution of e-NTPDases in the lungs [18].

The interpretation of functional test results should consider the action of e-NTPDases when endogenous purinergic agonists are used, but not with non-hydrolyzable agonists (e.g., α,β-meATP and 2-meSATP). For example, released ATP can induce vasoconstriction by P2X receptors and vasodilation by adenosine, which has been observed as a product of hydrolysis by e-NTPDases [15].

Expression and function of purinergic receptors in regulation the tone of pulmonary vessels

General considerations

The interpretation of studies on pulmonary vascular tone regulation is influenced by experimental heterogeneity. Among the variables that can determine differences in the results are as follows:

1. Vascular segment: To analyze the response of intrapulmonary vessels, these can be divided into vessels of small internal caliber (less than 500 μm approximately) and large internal caliber (greater than 1 mm approximately). Also, vessels can be divided into pulmonary arteries (PA) and pulmonary veins (PV). Thus, four different groups can be analyzed: small pulmonary arteries (SPA), large pulmonary arteries (LPA), small pulmonary veins (SPV), and large pulmonary veins (LPV). This classification has been based on histological and molecular differences between different types of vessels [19–21]. Despite this, most studies have used SPA [21–25] or LPA [20, 21, 24, 26, 27] and a few specific investigations have studied veins of small caliber [28], large caliber [27], or capillaries [29].

2. Experimental model: Differences in experimental results among species have been reported [7]; among them, models derived from rats, rabbits, cats, and guinea pigs are the most frequently used, consequently, leading to heterogeneous experimental designs. Moreover, for the same species, different responses have been reported depending on the experimental model, i.e., perfused lungs, vascular rings, and isolated cells [30]. For example, previous reports have shown different outcomes in studies with arterial rings and patch clamp isolated cells [25], possibly due to the activity of e-NTPDases, which is higher in arterial rings. In the perfused lung model, variables such as the flow rate used [31], the presence

of erythrocytes [32], and the physical barrier that represents the wall of vessels for agonists coming from the lumen to stimulate smooth muscle cells (SMC) may also affect the outcome [29].

3. Desensitization: Receptor desensitization is a biological phenomenon that occurs when a receptor's responsiveness to repeated or continuous stimulation decreases, and the rate of desensitization of purinergic receptors has been reported to vary [25, 33, 34]. For example, the $P2Y_2$ and $P2Y_4$ receptors desensitized faster (minutes) than the $P2Y_6$ receptors (hours) [35–37]. Some P2X receptors can be classified as fast desensitizers (less than 1 s), such as P2X1 and P2X3 receptors, and slow desensitizers (several seconds), such as P2X2, P2X4, P2X5, and P2X7 receptors [9, 38–40]. These differences in P2Y and P2X receptors can determine the outcome in similar experimental conditions.

4. Basal vessel tone: This can be modulated by pathways other than purinergic [41, 42] and determines the response of a vessel to the same agonist.

5. Agonist administration route: Although in perfused lung models, agonists are mainly administered intravascularly, administration by the airway has also been reported as a delivery route, resulting in effects on capillary vessels [29]. In addition, the models based on isolated vessels allow the perfusion with drugs directly reaching the SMC, avoiding physiological barriers, such as endothelial cells (ECs).

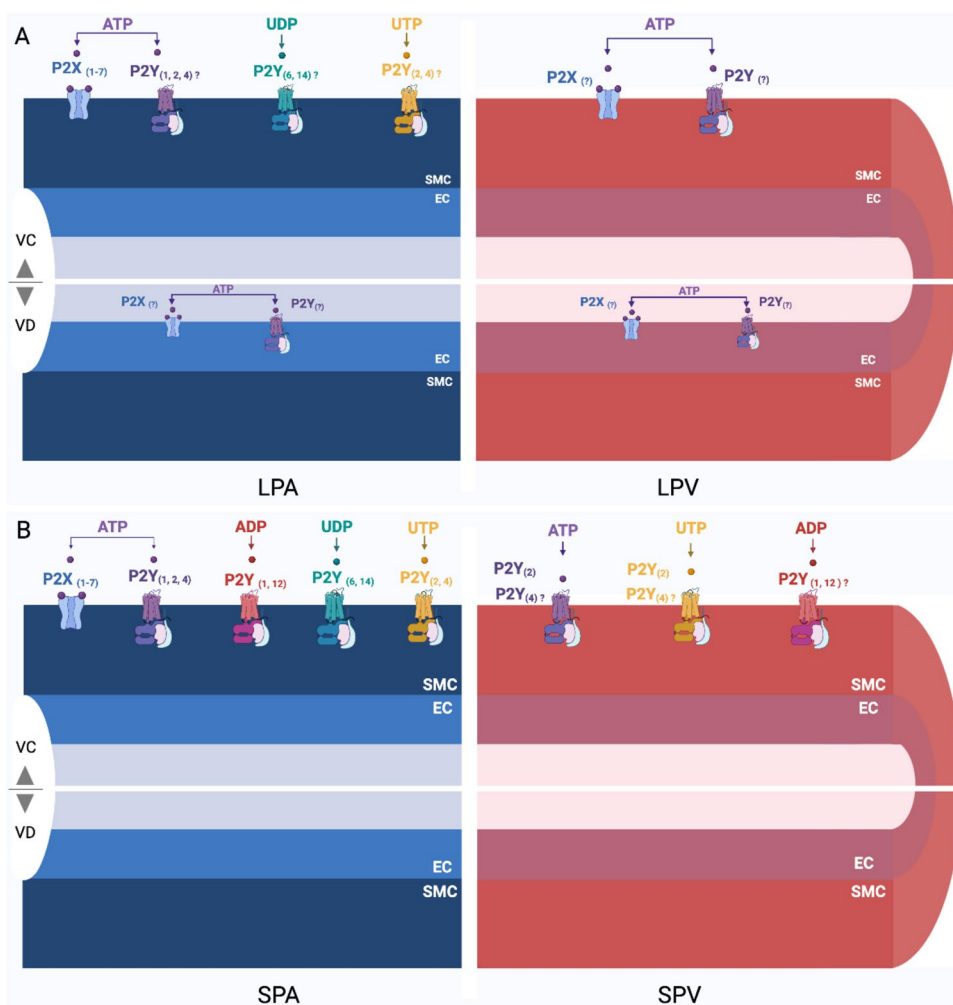## Purinergic receptors in pulmonary vessels

PRs are distributed in the pulmonary vessels differently depending on their caliber. In SPA, higher expression of $P2Y_1$, $P2Y_2$, and $P2Y_4$ receptors has been reported [22]. These receptors have been functional [23], although inhibition of the $P2Y_1$ receptor did not affect the contraction induced by ATP, so this receptor does not seem to be relevant for SPA contraction [22, 23]. In rat SPV, $P2Y_2$ and $P2Y_4$ receptors have been reported, although ATP-induced contraction mainly depended on the $P2Y_2$ receptor [28]. One study showed rat LPA contractions following stimulation with UTP and UDP, both in endothelium-intact and endothelium-denuded vessels, suggesting expression of P2Y receptors in LPA, specifically in SMC [20] (Table 1 and Fig. 1). More studies are required to elucidate the expression of P2Y-specific receptors in each vascular segment.

Regarding the P2X receptors, all receptor subtypes have been reported to be expressed in rat SPA and LPA [22, 24] (Table 1 and Fig. 1), being P2X1 the most expressed subtype in both structures [21, 22, 24]. In addition, the use of P2X1 receptor antagonists has been reported to exert an inhibition of agonist-induced contractions [24]. In the endothelium of mouse lung micro-vessels, P2X4 was the most expressed receptor [43], which resembles humans, where P2X4 receptor mRNA has been described as the most expressed receptor in the endothelium of PA [44–47]. In rat endothelium-denuded PA, the expression of P2X4 receptors has been observed next to P2X1 receptors [24].

Table 1 Expression of P2 receptors according to vascular segment in rat lungs (21, 23, 24, 28, 29, 31, 34)

	LPA	SPA		Capillary	SPV	LPV
mRNA	Smooth muscle cell					
	P2X1 (24)	P2X1 (24, 34)	$P2Y_1$ (23, 34)	-		
	P2X2 (24)	P2X2 (24, 34)	$P2Y_2$ (34)	-		
	P2X3 (24)	P2X3 (24, 34)	$P2Y_4$ (34)	-		
	P2X4 (24)	P2X4 (24, 34)	$P2Y_6$ (23, 31)	-		
	P2X5 (24)	P2X5 (24, 34)	$P2Y_{12}$ (23)	-		
	P2X6 (24)	P2X6 (24)	$P2Y_{13}$ (34)	-		
	P2X7 (24)	P2X7 (24, 34)	$P2Y_{14}$ (34)	-		
Immunofluorescence	Smooth muscle cell					
	P2X1 (24)	P2X1 (24)		-	$P2Y_2$ (28)	
	P2X2 (24)	P2X2 (24)		-		
	P2X4 (24)	P2X4 (24)		-		
	P2X5 (24)	P2X5 (24)		-		
	Smooth muscle cell + endothelial cell					
	P2X1 (21)	P2X1 (21)		$P2Y_2$ (29)		
	P2X3 (21)	P2X4 (21)				
	P2X4 (21)	P2X5 (21)				
	P2X5 (21)	P2X7 (21)				
	P2X7 (21)					

Fig. 1 Schematic representation of the presence of purinergic receptors and respective physiological agonists response in pulmonary vessels. **A** Vasoconstriction or vasodilation has been described in response to ATP in LPA and LPV, while vasoconstriction has been reported to UTP and UDP in LPA. **B** Vasoconstriction has been demonstrated in response to ATP, UTP, UDP, and ADP. Vasodilator response needs further studies in SPA and SPV. In both figures (**A** and **B**), the specific receptors that mediate the response to agonists are mainly unknown. LPA large pulmonary arteries, LPV large pulmonary veins, SPA small pulmonary arteries, SPV small pulmonary veins, SMC smooth muscle cells, EC endothelium, VC vasoconstriction, VD vasodilation. Figure created using BioRender software (21, 23, 24, 28, 29, 31, 34)

No reports have been found of the presence of P2X receptors in PV.

Expression of P1 receptors has also been described in pulmonary vessels, although the evidence only includes human pulmonary vessels. The expression of A_1, A_{2A}, A_{2B}, and A_3 receptors mRNA has been observed in smooth muscle cells isolated from the human pulmonary artery, accordingly with the protein levels of A_{2A} and A_{2B} in healthy individuals. However, this remains unknown for different species [48].

Role of P2 receptor signaling in the regulation of pulmonary vascular tone

ATP is one of the most studied purinergic ligands in the regulation of pulmonary vascular tone. As reported in rats PA and PV, the direct stimulation of SMC with ATP leads to an increase in vascular tone and an increased intracellular Ca^{2+} concentration [28, 22]; similar effects have been observed after the stimulation of EC from PA with ATP in different species [34, 49, 50]. These effects have also been

demonstrated for other P2 receptor agonists, such as UTP and ADP [34, 49, 50].

Ca^{2+} dynamics downstream of P2 receptors

Movement of Ca^{2+} has been related to the type of activated PR. Thus, P2X receptors (ionotropic) have been associated with the entry of Ca^{2+} from the extracellular medium through ion channels [51]. On the other hand, P2Y receptors have been related to the release of Ca^{2+} from the endoplasmic reticulum to the cytosol [52]. The second mechanism induces an increase in Ca^{2+} concentration which can be independent of extracellular Ca^{2+} and that has been recorded as Ca^{2+} oscillations [53].

Continuous stimulation of arteries with ATP for 30–50 s has shown oscillations of intracellular Ca^{2+} concentration with decreasing amplitude, which was slightly decreased but not abolished by the removal of external Ca^{2+} and strongly affected by inhibition of sarcoplasmic reticulum Ca^{2+} pump [53]. Therefore, oscillations of intracellular Ca^{2+} depended on the entry of Ca^{2+} from the extracellular medium via P2X

receptors, followed by release of Ca^{2+} from intracellular reservoirs after activation of P2Y receptors and IP3 formation [53]. The phenomenon has also been induced by UTP, but in this case, all the effects have been attributed to the Ca^{2+} released from the sarcoplasmic reticulum [53].

The role of ATP concentration in Ca^{2+} response has been described in SMC of rat PA. Low ATP concentrations (10–300 nM) only induced a transient response with an increase in intracellular Ca^{2+} concentration, which dropped to baseline values in 20–40 s, while high ATP concentrations (1 μM–1 mM) induced a biphasic response, with a transient response followed by a sustained elevation phase, which was maintained for several minutes [22]. This biphasic response was also induced by ADP, although ATP was more potent [22]. The transient response to low ATP concentrations depended on both P2X receptor-mediated Ca^{2+} mobilization from the extracellular medium and P2Y receptor-mediated Ca^{2+} mobilization from the endoplasmic reticulum [22, 30], whereas the transient response to high ATP concentrations was mainly mediated by P2Y receptors [28, 22, 50, 53]. The sustained response was mediated by P2X and P2Y receptors [22], although one study showed that stimulation with ATP in a Ca^{2+}-free medium was not able to affect the sustained phase [30], suggesting that it depended on P2Y receptors. The biphasic response to the single stimulus was also observed using UTP, where the intracellular Ca^{2+} concentration was increased in a concentration-dependent manner [50].

In the pulmonary vessels, the Ca^{2+} dynamics described above have been reported in bovine EC of PA [34, 48], rabbit SMC, rat SPA and LPA [50], and SMC of rat SPV [28]. A higher amplitude of transient phase in rabbit SPA compared to LPA was found [50], and a greater response to ATP than UTP and UDP was observed in rat SPA [54]. In the SMC of rat SPV, a sustained stimulation with ATP induced a transient increase in intracellular Ca^{2+} concentration, followed by immediate oscillations, which ceased rapidly after ATP withdrawal. This response was not dependent on P2X receptors, but instead P2Y receptors, since IP_3 receptor inhibition led to a single low-amplitude Ca^{2+} signal that quickly returned to baseline values, and phospholipase C-β inhibition blocked the contraction [28]. In the SMC of rat PA, biphasic Ca^{2+} currents were induced by ATP and UTP [54, 55]. Similarly, in SMC of human PA, ATP also induced a biphasic response [56], and the sustained phase in EC was mediated by Ca^{2+} influx via P2X4 receptors [47]. The latter was observed especially following an increase in shear stress and the concomitant ATP release [44].

Studies have shown that P2Y receptors can interact with ion channels [57]. Thus, the activation of both, P2X and P2Y receptors, led to membrane depolarization with the activation of voltage-dependent ion channels, triggering or enhancing muscle contraction, or the production of

vasodilation mediators in the endothelium. In this regard, the participation of the $Ca_v1.2$ channel in the Ca^{2+} entry after activation of P2Y receptors in SMC of rats SPA has been previously observed [33]. Additionally, a study has shown that the SMC contraction involves Cl^- channels activated by Ca^{2+} (CaCC), where the opening of these channels led to membrane depolarization by Cl^- output. This induced the opening of voltage-dependent Ca^{2+} channels and subsequent contraction. The initial stimulus was the increase in intracellular Ca^{2+} concentration induced by an agonist. The opening of CaCC has also been observed after an increase in the concentration of intracellular Ca^{2+} triggered by the release of Ca^{2+} from intracellular reservoirs, which created a spontaneous transient inward currents (STICs, a Ca^{2+}-activated chloride current), leading to a state of increased contractility [58]. Thus, the induction of oscillating input currents of Cl^- after stimulation with ATP, UTP, and UDP has been reported in rat SMC of SPA and LPA, which were dependent on the release of Ca^{2+} from the sarcoplasmic reticulum [54].

Vascular dynamics in response to the stimulation of P2 receptor

In experiments of vascular functional dynamics, the rat has been the most studied species, followed by rabbit, cat, human, and guinea pig. The effect of purinergic agonists on P2 receptors usually has been vasoconstriction if the vessel is basally relaxed, or vasodilation if it is previously constricted [27]. Predominantly, vasoconstriction depends on the agonist action on SMC, and the relaxation depends on the action on EC [12].

In rats, the most potent vasoconstrictor described in SPA with intact endothelium (E+), SPA with endothelium-denuded (E-), LPA E-, and LPV E- has been α,β-meATP, a non-selective P2 agonist [38, 59] (Table 2 and Fig. 1). In LPA E+, UDP has shown the highest vasoconstrictor potency [20]. In SPV E+, ATP-γ-S has shown the highest vasoconstrictor potency [28], although α,β-meATP effect on these vessels has not been described. ATP has shown greater potency in SPA than LPA [20], similar to UDP which had a slightly higher potency in SPA than LPA, although in LPA E+, studies have observed differences in UDP potency [20, 26]. UTP has shown a high potency in LPA E- and SPA, except for LPA E+, where a low potency is observed [20, 26]. The agonist with the lowest vasoconstrictor potency reported has been ADP in SPA/LPA E+ and SPA/LPA E- compared to α,β-meATP, UDP, UTP, ATP, and 2-meSATP [20, 28].

Conversely, in pre-constricted rat lung vessels, ATP has been able to induce endothelium-dependent vasodilation [26], while UTP and UDP seem to have no effects in these vessels [26] (Table 2 and Fig. 1). This is more prominent in arteries than in veins [42, 60], and it was observed

Table 2 Purinergic agonist potency for vasoconstriction or vasodilation according to vascular segment in rat lungs (20, 26, 27, 28)

	Potency	LPA		SPA		SPV		LPV	
		E+[a]	E-	E+	E-	E+	E-	E+	E-
Vasoconstriction	Higher potency			α,β-meATP (20)	α,β-meATP (20)				
			α,β-meATP [0.5 μM][b] (20, 27) UTP (20)	UTP (20) ATP (20) 2-meSATP (20)	UDP (20) UTP (20) ATP (20) 2-meSATP (20)				α,β-meATP [0.1 μM] (27)
		UDP (20)	β,γ-meATP [2.8 μM] (27) UDP (20)	UDP (20)		ATPγ-S [2.5 μM] (28)			
			2-meSATP [17.8 μM] (20, 27)			UTP [16,4 μM] (28) ATP [28.5 μM] (28)			2-meSATP [58.9 μM] (27) β,γ-meATP [95.4 μM] (27)
			ATP [169.8 μM] (20, 27)			ADP [117 μM] (28)			ATP [245.5 μM] (27)
		α,β-meATP (20) UDP (26) UTP (20, 26)		ADP (20)	ADP (20)				
	Lower potency/no effect	ATP (20, 26) 2-meSATP (20) [ADP] (20)	[ADP] (20)						
Vasodilation	Higher potency	2-meSATP [0.04 μM] (27) ATP [0.4 μM] (27) β,γ-meATP [0.9 μM] (27)							
								2-meSATP [1.6 μM] (27)	
		α,β-meATP [0.1 μM] (27)						ATP [16.6 μM] (27) β,γ-meATP [55.0 μM] (27)	
	Lower potency/no effect	UTP (26) UDP (26)						α,β-meATP (27)	

[a] Abbreviations: *E+* presence of endothelium, *E-* absence of endothelium

[b] EC_{50} or EC_{40} is shown in brackets when data was available

following pre-constriction using different stimuli, such as hypoxia [42], thromboxane A2 receptor agonists [41, 42, 60], and sympathomimetics such as methoxamine [26], phenylephrine [27], and serotonin [27]. In rabbit models, endothelium-dependent vasodilation in pre-constricted LPA using the alpha-1 adrenergic agonist, phenylephrine [50], or with U-46619 [32] has been observed with UTP stimulus [32, 50]. Additionally, vasodilation has been recorded in endothelium-denuded and pre-constricted PA of rabbits in response to ATP and 2-meSATP, suggesting

a SMC intrinsic vasodilation mechanism [50]. In rats, a greater ATP-induced vasodilation has been demonstrated in endothelium-intact arteries compared to endothelium-denuded, while vasodilation induced by 2-meSATP has been similar in both situations [50]. Strikingly, in pre-constricted LPV E + , α,β-meATP induced greater vasoconstriction rather than vasodilation [61].

In models of human endothelium-denuded SPA, the order of vasoconstrictor potency was described as follows: α,β-meATP = β,γ-meATP > ATP > 2-meSATP. On the other hand, in endothelium-intact SPA and pre-constricted with prostaglandin F_{2a}, the vasodilator potency follows a slightly different order: 2-meSATP > ATP > > β,γ-meATP = α,β-meATP [62]. However, the removal of the endothelium did not modify the vasodilator response in SPA to ATP and ATP analogs; therefore, a SMC intrinsic vasodilation mechanism has been suggested in humans [62]. This has also been reported in LPA, where relaxation of LPA induced by ATP partially decreased with the removal of endothelium [62, 63]. On the other hand, vasodilation in response to ADP was described in arterial rings pre-constricted with adrenaline; however, this was abolished in endothelium-denuded PA and was independent of prostaglandin production [64].

A major mechanism that mediates endothelium-dependent vasodilation is the release of nitric oxide (NO) [65]. In this sense, studies have described a reduction in vasodilation induced by ATP and 2-meSATP in previously constricted rat pulmonary artery and a reduction in vasodilation induced by ATP and UTP in previously constricted rabbit pulmonary artery, after treatment with L-NAME, a nitric oxide synthesis inhibitor [41, 42, 50]. On the other hand, the release of prostacyclin (PGI_2) from EC after perfusion with ATP and UTP was reported for the bovine pulmonary artery [49], although the functional impact of this finding has not been evaluated. In addition, endothelium-dependent hyperpolarization has been proposed as an alternative mechanism of vasodilation [66]. Within this context, a study reported that endothelium-dependent hyperpolarization was activated by $P2Y_2$ receptors and mediated by K^+ channels, myoendothelial gap junctions, and connexins 37 and 40 [66]. Another mechanism of vasodilation, the intrinsic mechanism of vascular SMC relaxation, has been suggested [67, 68]. This has been supported by experiments where the removal of endothelium did not change the vasodilator response to ATP and ATP analogs [67, 68].

Regarding the specific P2 receptor involved in the regulation of vascular tone, P2X1 receptor has been described as the main mediator of vasoconstriction induced by ATP in rat SPA [69] and LPA [24]. $P2Y_{12}$ receptor may also have a role in vascular tone control. This has been suggested by experiments that observed a reduced contraction in response to ATP when rat intrapulmonary artery is treated with cangrelor, a $P2Y_{12}$ antagonist [22, 23].

Combined exposure to $P2Y_{12}$ and P2X1 receptor antagonists abolished vasoconstriction induced by ATP [23]. $P2Y_1$ receptor was present and functional in studies using rat SPA; however, they did not seem to be involved in vascular tone regulation [22, 23]. In veins, a study reported that vasoconstriction of rat SPV was mediated by $P2Y_2$ receptors, based on the inhibitory effect observed by selective antagonists, the affinity of the agonists, and receptor expression in the tissue [28].

Role of P1 receptor signaling in the regulation of pulmonary vascular tone

Conversion of ATP, which is released by stressed, apoptotic, and/or necrotic cells, to adenosine is performed by ecto-5′-mucleotidase, and activation of adenosine receptors allows biological effects of adenosine. The balance between ATP and ADP determines the final effect. In this sense, studies have observed that the regulation of adenosine signaling depends on ATP release, enzymatic conversion, expression of adenosine receptors, and termination of extracellular adenosine signaling by equilibrative nucleoside transporters (ENTs) [70–72].

In guinea pig vessels pre-constricted with noradrenaline, adenosine presents a biphasic action consisting of a fast constrictor response[73, 74] and a slow relaxation phase [74]. The vasoconstrictor response was inhibited by a selective A_1 receptor antagonist and an A_1 receptor agonist-induced vasoconstriction, suggesting that the smooth muscle contraction phase is A_1 receptor-dependent [74]. On the other hand, the vasodilator response was inhibited by A_1 and A_2 receptor antagonists, in the presence of complete A_1 receptor blockade by a selective antagonist and remained present after the addition of the selective A_{2A} receptor antagonist, CP66713, after endothelium removal and in the presence of NO synthase inhibitor, L-NOARG [74]. This suggests that vasodilation is A_{2B} receptor-dependent and A_{2A} receptor-independent, with no participation of an endothelium-mediated mechanism or production of NO [74].

In rats, a dose-dependent vasodilator response to adenosine has been described in perfused lungs and in PA pre-constricted with norepinephrine. In endothelium-intact arterial rings, 10^{-6} M adenosine induced an increase in tension; however, vasodilation was observed if the concentration was increased to 10^{-4} M [75]. In endothelium-denuded arterial rings, the addition of adenosine induced vasodilation [75]. Similarly, adenosine induced vasodilation in perfused lungs where previous vasoconstriction was induced by hypoxia [76]. This process was mediated by A_{2B} receptors and independent of NO generation [76].

A vasodilator response to adenosine has also been reported in rabbit PA pre-constricted with the thromboxane

mimetic U-46619 [77, 78], phenylephrine [78], serotonin [79], and acetylcholine [79]. Interestingly, exposure to adenosine receptor antagonists increased the contraction force induced by phenylephrine [78]. The adenosine vasodilator response was partially dependent on the endothelium in a study using rabbit PA, since vasodilation induced by adenosine was attenuated in endothelium-denuded arteries [78]. The effect of adenosine was partially mediated by NO, since arteries pre-treated with a nitric oxide synthase inhibitor shifted rightward the adenosine concentration–response curve [78]. A greater vasodilator response induced by adenosine has been reported in endothelium-denuded vessels, possibly because this can decrease the release of endothelium-derived constrictor factors [79]. Additionally, the action of adenosine could be mediated by cyclic guanidine monophosphate (cGMP), since its vasodilator effect was inhibited by methylene blue, a guanylyl cyclase inhibitor [79].

Vasodilation after stimulation of ventilated dogs with adenosine administered by infusion has been observed [80]. However, vasoconstriction induced by adenosine administered by infusion was reported in sheep, an effect that was associated with an increase in plasma levels of thromboxane A_2 [81].

Vasoconstriction induced by infusion of adenosine under low resting tone conditions in intact-chest cats has been reported [82–84], and this may depend on A_1 receptors [84], cyclooxygenase activity [82–84], and thromboxane receptors [82, 83]. On the other hand, a vasodilator effect of adenosine has also been reported in vessels pre-constricted with U-46619 [82, 83], which appears to involve A_2 receptors [84], but not NO, guanylyl cyclase nor K^+_{ATP} channels [84]. ATP also induced vasoconstriction in the same experiment, which may partly be due to its degradation to adenosine, since A_1 antagonists inhibited the ATP effect [83], and this inhibition was less when the degradation was blocked [83].

In human PA pre-constricted with serotonin, vasodilation induced by adenosine has been observed, with no significant differences between endothelium-denuded and endothelium-intact vessels, nor between SPA (200–400 μm internal diameter) and LPA (7–10 mm internal diameter) [85], suggesting that the vasodilation was not mediated by the release of relaxant factors from endothelial cells. In male subjects, intravenous administration of adenosine reduced pulmonary vascular resistance and mean pulmonary arterial pressure, without affecting systemic circulation [86]. No changes in arterial O_2 partial pressure were found in this study, so it is suggested that adenosine-induced vasodilation does not significantly affect the ventilation-perfusion ratio [86]. In conscious subjects, a decreased pulmonary vascular resistance has been reported after a low dose of adenosine, with no hemodynamic effects on systemic circulation, while the administration of a high dose of adenosine reduced both pulmonary and systemic vascular resistance. It is worth mentioning that this study reported an increase in the end pressure of the left ventricular diastole, pulmonary capillary pressure, and mean pulmonary arterial pressure [87].

Innervation and purinergic regulation

Simultaneous release of ATP and catecholamines at sympathetic nerve terminals of pulmonary vessels has been reported in rabbits [88, 89]. A study observed a reduction in contractions of rabbit pulmonary artery induced by field stimulation when the artery was treated with ATP and ADP [90]. In this sense, in fixed blood vessels of rats, clusters of P2X1 and P2X2 receptors in SMC were colocalized with nerve varicosities [91]. On the other hand, in rings of guinea pig pulmonary artery, a vasoconstrictor effect of adenosine and P_1 receptor agonists has been reported, which potentiated the vasoconstriction in response to nerve stimulation or noradrenaline, and this effect was mediated by postsynaptic receptors [74]. Thus, in the neuromuscular synapses, there are regulatory mechanisms where purinergic signaling seems to participate, and this determines the physiological response to the stimulus of neurotransmitters.

Perspectives of purinergic signaling in pulmonary vascular disease

In the pulmonary circulation, purinergic signaling could play an important role in regulating vascular tone and preventing the development of pulmonary vascular diseases (PVD). For example, adenosine released from endothelial cells can activate A_{2A} receptors on smooth muscle cells, leading to vasodilation and decrease in pulmonary vascular resistance [92]. ATP released from platelets and other cells can activate $P2Y_1$ receptors on smooth muscle cells, leading to vasoconstriction [23]. However, purinergic signaling is often dysregulated in patients with PVD. For example, an increase in ATP release or a decrease in ecto-nucleotidase activity may lead to increased levels of extracellular ATP [93–95]. This can trigger P2 receptors on smooth muscle cells, inducing vasoconstriction and the development of pulmonary hypertension. In addition, purinergic signaling may also play a role in the inflammation which is associated with PVD. For example, ATP released from immune cells can activate P2X7 receptors on endothelial cells, leading to the release of pro-inflammatory cytokines. These cytokines could then further promote vascular remodeling and progression of PVD [96]. Adenosine and adenosine receptors, expressed in the epithelium and endothelium of different organs, have also been associated with inflammatory diseases and hypoxia, which have been shown to enhance the production and signaling effects of adenosine [97, 98]. While the

potential therapeutic benefits of targeting purinergic signaling in PVD are promising, further research is needed to fully understand this pathway and to develop safe and effective therapies. Some key research areas are as follows: specific mechanisms of purinergic signaling deregulation in PVD, the role of purinergic signaling in the development and progression of PVD, and the efficacy and safety of drugs that target purinergic signaling in PVD.

Conclusions

Research on the purinergic pathway is moving forward. Recent investigations describing endogenous and exogenous PR agonists and antagonists have been published [91, 92, 96, 99–102], expanding the knowledge in this field. In addition, there is growing evidence concerning the role of purinergic signaling in the vascular pathophysiology of lung diseases, such as pulmonary arterial hypertension [93, 94, 103–105] and chronic obstructive pulmonary disease [106, 107]. Remarkably, purinergic agonists can induce different responses depending on the previous condition of the tissue; thus, the use of compounds that target purinergic receptors as regulators of pulmonary vascular tone represents a challenge.

The current research efforts predominantly concentrate on arteries, with limited attention given to venous segments and distal circulation within the pulmonary vasculature. This imbalance underscores the critical need for studies that specifically address venous segments and small vessels to gain a comprehensive understanding of pulmonary vascular function and regulation. Additionally, there is a scarcity of evidence regarding vasodilation following different mechanisms of pre-contraction. Therefore, further research is essential to elucidate the purinergic mechanisms involved in regulating pulmonary vascular tone and to determine the contribution of purinergic signaling to pulmonary vascular diseases, such as pulmonary hypertension.

Declarations

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