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Review

Pharmacological Profile of Purinergic P2Y Receptors Involved in Neurogenic Cardiovascular Modulation Induced by ADP and Related Compounds in Rats

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Abstract

Cardiovascular modulation mediated by adenine-based purines is a very complicated and dynamic process driven by adenosine, adenosine diphosphate (ADP), and adenosine triphosphate (ATP). It is orchestrated through the interaction of these molecules with specific purinergic P1, P2Y, and P2X receptors, located throughout the central and peripheral nervous systems. This, along with the constant enzymatic interconversion of these compounds by ectonucleotidases, results in overlapping and highly variable cardiovascular responses. Activation of G protein-coupled purinergic P2Y receptors plays a fundamental role in cardiovascular homeostasis, modulating autonomic integration, neurogenic transmission, and vascular function. Within this context, ADP-sensitive P2Y receptors, particularly the $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$ subtypes, participate in distinct sensory, autonomic, and functional cardiovascular mechanisms, as identified in several *in vitro* and *in vivo* experimental models. Accordingly: (i) the stable and non-hydrolysable ADP analogue, adenosine 5′-O-(β-thio)-diphosphate (ADPβS), has been widely used as a pharmacological tool to investigate P2Y receptor-mediated cardiovascular responses; and (ii) the specific role of P2Y receptors has been confirmed using selective P2Y receptor antagonists, including MRS2500 ($P2Y_1$), PSB0739 ($P2Y_{12}$) or MRS2211 ($P2Y_{13}$). The cardiovascular effects of ADP are complex; depending on the experimental conditions, it can either decrease or increase heart rate, myocardial contractility, vascular tone, and/or systemic blood pressure. These effects are mediated by several P2Y receptors that act at different levels of cardiovascular control, encompassing crucial integration sites within the central nervous system, as well as peripheral autonomic and cardiovascular effector mechanisms such as preganglionic nerves, autonomic ganglia, postganglionic nerves, sensory fibres, vascular smooth muscle, vascular endothelium, and cardiac tissues. In this review, we: (i) summarize the pharmacological profile of the P2Y receptors that mediate cardiovascular responses, with special emphasis on peripheral autonomic, sensory, and vascular mechanisms, revealed in *in vivo* experimental models; and (ii) highlight the potential of P2Y receptors as therapeutic targets for the treatment of cardiovascular pathologies, including hypertension, coronary vasospasm, ischemic injury, and coagulation problems.

Keywords: adenosine diphosphate (ADP); autonomic nervous system; blood pressure regulation; neurogenic control; sensory vasodilatation; purinergic P2Y receptors

1. Introduction

In recent decades, purinergic signalling has become established as a key modulatory system in cardiovascular physiology, integrating endothelial, autonomic, and sensory mechanisms that, together, contribute to the maintenance of arterial blood pressure and vascular homeostasis [1]. This system operates through extracellular nucleotides such as adenosine diphosphate (ADP) and adenosine triphosphate (ATP), which act on three major families of purinergic receptors, namely, P1, P2Y, and P2X.

P1 receptors are G protein-coupled receptors (GPCRs) activated by adenosine, of which four subtypes are known, namely: A_1 (G_i-coupled), A_{2A} (G_s-coupled), A_{2B} (G_s-coupled), and A_3 (G_i-coupled) receptors. Interestingly, A_{2B} and A_3 receptors may also be coupled to G_q [2]. These receptors modulate cardiovascular and neural function [3].

P2Y receptors are metabotropic GPCRs and include eight subtypes ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$), which are activated by ATP, ADP, uridine triphosphate (UTP), uridine diphosphate (UDP) or UDP-glucose, in a subtype-specific manner [4]. $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$ are G_q-coupled receptors ($P2Y_{11}$ may also be a G_s-coupled receptor); while $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ are $G_{i/o}$ coupled receptors [5]. These

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receptors, which are functional in mammals, play critical roles in the modulation of autonomic neurotransmission, vascular tone, platelet function, and endothelial signalling [6]. It is worthy of note that the $P2Y_3$ receptor has been cloned only in chickens, but not in humans or other mammals, and displays some homology with the human $P2Y_6$ receptor [7]. The $P2Y_5$, $P2Y_7$, $P2Y_9$, and $P2Y_{10}$ receptors have also been cloned and show some sequence homology. However, there is some doubt as to whether these receptors are nucleotide receptors [4].

In contrast, P2X receptors are ligand-gated cation channels, directly activated by ATP, and consist of seven subtypes ($P2X_1$-$P2X_7$), which mediate rapid excitatory responses in neurons, vascular smooth muscle, and immune cells, and contribute to fast synaptic transmission and neurogenic vascular modulation [1,2,8,9,10,11].

Within this receptor system, P2Y receptors mediate slower but finely regulated responses and play a prominent role in the modulation of vascular tone, cardiac output and autonomic balance [12]. Among these, ADP-sensitive P2Y receptor subtypes (particularly $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$) have attracted increasing attention due to their involvement in prejunctional modulation of autonomic neurotransmitter release and in sensory neurogenic mechanisms, including those mediated by calcitonin gene-related peptide (CGRP) [13,14,15,16].

An important aspect in interpreting purinergic actions on cardiovascular modulation concerns the accessibility of circulating nucleotides to the central nervous system (CNS). Under physiological conditions, the blood-brain barrier (BBB) effectively restricts the entry of highly charged hydrophilic molecules, such as ATP and ADP, thus limiting their direct central availability [9]. There is no evidence for specific transport mechanisms that allow nucleoside diphosphates to cross the BBB, and extracellular nucleotides in the cerebral circulation are rapidly degraded by endothelial ectonucleotidases [17]. Consequently, central purinergic signalling reflects locally generated extracellular nucleotides rather than direct actions of blood-borne purines.

Purinergic signalling within the CNS plays an active role in autonomic integration and in the modulation of sympathetic and parasympathetic outflows. Several brain regions involved in cardiovascular control, including the paraventricular nucleus of the hypothalamus (PVN), the nucleus tractus solitarii (NTS), and the rostral ventrolateral medulla (RVLM), utilize extracellular nucleotides as intrasynaptic and extrasynaptic modulators of neuronal activity [1,18]. In these regions, ATP has been the most studied central purinergic transmitter; however, its rapid extracellular degradation suggests that ADP may also act as a physiologically relevant mediator within autonomic circuits and at peripheral neurovascular junctions.

Beyond the CNS, purinergic mechanisms contribute importantly to peripheral autonomic and vascular modulation. At peripheral sites, extracellular nucleotides modulate the release of neurotransmitters and neuropeptides, influence sympathetic-sensory interactions, and directly regulate vascular and cardiac function [1,19]. The extracellular metabolism of ATP to ADP, adenosine monophosphate (AMP), and adenosine by ectonucleotidases such as CD39 (ectonucleoside triphosphate diphosphohydrolase 1 or ENTPD1) and CD73 (ecto-5′-nucleotidase) represents a key determinant of the magnitude, duration, and spatial localization of purine-mediated cardiovascular effects [18,20].

Overall, these observations indicate that purinergic signalling contributes to cardiovascular modulation at multiple levels of autonomic control and through distinct receptor-dependent mechanisms. However, the functional organization through which ATP and ADP coordinate central autonomic integration, peripheral autonomic transmission, and neurovascular effector responses are not yet completely defined. In particular, the specific contribution of individual ADP-sensitive P2Y receptor subtypes to neurogenic vasodepressor and vasopressor responses requires experimental approaches capable of isolating peripheral effector mechanisms without interference from central reflex integration. In this context, the pithed rat model represents a valuable experimental tool, as it abolishes supraspinal baroreflex control while preserving peripheral autonomic and sensory nerve function [21,22,23]. Studies using this preparation have demonstrated that the stable and non-hydrolysable ADP analogue adenosine 5′-O-(β-thio)-diphosphate (ADPβS) elicits complex cardiovascular responses [22], involving both sensory [16] and sympathetic [14] neurogenic components, making this model particularly well-suited for dissecting peripheral P2Y receptor-mediated mechanisms [23].

Accordingly, the present review aims to analyse the pharmacological characteristics and functional relevance of the P2Y receptor subtypes involved in the cardiovascular responses induced by ADP and ADPβS, integrating evidence from central autonomic modulation with peripheral neurogenic and vascular mechanisms. To facilitate this analysis, the following section introduces a hierarchical framework for purinergic signalling in cardiovascular control, which serves as the conceptual basis for the mechanistic and pharmacological discussions that follow. It is worth noting that this review focuses mainly on findings obtained from experimental rat models, including awake, anaesthetised, and pithed rats (see Table 1, Ref. [14,24,25,26,27,28,29,30,31]); however, it is recognized that the characterization of most functional mechanisms mediated by P2Y receptors has been obtained from a variety of mammals, including rodents, humans, and other species.

Table 1. Identified P2Y receptor subtypes and purinergic receptor-mediated signalling involved in central and peripheral cardiovascular control.

Receptor subtype	Anatomical site	Experimental model/species	Identification/functional method	Proposed cardiovascular role	Original reference
$P2Y_{12}$ (predominantly)/$P2Y_{13}$	Cardiac sympathetic pathway	Pithed male Wistar rat	ADPβS + selective antagonists: PSB0739 for $P2Y_{12}$, MRS2211 for $P2Y_{13}$, and MRS2500 for $P2Y_1$	Inhibition of the cardioaccelerator sympathetic drive; mainly $P2Y_{12}$ and probably, to a lesser extent, $P2Y_{13}$	[14]
$P2Y_1$ (ADP-mediated)/$P2Y_2$ (UTP-mediated)	Vascular endothelium	Rat mesenteric arterial bed	Pharmacological + NO/cGMP vascular assays	Endothelium-dependent vasorelaxation	[26]
$P2Y_1$	NTS, ventrolateral medulla and nodose ganglia	Rat and human nodose ganglia; rat brainstem	Immunohistochemistry, autoradiography and radioligand displacement assays	Purinergic signalling in vagal and brainstem regions associated with autonomic cardiovascular regulation	[27]
$P2Y_{12}$	Adrenal chromaffin cells	Rat adrenal medullary slices	Immunohistochemistry, Ca^{2+} imaging, ADP pharmacology, AZD1283 antagonism	Modulation of chromaffin cell excitability and secretion of catecholamines	[28]
$P2Y_2$	Primary sensory neurons	Rat DRG and trigeminal sensory neurons	*In situ* hybridization for *P2ry2* mRNA, CREB phosphorylation, electrophysiology, ATP/UTP pharmacological studies	Sensory purinergic signalling; purinergic sensory signalling associated with neurogenic vascular modulation	[29]
$P2Y_1$	C1 neurons of the RVLM	Adult Sprague-Dawley rats	RVLM microinjection of MRS2365 and MRS2179, sympathetic nerve activity recordings, arterial pressure recordings, phrenic nerve activity recordings, immunohistochemistry for tyrosine hydroxylase, and C1-lesion experiments	Modulation of peripheral chemoreceptor-evoked sympathetic activity and arterial blood pressure	[25]
$P2Y_2$ (UTP-mediated), $P2Y_6$ (UDP-mediated), and $P2Y_1$ (ADP-mediated)	Rat intrapulmonary artery endothelium	Rat intrapulmonary artery rings	Wire myography, endothelium removal, subtype-selective antagonists (AR-C118925XX, MRS2578, MRS2179, cangrelor), RT-PCR and vascular pharmacology	Endothelium-dependent pulmonary vasodilatation mediated selectively by distinct nucleotide-sensitive P2Y receptor subtypes	[30]
Functional purinergic evidence					
Purinergic receptor-mediated signalling (receptor subtype not fully characterized)	NTS	Awake rats	ATP microinjection and cardiovascular recordings	Modulation of cardiovascular reflex responses	[24]
Purinergic receptor-subtype signalling (not characterized)	Commissural NTS	Awake rats and working heart-brainstem preparation	ATP microinjection, glutamatergic antagonism, electrophysiology and cardiovascular recordings	Neurotransmission of the sympathoexcitatory component of the peripheral chemoreflex	[31]

Note.

Abbreviations: ADP, adenosine diphosphate; UTP, uridine triphosphate; UDP, uridine diphosphate; ATP, adenosine triphosphate; ADPβS, ADP analogue adenosine 5′-O-(β-thio)-diphosphate; NO, nitric oxide; cGMP, cyclic guanosine monophosphate; CREB, cAMP response element-binding protein; RVLM, rostral ventrolateral medulla; NTS, nucleus tractus solitarii; DRG, dorsal root ganglion; RT-PCR, reverse transcription polymerase chain reaction. In several central autonomic studies, purinergic signalling was characterized by using ATP-sensitive responses and non-selective P2 receptor antagonists; therefore, definitive pharmacological discrimination between P2X and P2Y receptor subtypes remains limited.

2. Purinergic Signalling in Cardiovascular Modulation: A Hierarchical Framework

To integrate the diverse and sometimes opposing cardiovascular effects of purines, purinergic signalling can be organized into a hierarchical framework that ranges from central autonomic integration to peripheral neurovascular effector mechanisms. Within this framework, extracellular nucleotides act at different anatomical and functional levels of cardiovascular control, and their effects depend on the receptor subtype, cellular localization, and organization of autonomic pathways rather than a single dominant site of action [1,17].

A key organizing principle of this framework is the sequential signalling role of ATP and its metabolites. ATP primarily mediates fast excitatory transmission through activation of ionotropic P2X receptors, while its extracellular degradation generates ADP, which preferentially binds to metabotropic P2Y receptors and exerts slower modulatory effects, such as negative feedback mechanisms. This temporal and functional separation allows purinergic signalling to fine-tune autonomic transmission and cardiovascular responses across multiple levels of control [13,32]. These levels of control can encompass the CNS, preganglionic neurons, autonomic ganglia, the adrenal medulla, postganglionic neurons, sensory neurons, and cardiovascular effectors (see Fig. 1).

At peripheral neuroeffector junctions, prejunctional G_q-coupled $P2Y_1$ receptors inhibit CGRP release from perivascular sensory neurons (C fibres) and, consequently, attenuate CGRP-mediated vasodepressor responses; whereas G_i-coupled $P2Y_{12}$ and $P2Y_{13}$ receptors inhibit noradrenaline release from postganglionic cardiac sympathetic neurons resulting in attenuation of sympathetic cardioaccelerator activity. Blood vessels and the heart represent the final effector targets that integrate these purinergic mechanisms (Fig. 1).

Purinergic signalling is increasingly viewed as a modulatory system operating in restricted extracellular microdomains, where it adjusts synaptic efficacy rather than acting as a primary fast neurotransmitter [33].

2.1 Central Autonomic Level

At the central level, purinergic signalling contributes to the integration of cardiovascular control within autonomic nuclei of the brainstem and hypothalamus (see Fig. 1). In these regions, locally released ATP and its metabolites modulate neuronal excitability and synaptic integration, thereby influencing sympathetic and parasympathetic outflow. In view that circulating nucleotides do not cross the BBB under physiological conditions, central purinergic actions reflect intrinsic nucleotide release within autonomic circuits rather than direct effects of blood-borne ATP or ADP [17,34].

Notwithstanding, BBB permeability may become altered under pathological conditions such as hypertension, ischemia, neuroinflammation, or cerebrovascular injury, potentially facilitating increased access of circulating mediators to central autonomic regions [34,35,36]. Experimental evidence indicates that hypertension may disrupt BBB integrity and permit the entry of circulating molecules into hypothalamic and brainstem autonomic nuclei involved in cardiovascular regulation [35]. Similarly, ischemic injury is associated with increased BBB permeability and alterations in tight junction organization, which may facilitate abnormal neurovascular exchange between the peripheral circulation and the CNS [36]. Under such pathological conditions, peripheral nucleotides or purinergic metabolites could potentially contribute more directly to central purinergic signalling. Nevertheless, the experimental evidence discussed throughout the present review primarily derives from controlled experimental models designed to differentiate peripheral from central purinergic mechanisms, particularly reflex-independent preparations such as the pithed rat model.

Although ATP has been more extensively characterized as a central purinergic transmitter, its rapid extracellular conversion suggests that ADP may also contribute to tonic and phasic modulation of central autonomic networks via metabotropic P2Y receptors. As depicted in Fig. 1, these central mechanisms provide the initial level of purinergic modulation that shapes autonomic commands transmitted to peripheral effector pathways [1,18].

2.2 Autonomic Ganglia: The Preganglionic-Postganglionic Interface

A second critical level of purinergic modulation is located in the autonomic ganglia, which function as the interface between central autonomic commands and peripheral neuroeffector mechanisms. In both sympathetic and parasympathetic ganglia, ATP is released from preganglionic nerve terminals and acts as a cotransmitter alongside acetylcholine. At this synaptic level, ATP contributes to fast excitatory transmission via P2X receptors expressed on postganglionic neurons, thereby enhancing the reliability and temporal precision of autonomic signal transmission [32,37,38].

Following its release, ATP within the ganglionic synaptic cleft is rapidly degraded to ADP by ectonucleotidases. In contrast to ATP-mediated fast excitation, ADP activates metabotropic P2Y receptors and modulates ganglionic transmission over longer timescales. Through this mechanism, ADP can fine-tune autonomic output by regulating neuronal excitability and synaptic efficacy before signals reach peripheral target tissues [13,19]. This dual ATP-ADP organization highlights the role of autonomic ganglia as an active modulatory checkpoint rather than a simple relay station in cardiovascular control.

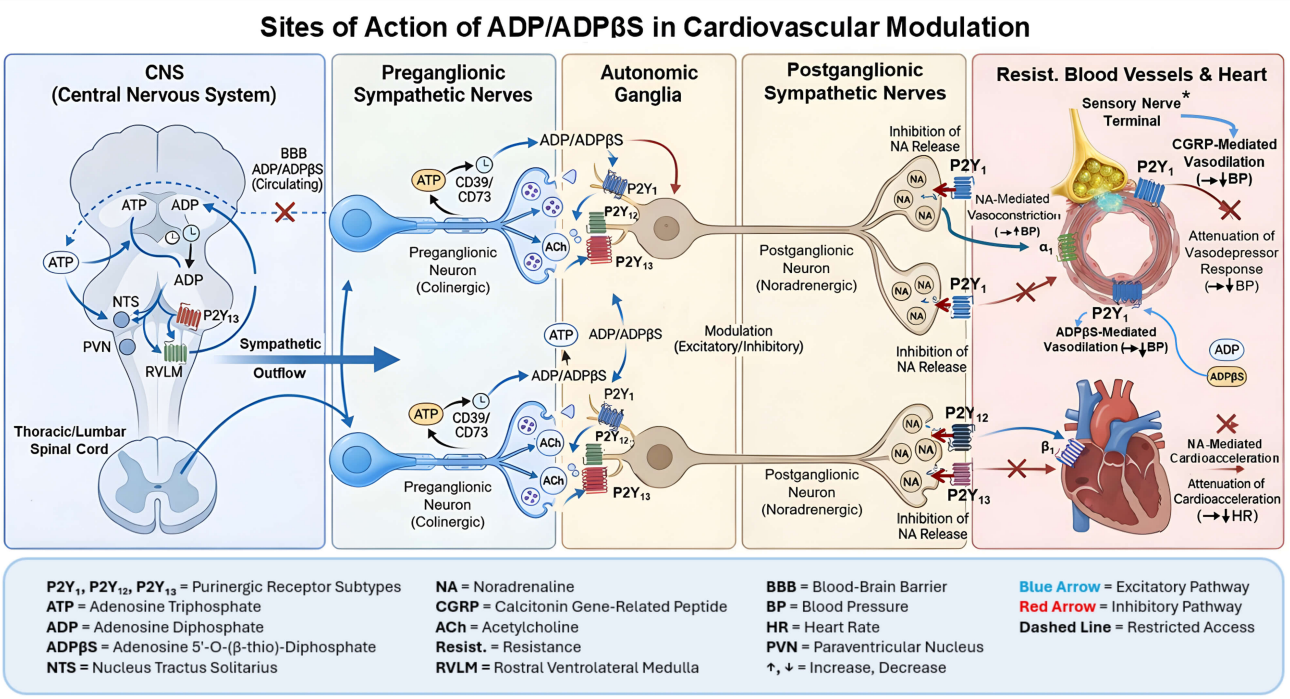


Fig. 1. Hierarchical organization of ADP/ADPβS sites of action in cardiovascular modulation. ADP and its stable analogue ADPβS modulate neurotransmitter release and cardiovascular function via a hierarchical P2Y receptor signalling network involving the central nervous system (CNS), preganglionic nerves, autonomic ganglia/adrenal medulla, postganglionic nerves, and sensory nerves. *The perivascular sensory nerves are C fibres originating in the spinal cord whose activation results in a vasodilator response through the release of calcitonin gene-related peptide (CGRP) mainly. Created by the authors, using BioRender.com (Toronto, ON, Canada; https://www.biorender.com) under license, based on the mechanisms described in this study.

2.3 Postganglionic Autonomic and Sensory Nerve Terminals

Beyond the ganglia, purinergic signalling extends to postganglionic autonomic and sensory nerve terminals (see Fig. 1), where ADP-sensitive P2Y receptors modulate neurotransmitter and neuropeptide release. For example, at sympathetic nerve endings, these receptors influence noradrenergic transmission and contribute to the modulation of cardiac and vascular autonomic tone [13,14,15]. In parallel, P2Y receptors expressed on sensory afferent fibres modulate the release of vasoactive neuropeptides, thereby activating or inhibiting sensory neurogenic (peptidergic) mechanisms involved in cardiovascular modulation [13,19], including perivascular sensory CGRPergic neurons that modulate systemic vascular tone [16] (see Fig. 1).

At this level, purinergic signalling plays a particularly important role in shaping the balance between sympathetic vasoconstrictor (noradrenergic) influences and sensory vasodilator (CGRPergic) mechanisms. The functional specialization of ADP-sensitive P2Y receptor subtypes allows purines to differentially regulate autonomic efferent and sensory afferent pathways, contributing to the complexity of cardiovascular responses observed *in vivo* [1,12].

2.4 Peripheral Vascular and Cardiac Effector Tissues

The final level of the hierarchical framework involves the direct actions of purinergic receptors expressed on vascular and cardiac effectors within the autonomic neuroeffector junction (see Fig. 1). In this context, P2Y receptors located on vascular smooth muscle cells and endothelial cells participate in the modulation of vascular tone, whereas those expressed in cardiac tissues influence chronotropic and inotropic responses. These peripheral actions represent the ultimate neuroeffector mechanisms through which purinergic signalling translates autonomic modulation into changes in arterial blood pressure and cardiac output [1,19].

The magnitude and duration of these responses are strongly influenced by extracellular nucleotide metabolism. The sequential conversion of ATP to ADP, AMP, and adenosine by ectonucleotidases such as CD39 and CD73 determines the spatial and temporal profile of purinergic receptor activation and ensures tight local control of cardiovascular effects [18,20].

2.5 Integrative Perspective

With the above lines of reasoning in mind, purinergic signalling in cardiovascular modulation is best understood as a multilevel system in which ATP and ADP act as sequential extracellular signals that coordinate central in-

tegration, autonomic transmission, and peripheral neuroeffector responses (see Fig. 1). This hierarchical organization provides a conceptual framework for interpreting the complex cardiovascular actions of ADP and related compounds, as well as for distinguishing central from peripheral mechanisms.

On this basis, the following sections will focus particularly on the role of purinergic signalling within specific levels of this neuroanatomical hierarchy (see Table 1), beginning with a detailed analysis of central autonomic mechanisms and, subsequently, addressing peripheral autonomic and neurogenic pathways that are specifically relevant to the function of ADP-sensitive P2Y receptors.

3. Central Purinergic Mechanisms in Cardiovascular Modulation

3.1 Purinergic Signalling in Central Autonomic Nuclei

Although the present review focuses on peripheral P2Y receptor-mediated mechanisms revealed by experimental animal models (including the pithed rat), an understanding of central purinergic modulation is essential to place these findings within the broader context of cardiovascular autonomic modulation.

Purinergic signalling constitutes a fundamental neuromodulatory system within the CNS, where purines participate in the modulation of neuronal excitability, synaptic integration, and autonomic output. Unlike classical fast neurotransmitters, extracellular nucleotides such as ATP and ADP are released in an activity-dependent manner and exert their effects through a combination of ionotropic and metabotropic purinergic receptors; this allows them to modulate cardiovascular reflexes and the sympathetic-parasympathetic balance in a context-dependent fashion [17,39]. Furthermore, in central autonomic circuits, purinergic signalling operates as an integrative modulatory system that interacts with glutamatergic, GABAergic, and monoaminergic neurotransmission, thereby fine-tuning cardiovascular homeostasis rather than generating discrete reflex responses.

ATP has been the most extensively studied purinergic mediator in the CNS and is now widely recognized as a neurotransmitter and cotransmitter, with substantial evidence supporting a modulatory contribution from glial sources under specific physiological conditions [40]. In central autonomic nuclei, ATP can be released from both neurons and astrocytes through vesicular and non-vesicular mechanisms, including hemichannels, ATP-binding cassette transporters, and P2X7 receptor-associated pathways [17,40]. Once released, ATP predominantly activates P2X receptors, producing fast excitatory responses that increase neuronal firing. These actions are particularly relevant in preganglionic regions of the hypothalamus and brainstem, where ATP-mediated excitation contributes to increases in sympathetic nerve activity, arterial blood pressure, and heart activity [1,18].

Importantly, ATP-driven signalling in the CNS is intrinsically transient and dynamically transformed by rapid extracellular metabolism. Indeed, ectonucleotidases expressed in neuronal and glial membranes hydrolyse ATP to ADP, AMP, and adenosine, thereby reshaping the purinergic signalling rather than disrupting it [17,20]. As a consequence, ATP release inevitably leads to the generation of secondary purinergic mediators with distinct receptor selectivity and temporal profiles. In this context, ADP emerges as a physiologically relevant signalling molecule capable of exerting sustained modulatory effects within central autonomic pathways.

Unlike ATP, which preferentially activates ionotropic P2X receptors, ADP predominantly stimulates metabotropic P2Y receptors, particularly the ADP-sensitive $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$ subtypes [13]. These receptors belong to the GPCR superfamily and modulate neuronal activity through second-messenger pathways, including phospholipase C (PLC) activation, intracellular Ca^{2+} mobilization, and inhibition of adenylate cyclase. As such, ADP-mediated P2Y signalling is well suited to modulate synaptic integration, neurotransmitter release, and firing patterns over longer periods, supporting its role in tonic autonomic modulation rather than fast excitatory transmission [17].

The functional distinction between P2X- and P2Y-mediated signalling represents a key organizing principle of central purinergic control. Thus, P2X receptors mediate rapid depolarizing responses that can synchronize neuronal activity, whereas P2Y receptors exert slower modulatory influences that shape the excitability and plasticity of the neuronal network [13,17]. Within central autonomic nuclei, this duality allows purinergic signalling to contribute both to acute adjustments in autonomic output and to sustained changes in cardiovascular control associated with physiological challenges or pathological conditions.

Although ATP has been most extensively studied as a central purinergic neurotransmitter, its rapid extracellular degradation to ADP suggests that the latter compound may represent a physiologically relevant mediator at multiple levels of cardiovascular control, including central autonomic nuclei and peripheral (autonomic and sensory) neurovascular junctions. Consequently, increasing attention has been directed toward ADP-sensitive P2Y receptors as key modulators of central autonomic function. These concepts provide the mechanistic framework for understanding how purinergic signalling within specific autonomic nuclei contributes to cardiovascular modulation and set the stage for examining the roles of individual brain regions and P2Y receptor subtypes in subsequent sections.

3.2 Nucleus Tractus Solitarii (NTS)

The NTS is the primary central integrative site for cardiovascular visceral afferent information and plays a pivotal role in the reflex modulation of arterial blood pres-

sure and heart rate. It receives convergent inputs from arterial baroreceptors, chemoreceptors, and cardiopulmonary afferents, thereby integrating peripheral sensory signals with central autonomic circuits that shape sympathetic and parasympathetic outflow. Within this framework, purinergic signalling has emerged as an important modulatory mechanism in the NTS, contributing to the complexity, plasticity, and context dependency of central cardiovascular control [1,18].

Experimental evidence demonstrates that extracellular nucleotides, particularly ATP, actively modulate neuronal activity within the NTS. Local microinjection of ATP into this nucleus elicits marked cardiovascular responses, including changes in arterial blood pressure and heart rate, whose direction and magnitude depend strongly on the physiological state of the animal and the experimental conditions employed. For example, in awake rats, ATP administration into the NTS predominantly induces vasopressor and tachycardic responses, whereas in anaesthetised animals, vasodepressor or biphasic responses have been reported, which underlines the highly state-dependent nature of purinergic signalling in this nucleus [24,41].

The heterogeneity of cardiovascular responses evoked by purinergic stimulation of the NTS reflects the involvement of multiple purinergic receptor subtypes as well as interactions with other neurotransmitter systems operating within this nucleus [24]. Indeed, activation of ionotropic P2X receptors is associated with fast excitatory effects on NTS neurons, facilitating the transmission of visceral afferent signals and modulating baroreflex gain. These fast excitatory actions are consistent with the role of ATP as a neurotransmitter and cotransmitter in central autonomic circuits [1,17,42].

As in other regions of the CNS, extracellular ATP in the NTS is rapidly metabolized by ectonucleotidases to ADP, AMP, and adenosine. The presence of ADP-sensitive P2Y receptors in the NTS suggests that purinergic modulation within this nucleus is not limited to fast excitatory transmission but also includes slower modulatory mechanisms capable of shaping synaptic integration and autonomic output over longer timescales [39].

Such dual purinergic signalling, involving ATP-P2X-mediated excitation and ADP-P2Y-mediated modulation, provides a mechanistic basis for the diverse cardiovascular responses observed following NTS stimulation with nucleotides. Moreover, this organization is consistent with the broader concept that central purinergic mechanisms are profoundly influenced by the functional state of autonomic circuits, including baseline sympathetic tone, synaptic integration, and neurotransmitter interactions, all of which can be markedly altered by anaesthesia or pathological conditions [17].

Taken together, these findings identify the NTS as a critical site for central purinergic modulation of cardiovascular reflexes, where ATP and its metabolites, including ADP, dynamically regulate afferent processing and baroreflex function. This conceptual framework establishes a direct link between central nucleotide signalling and the complexity of cardiovascular responses observed *in vivo*, and provides a foundation for examining purinergic mechanisms in other autonomic nuclei, as well as for the transition to peripheral neurogenic and vascular actions mediated by ADP-sensitive P2Y receptors.

3.3 Paraventricular Nucleus (PVN)

The PVN is a higher-order integrative centre for autonomic and neuroendocrine control, and exerts a major influence on cardiovascular function by shaping sympathetic outflow. PVN preautonomic neurons project to key downstream autonomic regions, including brainstem presympathetic circuits and the spinal intermediolateral cell column, thereby contributing to the regulation of arterial blood pressure and heart rate through coordinated changes in vasomotor and cardiac autonomic drive [1,18,43].

Within this integrative framework, purinergic signalling has been recognized as a functionally relevant mechanism for central cardiovascular modulation at the hypothalamic level. Experimental evidence in awake rats indicates that activation of P2 purinoceptors within the PVN modulates arterial pressure, supporting the concept that extracellular nucleotides act not only as neurotransmitters, but also as local integrators of autonomic output [44]. These observations place purinergic signalling among the neuromodulatory systems that shape PVN-driven sympathetic control of the cardiovascular system [45].

From a mechanistic point of view, purinergic signalling of the PVN is particularly relevant to sympathetic drive [43,46]. *In vivo* studies demonstrate that microinjection of ATP into the PVN produces a robust increase in lumbar sympathetic nerve activity (LSNA), and that this sympathoexcitation is significantly attenuated by local antagonism of P2 receptors with pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS), consistent with a direct contribution of purinergic receptor activation to the recruitment of sympathetic outflow [45,47,48].

It is noteworthy that purinergic excitation in the PVN does not operate in isolation, but it also interacts with excitatory amino acid transmission [45,49]. Evidence indicates that ATP and glutamate can function as cotransmitters within central autonomic circuits, and that PVN-driven sympathoexcitation is shaped by ionotropic glutamatergic mechanisms. Specifically, blockade of non-N-methyl-D-aspartate (non-NMDA) glutamate receptors with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) significantly attenuates ATP-evoked sympathoexcitation, whereas NMDA receptor antagonism with 2R-amino-5-phosphonopentanoic acid (AP5) does not, suggesting a preferential coupling of purinergic excitation to non-NMDA glutamatergic components in PVN-related sympathetic recruitment [45,47,48].

As in other central autonomic nuclei, extracellular ATP in the PVN is rapidly metabolized by ectonucleotidases to ADP and other purine derivatives, implying that metabotropic P2Y receptor signalling may contribute to slower modulatory effects on neuronal integration and synaptic efficacy [1,17]. Certainly, the precise involvement of ADP-sensitive P2Y receptor subtypes in PVN-mediated cardiovascular control remains less well characterized than in peripheral neurovascular junctions; however, the established capacity of PVN purinergic signalling to alter sympathetic activity provides a mechanistic basis for considering ADP as a physiologically relevant mediator within hypothalamic autonomic circuits [39,45].

Overall, these findings indicate that purinergic signalling within the PVN contributes significantly to the modulation of sympathetic cardiovascular control by modulating neuronal activity and by interacting with excitatory glutamatergic transmission. Instead of acting as an isolated excitatory mechanism, purinergic signalling in this nucleus functions as an integrative modulatory system capable of shaping both acute and sustained sympathetic responses. This hypothalamic level of purinergic control provides a functional bridge between central autonomic integration and the downstream execution of sympathetic output in brainstem circuits, particularly within the rostral ventrolateral medulla [18,50], where: (i) presympathetic neurons regulate basal and reflex control of arterial blood pressure and heart rate; and (ii) purinergic mechanisms have been implicated in both physiological modulation and pathological states.

3.4 Rostral Ventrolateral Medulla (RVLM)

The RVLM is a critical region of the brainstem responsible for the generation and maintenance of basal sympathetic vasomotor tone and for the execution of cardiovascular reflex responses. The neurons of the RVLM, including the well-characterized C1 presympathetic population, provide a tonic excitatory drive to the sympathetic preganglionic neurons of the spinal cord and thus play a central role in the modulation of arterial blood pressure and heart rate [18,50]. In this context, the RVLM represents a final common pathway through which higher-order central autonomic signals are translated into peripheral sympathetic cardiovascular activity.

Within this functional framework, purinergic signalling has been identified as an important modulatory mechanism in the RVLM. Experimental evidence indicates that extracellular nucleotides influence the excitability of presympathetic neurons and contribute to the modulation of sympathetic nerve activity. Indeed, previous studies have demonstrated that local application of ATP within the RVLM causes vasopressor responses and increases sympathetic activity, supporting a role for purinergic mechanisms in the direct control of cardiovascular function at the brainstem level [17,18,50].

More recent work has provided mechanistic insight into the functional relevance of purinergic signalling in the RVLM under pathological conditions. In experimental models of heart failure following myocardial infarction, purinergic signalling within the RVLM has been shown to contribute to enhanced sympathetic drive [51]. In particular, experimental interventions aimed at increasing extracellular ATP breakdown within the RVLM attenuate indices of sympathoexcitation and improve cardiovascular outcomes, indicating that endogenous nucleotide signalling participates in the maintenance of pathological sympathetic hyperactivity [51].

At the receptor level, both the ionotropic P2X and metabotropic P2Y receptors have been linked to RVLM function, with the receptor transduction mechanisms previously pointed out. Although the specific contribution of ADP-sensitive P2Y receptor subtypes in the RVLM is not yet fully defined, their expression within central autonomic circuits supports a role for nucleotide metabolites in shaping sympathetic output on longer timescales [1,13,52]. Indeed, some findings support the presence of functional $P2Y_1$ receptor signalling within the RVLM, particularly in presympathetic C1 neurons involved in cardiovascular and sympathetic regulation [25]. However, the contribution of other ADP-sensitive P2Y receptor subtypes in this region has not yet been completely characterized.

Beyond receptor-level effects, purinergic signalling in the RVLM must be understood within the broader context of extracellular nucleotide dynamics in the CNS. Neuronal activity, metabolic stress, and sustained autonomic drive are associated with activity-dependent release of ATP from neurons and glial cells through multiple mechanisms, including vesicular release, hemichannels, pannexins, and volume-regulated anion channels [17,40,52]. Such dynamic modulation of extracellular purines provides a mechanistic framework by which purinergic signalling in presympathetic regions such as the RVLM can fine-tune neuronal excitability and sympathetic output during sustained autonomic challenges and pathological states characterized by chronic sympathoexcitation [17,40].

As a whole, these observations identify the RVLM as a critical site for purinergic modulation of sympathetic cardiovascular control. By influencing presympathetic neuronal activity, purinergic signalling within this region contributes to the modulation of arterial blood pressure and heart rate in both physiological and pathological contexts [17,40]. Importantly, the role of purinergic mechanisms at the level of the RVLM establishes a direct mechanistic link between higher-order central integration (e.g., within the NTS and PVN) and peripheral autonomic effector pathways (see Table 1). This organization provides a logical transition toward the analysis of ADP-mediated actions at peripheral autonomic and sensory sites, where P2Y receptor-dependent mechanisms play a prominent role in neurogenic and cardiovascular modulation.

The available evidence regarding P2Y receptor subtypes involved in cardiovascular control is summarized in Table 1.

4. Purinergic Signalling in Autonomic Ganglia, as well as in Preganglionic and Postganglionic Nerve Terminals

Autonomic ganglia constitute a critical integrative interface between central autonomic commands and peripheral effector mechanisms (see Fig. 1). Traditionally viewed as passive relay stations, autonomic ganglia are now recognized as active modulatory sites where synaptic transmission is dynamically regulated by multiple neurotransmitters and neuromodulators [53]. In this context, purinergic signalling plays a fundamental role in shaping autonomic output before it reaches postganglionic nerves and cardiovascular target tissues [1,17,32,38]. Rather than functioning as simple relay stations, autonomic ganglia are now recognized as sites of complex synaptic integration, where cotransmission involving ATP and neuropeptides plays a critical role in shaping postganglionic output [53,54]. The concept of cotransmission established by Burnstock [54] provides a unifying framework to understand how fast and slow signalling modalities coexist within the same synaptic microenvironment, enabling precise temporal and functional control of autonomic output.

4.1 ATP as a Preganglionic Cotransmitter in Autonomic Ganglia

In both sympathetic and parasympathetic ganglia, ATP is released from preganglionic nerve terminals and acts as a cotransmitter alongside acetylcholine (see Fig. 1). This phenomenon has been demonstrated in several autonomic ganglia relevant to cardiovascular modulation, including the superior cervical ganglion, stellate ganglion, and celiac ganglion [32,37,38,54]. In the ganglionic synapse, ATP mediates fast excitatory postsynaptic potentials by activation of ionotropic P2X receptors expressed on postganglionic neurons, thereby contributing to fast and reliable synaptic transmission [19,37].

From a cotransmission perspective, ATP provides a rapid excitatory signal that complements nicotinic cholinergic transmission, particularly during periods of increased preganglionic activity. Importantly, ATP-mediated responses may occur independently of acetylcholine-induced depolarization or synergistically with it, indicating that purinergic signalling contributes distinct functional properties at the ganglionic level, rather than simply duplicating cholinergic mechanisms [32,38,54].

4.2 Extracellular Nucleotide Metabolism and Generation of ADP in Ganglionic Synapses

A defining feature of purinergic cotransmission is the rapid extracellular metabolism of ATP following its release. Within autonomic ganglia, ATP is hydrolysed by ectonucleotidases expressed in neuronal and glial elements, generating ADP as a biologically active extracellular signalling molecule rather than an inactive metabolic byproduct [17,18,19]. This enzymatic cascade ensures that purinergic signalling does not end abruptly, but rather transforms into a sequential signalling process in which distinct purine species exert temporally and functionally differentiated effects [17,32].

Recent methodological advances have provided direct evidence that ATP-to-ADP conversion can occur rapidly and efficiently within extremely confined extracellular microdomains. For example, using femtolitre-scale reactor array devices, Ueno et al. [55] demonstrated that enzymatic ATP- and ADP-producing reactions can be detected and quantified in microenvironments comparable in volume to synaptic clefts. Although these experiments were not performed in autonomic tissues, their findings support the concept that extracellular nucleotide metabolism in ganglionic synapses is likely to be highly localized and dynamically regulated, enabling ATP released from preganglionic terminals to be rapidly transformed into ADP with functional consequences for the activation of metabotropic P2Y receptors.

The local generation of ADP within the ganglionic synaptic cleft creates favourable conditions for the activation of metabotropic P2Y receptors, allowing purinergic signalling to shift from fast ionotropic excitation mediated by P2X receptors to slower modulatory control of synaptic transmission. From the perspective of cotransmission, this sequential ATP-ADP signalling provides an efficient mechanism by which autonomic ganglia can integrate rapid preganglionic inputs with longer-lasting modulatory influences, thereby shaping the magnitude and temporal pattern of postganglionic activation [13,17,54].

Through this mechanism, ADP-sensitive P2Y receptors contribute to the fine-tuning of ganglionic output during sustained or repetitive autonomic activation. Consequently, extracellular nucleotide metabolism in autonomic ganglia represents a critical determinant of autonomic signal processing, positioning ADP not only as a degradation product of ATP, but also as a functionally relevant mediator in the modulation of cardiovascular autonomic transmission [1,54].

4.3 P2Y Receptor-Mediated Modulation of Ganglionic and Postganglionic Transmission

Metabotropic P2Y receptors expressed in autonomic ganglia contribute to the fine modulation of synaptic efficacy and neuronal excitability. Several subtypes of P2Y receptors, including $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$, have been identified in autonomic neurons and associated structures, where they activate different intracellular signalling pathways [13,19].

Activation of G_q-coupled $P2Y_1$ receptors can facilitate intracellular Ca^{2+} signalling and trigger a neuronal re-

sponse, whereas activation of G_i-coupled $P2Y_{12}$ and $P2Y_{13}$ receptors inhibits adenylate cyclase activity and reduces cyclic adenosine monophosphate (cAMP) levels, leading to inhibitory modulation of synaptic transmission [1,13]. Through these mechanisms, ADP-sensitive P2Y receptors can either facilitate or restrict ganglionic output depending on receptor subtype expression, local nucleotide concentrations, and the functional state of the autonomic network [1,17,19].

Functionally, this modulatory control allows autonomic ganglia to adjust the strength and pattern of postganglionic firing in response to sustained or repetitive preganglionic activity (see Fig. 1). As a result, ganglionic purinergic signalling contributes to the adaptability of autonomic cardiovascular responses under varying physiological and pathophysiological conditions [1,38,54]. For example, Villanueva-Castillo et al. [14] have shown that ADPβS can inhibit the cardioaccelerator sympathetic outflow (reflecting inhibition of cardiac postganglionic sympathetic neurons) by stimulation of prejunctional $P2Y_{12}$ and $P2Y_{13}$ receptors within the neurocardiac junction [14].

ADPβS inhibits the cardioaccelerator sympathetic outflow by activating prejunctional $P2Y_{12}$ and $P2Y_{13}$ receptors at the neurocardiac junction, thereby reducing the activity of cardiac postganglionic sympathetic neurons.

4.4 Functional Implications for Cardiovascular Autonomic Modulation

The presence of both fast ATP-mediated excitation and slower ADP-mediated modulation in autonomic ganglia supports the concept that these structures act as dynamic filters rather than simple transmission relays. Thus, by modulating ganglionic transmission, purinergic signalling influences the balance between sympathetic and parasympathetic outflow and shapes the autonomic tone that reaches cardiovascular effectors [1,17,32].

This ganglionic level of control is particularly relevant for interpreting cardiovascular responses to purinergic stimulation observed *in vivo*. Modulation of ganglionic transmission by ADP-sensitive P2Y receptors can amplify, dampen, or reshape autonomic signals originating in central nuclei before they reach postganglionic nerves. As illustrated in Fig. 1, ganglionic purinergic mechanisms represent a critical intermediate layer linking central autonomic integration with peripheral neurogenic and vascular/cardiac responses [1,17,19].

4.5 Position of Ganglionic Mechanisms Within the Neuroanatomical Hierarchical Framework

Within the hierarchical organization of purinergic cardiovascular modulation, autonomic ganglia occupy a strategic intermediate position between central autonomic nuclei and postganglionic effector pathways (see Fig. 1). At this level, ATP functions primarily as a fast excitatory cotransmitter, whereas ADP acts as a modulatory signal capable of fine-tuning autonomic output through P2Y receptor activation [13,17,32].

Recognizing the contribution of ganglionic purinergic signalling is fundamental to understanding how purines coordinate cardiovascular control at multiple levels of the autonomic axis. Fig. 1 illustrates that this perspective provides a mechanistic link between central purinergic modulation and the peripheral postganglionic and sensory mechanisms discussed in the following section [1,18].

5. Postganglionic Autonomic and Sensory Mechanisms of ADP-Sensitive P2Y Receptors

Following ganglionic integration, autonomic signals are conveyed to cardiovascular effector systems by postganglionic sympathetic nerve terminals, the adrenal medulla (as a modified sympathetic ganglion; [8]), as well as by primary sensory nerves [56] (see Fig. 1). Indeed, peripheral vascular resistance is modulated by perivascular sympathetic (noradrenergic) and primary sensory (peptidergic) nerves [23,57]. These perivascular sensory nerves are C fibres originating in the spinal cord [58] whose activation results in a vasodilator response primarily through the release of CGRP [56], which is located together with neurokinin A and substance P in neuronal vesicles [59]. At this level, purinergic signalling plays a critical role in shaping the neurogenic control of vascular tone and cardiac function by modulating the release of neurotransmitters and neuropeptides rather than acting as a primary fast excitatory system [1,19].

A previous physiological study has demonstrated that systemic administration of ADP elicits marked cardiovascular responses, including changes in arterial blood pressure and heart rate, indicating that ADP is a biologically active nucleotide capable of modulating peripheral autonomic control in a dose-dependent manner [60]. The subsequent identification of metabotropic P2Y receptors provided the necessary mechanistic framework for interpreting these observations.

In peripheral autonomic neurons, ADP-sensitive P2Y receptors (particularly the $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$ subtypes) are expressed at multiple levels of autonomic transmission, including sympathetic and parasympathetic ganglia, as well as postganglionic nerve terminals [13,19] (see Fig. 1). These receptors belong to the GPCR family and modulate neuronal excitability and neurotransmitter release via second messenger signalling cascades, rather than fast synaptic depolarization.

5.1 Modulation of Sympathetic Postganglionic Neurotransmission

Postganglionic sympathetic nerve terminals that innervate the heart and blood vessels express multiple purinergic receptor subtypes that modulate noradrenaline (NA) release and sympathetic efferent activity. In particular, G_i-coupled ADP-sensitive $P2Y_{12}$ and $P2Y_{13}$ recep-

tors inhibit adenylate cyclase activity, reduce intracellular cAMP levels, and limit Ca^{2+} availability at presynaptic terminals, resulting in inhibitory modulation of neurotransmitter release [13,52].

Activation of these receptors leads to attenuation of sympathetic vasopressor and cardioaccelerator responses, providing a mechanism for fine control of autonomic output rather than a complete suppression of neurotransmission. This inhibitory purinergic control becomes particularly relevant under conditions of sustained sympathetic activation, where excessive noradrenergic signalling could otherwise lead to maladaptive increases in heart rate, myocardial oxygen demand, and vascular resistance [14,61].

5.2 Sensory Mechanisms and Neuropeptide Modulation

In parallel with sympathetic efferent control, purinergic signalling also modulates sensory pathways involved in cardiovascular modulation (see Fig. 1). $P2Y_1$ receptors, which are primarily coupled to G_q proteins, activate PLC-dependent signalling cascades leading to intracellular Ca^{2+} mobilization. In sensory neurons, this signalling profile is consistent with facilitatory effects on neuropeptide release and supports a role for $P2Y_1$ receptors in the modulation of sensory components of autonomic cardiovascular control [10,13,62].

Through this mechanism, ADP-sensitive P2Y receptors contribute to the integration of sensory and postganglionic sympathetic influences, allowing peripheral neural circuits to dynamically adjust cardiovascular output in response to local metabolic and neuronal signals.

5.3 Temporal Organization of Postganglionic Purinergic Signalling

As previously described, peripheral purinergic signalling generates a sequence of events in which fast P2X-mediated excitation is followed by slower, modulatory P2Y receptor activation [17,63]. This temporal separation allows ADP to act as a sustained modulatory signal that fine-tunes postganglionic autonomic neurotransmission rather than triggering fast excitatory responses. As a consequence, ADP-sensitive P2Y receptors function as modulators of autonomic gain, adjusting the magnitude and duration of sympathetic and sensory nerve activity before the signals reach vascular and tissue effectors.

5.4 Position of Autonomic Postganglionic and Sensory Mechanisms Within the Hierarchical Framework

Within the hierarchical organization of purinergic cardiovascular modulation, postganglionic and sensory mechanisms represent a critical intermediate stage between ganglionic integration and tissue effector responses (see Fig. 1). At this level, ADP-sensitive P2Y receptors coordinate sympathetic inhibition and sensory facilitation to shape cardiovascular output under physiological and pathophysiological conditions.

This autonomic postganglionic framework provides the mechanistic basis for understanding how ADP-mediated purinergic signalling influences downstream neurovascular and tissue responses, which are addressed in the following section using reflex-independent experimental approaches [1,13,17,19].

6. The Blood Vessels and Heart as Effectors Activated by ADP and ADPβS *In Vivo* and *In Vitro* Using Diverse Experimental Models

ADP, along with stable analogues such as ADPβS, exert complex cardiovascular effects that ultimately manifest at the level of blood vessels and the heart as final effector organs. Since Burnstock's original proposal of the purinergic neurotransmission concept in 1972 [64], a substantial body of experimental evidence has demonstrated that purines modulate cardiovascular function predominantly through peripheral mechanisms, involving vascular smooth muscle, endothelium, sensory afferent fibres, autonomic nerve terminals and cardiac tissues [6,65].

In view that circulating nucleotides such as ADP and ADPβS do not readily cross the BBB under physiological conditions, the cardiovascular responses elicited by their systemic administration must arise primarily from peripheral sites of action, rather than from direct activation of central P2Y receptors. Consequently, blood vessels and the heart represent the main effector targets through which ADP-sensitive purinergic signalling is translated into functional changes in arterial blood pressure, vascular resistance, and cardiac activity. This peripheral localization provides a clear conceptual framework for interpreting the cardiovascular actions of ADP and related compounds across different experimental settings.

The identification and characterization of these vascular and cardiac effector mechanisms have relied on the use of diverse experimental models, each offering complementary levels of mechanistic resolution. *In vivo* preparations, including anaesthetised animals [60,66], allow the evaluation of cardiovascular responses under conditions where reflex integration is preserved; whereas reflex-independent models, such as the pithed rat, enable isolation of peripheral sensory and autonomic mechanisms by eliminating supraspinal and spinal reflex influences [67]. In parallel, *in vitro* and *ex vivo* preparations of vascular and cardiac tissues allow direct questioning of effector responses at the tissue level, independent of neuronal inputs [18,19,68].

Overall, these experimental approaches provide a hierarchical and integrative framework for dissecting how ADP and ADPβS activate peripheral purinergic pathways to modulate cardiovascular function. By combining evidence obtained *in vivo* and *in vitro*, it is possible to link receptor activation at peripheral neurovascular sites with functional outcomes at the level of blood vessels and the heart, thereby establishing a coherent basis for the mechanistic and pharmacological analyses developed in the following sections.

6.1 Experimental Models to Identify Vascular and Cardiac Effectors

The identification of blood vessels and the heart as peripheral effectors of ADP- and ADPβS-mediated cardiovascular actions has relied on the use of complementary experimental models that differ in the degree of neural integration they preserve. These models range from (i) *in vivo* preparations with intact reflex control, to (ii) reflex-independent *in vivo* preparations, and (iii) isolated *in vitro* or *ex vivo* tissues, each providing distinct insights into the mechanisms by which purinergic signalling is translated into functional cardiovascular responses.

6.1.1 Anaesthetised *In Vivo* Preparations

The first investigations conducted in anaesthetised animals established that systemic administration of ADP induces pronounced changes in arterial blood pressure and heart rate, thereby identifying the peripheral cardiovascular system as a primary target of circulating nucleotides [60,66]. In these preparations, cardiovascular responses reflect the integrated contribution of peripheral vascular and cardiac actions along with intact baroreflex and chemoreflex modulation. Subsequent pharmacological studies confirmed that many of these responses are mediated by P2Y receptors located throughout the peripheral cardiovascular system, including the heart, blood vessels, and endothelial cells [65].

6.1.2 Reflex-Independent *In Vivo* Preparations

While anaesthetised models provide important physiological context, the presence of intact baroreflex pathways can overshadow the direct contribution of peripheral sensory and autonomic mechanisms to nucleotide-induced cardiovascular effects. To overcome this limitation, reflex-independent *in vivo* preparations have been extensively employed. Among these, the pithed rat model, originally described by Shipley and Tilden in 1947 [67], abolishes supraspinal and spinal reflex integration while preserving the functional integrity of peripheral sympathetic and sensory nerves. This unique characteristic allows cardiovascular responses to be attributed exclusively to peripheral neuroeffector mechanisms [23].

The pithed rat model has proven particularly valuable for the study of purinergic cardiovascular modulation, as it enables clear discrimination between sensory vasodilator and sympathetic vasoconstrictor or cardioaccelerator components that may otherwise be masked by baroreflex compensatory mechanisms in intact animals. Comparative studies have demonstrated that the cardiovascular responses to ADPβS differ markedly between anaesthetised and pithed animals, revealing specific contributions from receptor subtypes that depend on the integrity of central baroreflex circuits [22,23]. These observations highlight the importance of baroreflex-independent models for isolating the peripheral pharmacology of ADP-sensitive P2Y receptors.

6.1.3 *In Vitro* and *Ex Vivo* Tissue Preparations

In addition to *in vivo* approaches, *in vitro* and *ex vivo* preparations of vascular and cardiac tissues provide a complementary level of analysis by allowing direct investigation of neuroeffector responses in the absence of physiological neurogenic influences. In this context, isolated resistance arteries, conduit vessels, and cardiac preparations have been widely used to examine endothelium-dependent and smooth muscle-mediated responses to ADP and ADPβS, thereby defining the intrinsic effector properties of vascular and cardiac tissues [18,19,68]. These models are particularly useful for identifying receptor localization and signalling pathways at the tissue level.

Taken together, anaesthetised animals, reflex-independent *in vivo* preparations such as the pithed rat, and isolated tissue models constitute a hierarchical experimental framework for the study of purinergic cardiovascular modulation. By integrating the evidence obtained from these models, it is possible to elucidate how ADP-sensitive signalling pathways operate at the peripheral neurovascular and tissue levels to activate blood vessels and the heart as final effectors, thus providing a solid foundation for the mechanistic analyses developed in the subsequent sections.

6.2 Peripheral Neurovascular and Tissue-Level Actions of ADP

At the peripheral level, ADP exerts pronounced cardiovascular effects through actions at neurovascular junctions, as well as in vascular and cardiac tissues, establishing this nucleotide as an active physiological modulator of peripheral cardiovascular function. Indeed, early *in vivo* studies demonstrated that systemic administration of ADP produces marked reductions in arterial blood pressure, decreases in peripheral vascular resistance, and compensatory changes in cardiac output and heart rate; these findings provided the first evidence that ADP modulates cardiovascular function independently of direct CNS involvement [60,66].

Although these pioneering observations preceded the molecular identification of purinergic receptors, they laid the conceptual groundwork for recognizing ADP as a biologically relevant extracellular mediator capable of influencing vascular and cardiac function. Subsequent pharmacological studies confirmed that many of these peripheral cardiovascular effects are mediated by P2Y receptors located on vascular smooth muscle cells, endothelial cells, and peripheral nerve terminals, thereby consolidating the role of ADP as a modulator of peripheral effectors within the cardiovascular system [65].

On the other hand, peripheral sensory nerves represent a critical component of ADP-mediated cardiovascular modulation. In this respect, perivascular sensory afferents, particularly those containing CGRP, densely innervate resistance blood vessels and play a fundamental role in the modulation of vascular tone [23,69]. Activation of these perivascular sensory nerves leads to the release

of CGRP and other neuropeptides (neurokinin A and substance P), resulting in a robust vasodilatation/vasodepressor response that helps attenuate sympathetic vasoconstrictor influences and fine-tune peripheral vascular resistance [23,70,71,72]. Within the sensory neurovascular junction, ADP is well-positioned to indirectly influence vascular function by modulating sensory neurotransmission at peripheral sites [15,16]. Furthermore, in certain blood vessels, such as the dura mater, the action of ADP may involve the release of prostaglandin E_2 [73]. However, it is still unclear whether this mechanism is operative in resistance blood vessels.

In addition to its actions on sensory afferents, ADP can act directly on endothelial cells and vascular smooth muscle, which express multiple P2Y receptor subtypes capable of responding to extracellular nucleotides, allowing ADP to modulate vascular tone through both endothelium-dependent and endothelium-independent mechanisms. These include vasodilatation mediated by nitric oxide (NO), changes in endothelial hyperpolarization, and direct modulation of smooth muscle contractility, depending on the vascular bed and receptor expression profile [17,19].

Importantly, the cardiovascular actions of ADP at the peripheral level reflect the integration of neurogenic and effector mechanisms, rather than a single uniform pathway. Thus, by acting simultaneously at sensory nerve terminals, endothelial cells, and vascular smooth muscle, ADP-sensitive purinergic signalling converges on blood vessels as central integrators of autonomic, sensory, and endothelial inputs. This multifaceted organization provides the physiological basis upon which more selective pharmacological approaches (such as the use of stable ADP analogues) have been developed to dissect receptor subtype-specific contributions to cardiovascular modulation.

Collectively, these observations establish ADP as a physiologically relevant extracellular mediator that modulates cardiovascular function through coordinated actions at the peripheral neurovascular and tissue levels. Understanding these basic mechanisms is essential for interpreting the effects of stable ADP analogues and for elucidating the specific receptor subtype pathways that underlie the cardiovascular responses elicited by ADPβS, which are discussed in the following section.

6.3 ADPβS as a Pharmacological Tool for Dissecting Peripheral P2Y Receptor Mechanisms

A major limitation in the experimental analysis of ADP-mediated cardiovascular signalling *in vivo* is the extremely short extracellular half-life of endogenous ADP, which is rapidly degraded by ectonucleotidases expressed on endothelial cells and vascular smooth muscle, as well as in circulating blood. Enzymes such as CD39 (ENTPD1) efficiently hydrolyse ATP and ADP to downstream metabolites, thereby restricting both the spatial and temporal resolution of purinergic signalling under physiological conditions [17,74]. This rapid metabolism complicates the attribution of cardiovascular effects to specific populations of ADP-sensitive receptors.

To overcome this limitation, the stable ADP analogue, ADPβS, has been widely used as a pharmacological tool to investigate peripheral mechanisms mediated by P2Y receptors (see Fig. 2, Ref. [19]). ADPβS is a chemically modified, non-hydrolysable analogue of ADP in which a non-bridging oxygen atom in the β-phosphate moiety is replaced by a sulphur. This structural modification confers marked resistance to ectonucleotidase-mediated degradation while preserving high affinity for ADP-sensitive P2Y receptor subtypes [13,63]. As a result, ADPβS behaves as a relatively long-acting agonist in extracellular environments where endogenous ADP would otherwise be rapidly inactivated.

The enhanced stability of ADPβS is particularly advantageous for *in vivo* studies aimed at dissecting peripheral purinergic mechanisms, as it allows for sustained activation of ADP-sensitive P2Y receptors and facilitates the systematic use of selective antagonists to elucidate the specific contributions of each receptor subtype. This approach is especially valuable in experimental settings designed to minimize or eliminate central baroreflex influences, where peripheral sensory, autonomic, and effector mechanisms can be functionally separated [1,74].

It is important to note that ADPβS, which preferentially activates metabotropic P2Y receptors (including $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$), is not a substrate of adenylate kinase for synthesizing ATP from ADP [74]. This structural difference with ADP (see Fig. 2) minimizes confounding contributions from fast ionotropic P2X receptor-mediated responses that are more prominently elicited by ATP [1,13]. This pharmacological profile is particularly advantageous in cardiovascular tissues, where mixed purinergic receptor expression can otherwise complicate interpretation of functional responses.

In *in vivo* experimental models, especially in reflex-independent preparations such as the pithed rat, systemic administration of ADPβS elicits reproducible cardiovascular responses that can be pharmacologically distinguished using selective P2Y receptor antagonists. This approach has allowed the identification of distinct sensory, autonomic, and effector components of purinergic cardiovascular modulation, providing robust evidence of the specific functional organization of the receptor subtype at the peripheral level [14,16,22].

Specifically, pharmacological dissection has shown that G_q-coupled $P2Y_1$ receptors primarily activate sensory neurogenic mechanisms associated with vasodepressor responses, whereas G_i-coupled $P2Y_{12}$ and $P2Y_{13}$ receptors selectively modulate sympathetic efferent activity, including the vasopressor and cardioaccelerator components [14,16,22]. The ability of ADPβS to maintain receptor ac-

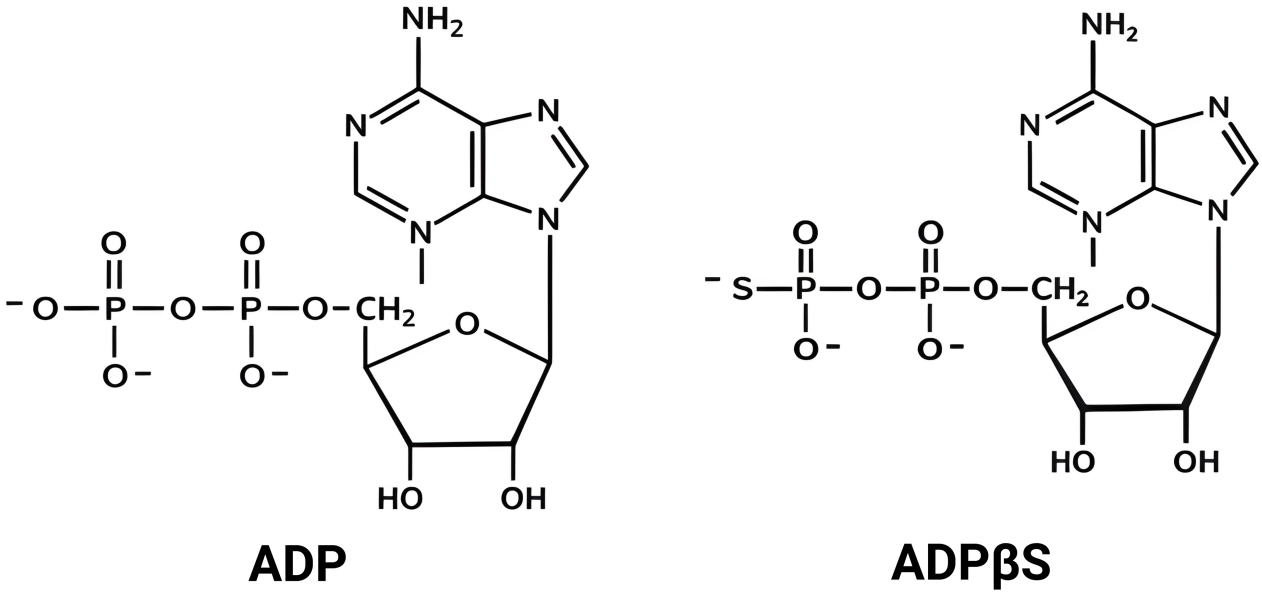


Fig. 2. Chemical structures of adenosine diphosphate (ADP) and adenosine 5′-O-(β-thio)-diphosphate (ADPβS). ADPβS differs from ADP by the substitution of a non-bridging oxygen atom by a sulphur in the β-phosphate moiety, which confers resistance to hydrolysis mediated by ectonucleotidases, while retaining affinity for ADP-sensitive P2Y receptors. Structures adapted from Ralevic and Burnstock [19].

tivation is essential for revealing this functional organization, as it allows for the clear attribution of cardiovascular effects to discrete populations of peripheral P2Y receptor by the use of selective antagonists [14,16,22,61].

In summary, ADPβS constitutes a powerful experimental tool for the mechanistic analysis of peripheral purinergic cardiovascular signalling. By overcoming the limitations imposed by rapid nucleotide degradation and by selectively activating ADP-sensitive P2Y receptors, ADPβS allows for precise dissection of the pathways through which purinergic signalling is translated into vascular and cardiac effector responses. The distinct phenotypic cardiovascular effects revealed through this approach, and their relationship to specific P2Y receptor subtypes, are examined in detail in the following section.

6.4 Cardiovascular Responses Induced by ADPβS

Systemic administration of ADPβS elicits a set of distinct cardiovascular responses that reflect the activation of different peripheral purinergic mechanisms converging on blood vessels and the heart as final effector organs [14,15,16,18,22]. In contrast to a unitary cardiovascular action, ADPβS produces a combination of vasodepressor, vasopressor, and cardiac modulatory effects, whose relative expression depends on the activation of the specific P2Y receptor subtype, anatomical localization, and the integrity of autonomic reflex pathways [14,15,16,18,22].

In reflex-independent preparations, such as the pithed rat model, these blood pressure components can be clearly distinguished and attributed exclusively to peripheral sensory and autonomic mechanisms, free from supraspinal and spinal modulation [22]. This experimental context has been instrumental in revealing that ADPβS-sensitive signalling pathways do not operate redundantly, but rather activate functionally segregated effector circuits within the peripheral cardiovascular system [14,15,22,23].

6.4.1 Vasodepressor Responses to ADPβS and Inhibition of Sensory CGRPergic Mechanisms

Vasodepressor responses represent one of the most prominent and reproducible cardiovascular effects elicited by ADPβS in *in vivo* experimental models, particularly in the reflex-independent pithed rat preparation [22]. These responses are characterized by marked reductions in blood pressure and peripheral vascular resistance, indicating activation of potent vasodilator mechanisms operating predominantly at the level of resistance blood vessels [22].

Experimental evidence demonstrates that the vasodepressor component of the cardiovascular response to ADPβS depends critically on the integrity of the vascular tone (both neurogenic and non-neurogenic). Indeed, as previously reported by our group [22], in the pithed rat model (which is devoid of neurogenic vascular tone): (i) the decreases in diastolic blood pressure (a more precise index of peripheral vascular resistance) produced by ADPβS are markedly attenuated compared to those in anaesthetised rats; and (ii) these attenuated ADPβS responses are increased when the non-neurogenic vascular tone is restored by a continuous i.v. infusion of methoxamine. These ADPβS-induced vasodepressor responses, which clearly

differ from the reflex-mediated hypotension observed in intact or anaesthetised animals, are mediated by $P2Y_1$ receptors, probably located at the vascular endothelial level [22], as these responses were: (i) markedly blocked by the selective $P2Y_1$ receptor antagonist MRS2500; and (ii) resistant to blockade by antagonists at $P2Y_{12}$ (PSB0739) or $P2Y_{13}$ (MRS2211) receptors.

On the other hand, we have also demonstrated in pithed rats that ADPβS inhibits the decrease in blood pressure produced by electrical stimulation of the vasodepressor sensory CGRPergic outflow, without affecting those to exogenous CGRP [16]. This response to ADPβS implies a prejunctional inhibition of CGRP release from perivascular sensory nerves that innervate resistance blood vessels and has been shown [16]: (i) to be unrelated to activation of ATP-sensitive K^+ channels, as it was resistant to blockade by glibenclamide; and (ii) to be mediated by $P2Y_1$ and probably $P2Y_{13}$, but not by $P2Y_{12}$, receptors, as it was attenuated by MRS2500, but not by blocking doses of PSB0739 or MRS2211. However, when a very high dose of MRS2211 (3000 µg/kg, i.v.) was used, this sensory inhibition was reversed [16]. Although this latter finding may suggest the possible involvement of $P2Y_{13}$ receptors, it is equally possible that this dose level is also blocking $P2Y_1$ and $P2Y_{12}$ receptors in the pithed rat model, as the selectivity of MRS2211 for these receptors is limited (with pK_i values of 5.0, 5.0 and 6.3 for $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$, respectively [16,75]). Taken collectively, these lines of evidence confirm the existence of a functionally organized $P2Y_1$-CGRP sensory axis that mediates ADPβS-induced vasodilatation/vasodepressor responses [16,22].

The persistence of vasodepressor responses under reflex-independent conditions (pithed rat model) confirms that this mechanism operates irrespective of central autonomic integration and does not depend on the baroreflex or supraspinal modulation. Within this organization, blood vessels stand out not only as passive targets but also as active effectors within a hierarchically organized neurovascular signalling axis.

Although other purinergic receptor subtypes have been implicated in vascular modulation in isolated arterial preparations and specific vascular beds, the available *in vivo* evidence supports a dominant contribution of the sensory $P2Y_1$-CGRP pathway to ADPβS-induced vasodepressor responses in the pithed rat model. Endothelial and smooth muscle contributions may modulate the magnitude or duration of vasodilatation, but these appear secondary to the primary sensory neurogenic mechanism revealed under reflex-independent conditions [15,22].

On the whole, these findings: (i) establish the vasodepressor response as a distinct and hierarchically organized phenotypic manifestation of peripheral purinergic signalling; and (ii) provide a robust functional basis for subsequent analyses of receptor subtype-specific cardiovascular responses, which are addressed in the following sections.

6.4.2 Vasopressor Responses to ADPβS

In contrast to its vasodepressor effects, ADPβS can also elicit secondary vasopressor responses characterized by increases in systolic blood pressure (an index of cardiac left ventricular contractility), which can only be observed in pithed rats when the non-neurogenic vascular tone is restored by a continuous i.v. infusion of methoxamine [22]. It is noteworthy that these vasopressor responses by ADPβS are not produced in anaesthetised rats or in pithed rats without a methoxamine infusion. As discussed in detail by our group [22], these findings overall may imply the potential role of: (i) baroreflex compensatory mechanisms of central origin triggered by blood pressure changes, which could have overshadowed the ADPβS-induced increases in systolic blood pressure in anaesthetised rats, as the CNS is intact; and (ii) peripheral vascular mechanisms, which are absent in pithed rats without restoration of systemic vascular tone.

On this basis, the ADPβS-induced increases in systolic blood pressure in pithed rats with restored vascular tone may reflect activation of peripheral pathways at the level of the cardiac left ventricle [22]. Moreover: (i) the absence of centrally originating baroreflex compensatory mechanisms is a *conditio sine qua non* for avoiding the masking of these responses, which arise exclusively from peripheral mechanisms [14,16,67]; and (ii) the induction of these vasopressor responses, which do not represent a simple rebound or inverse manifestation of vasodepression, indicates that ADPβS can influence sympathetic efferent activity and vascular tone in a mechanistically distinct manner from the sensory CGRPergic vasodepressor pathway described above. Thus, rather than encoding a single vasodepressor or vasopressor command, ADPβS-sensitive pathways operate as a flexible modulatory system in which different P2Y receptor subtypes exert opposing influences on vascular tone depending on their cellular localization and their coupling to downstream signalling pathways [1].

Altogether, these findings establish vasopressor responses as a distinct and physiologically relevant component of ADPβS-induced cardiovascular effects. This vasopressor component provides a functional framework for a more in-depth analysis of receptor subtype-specific modulation. Mechanistically, the vasopressor responses to ADPβS are mediated by activation of $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$ receptor subtypes, most likely at the level of cardiac left ventricle, as these responses were blocked by MRS2500, PSB0739 or MRS2211 [22]. Furthermore, as described in detail below, $P2Y_{12}$ and $P2Y_{13}$ receptors also modulate cardiac sympathetic noradrenergic transmission at the peripheral level, producing an inhibition of the sympathetic cardioaccelerator outflow rather than having direct effects on cardiac tissues [14].

6.4.3 Inhibition by ADPβS of the Sympathetic Cardioaccelerator Outflow

In addition to the above effects, ADPβS can modify cardiac function by influencing heart rate responses mediated by peripheral autonomic mechanisms. For example, in baroreflex-independent preparations such as the pithed rat model, the increases in heart rate elicited by stimulation of the sympathetic cardioaccelerator outflow (but not those to exogenous noradrenaline) can be dose-dependently inhibited by ADPβS [14]. This response, which involves prejunctional sympatho-inhibitory mechanisms, is mediated by $P2Y_{12}$ and $P2Y_{13}$ receptors as it was blocked by PSB0739 or MRS2211, but not by MRS2500 [14]. Since these receptors are, by definition, coupled to G_i proteins [5], their activation inhibits adenylate cyclase activity, leading to a decrease in intracellular cAMP levels and an attenuation of cAMP-dependent signalling pathways, which are critical for neurotransmitter release and excitability in sympathetic efferent neurons [12,13]. This signalling profile is consistent with a modulatory sympatho-inhibitory function, rather than a direct effect on cardiac tissues.

6.4.4 The Role of Purinergic Modulation on the Release of Catecholamines From the Adrenal Medulla

As established in the classical studies of stimulus-secretion coupling in the adrenal medulla by Douglas and Rubin in 1961 [76], and Neher and Marty in 1982 [77], the exocytosis of catecholamines in chromaffin cells is critically dependent on Ca^{2+} influx through voltage-operated calcium channels. Hence: (i) modulation of intracellular Ca^{2+} dynamics provides a direct mechanism for regulating secretory output [76,77]; and (ii) extracellular ATP and ADP may participate in the fine regulation of chromaffin cell excitability and catecholamine secretion through purinergic signalling mechanisms [76,77].

Subsequently, purinergic signalling was recognized as an important modulatory component of: (i) neurotransmitter release in peripheral autonomic tissues, where G_i-coupled P2Y receptors act as presynaptic/prejunctional inhibitory modulators [19]; and (ii) neuroendocrine secretion and autonomic integration in peripheral tissues [78]. Likewise, activation of G_i-coupled P2Y receptors has been shown: (i) to inhibit voltage-dependent Ca^{2+} channels and to reduce exocytosis in adrenal chromaffin cells [77]; and (ii) to modulate neurotransmitter release and cardiovascular autonomic function in peripheral tissues [1]. Interestingly, some studies in adrenal chromaffin cells further indicate that extracellular nucleotides modulate catecholamine secretion through metabotropic purinergic mechanisms involving intracellular Ca^{2+} mobilization and regulation of exocytotic activity [79].

More recently, an elegant study reported by Maesawa et al. [28] demonstrated in rat adrenal medullary slices that extracellular ADP can attenuate carbachol-induced intracellular Ca^{2+} elevations in a subset of chromaffin cells; since this effect was partially reversed by selective $P2Y_{12}$ receptor antagonism, a G_i-coupled inhibitory modulation of stimulus-secretion coupling was suggested [28]. This finding clearly established a direct role of ADP-sensitive purinergic signalling in the modulation of catecholamines secretion within the adrenal medulla. Maesawa et al. [28] also showed that NTPDase2-positive sustentacular cells surrounding chromaffin cells may contribute to extracellular ATP hydrolysis and local ADP formation. These results confirm the existence of a purinergic microenvironment capable of modulating sympatho-adrenal transmission by a local purinergic negative feedback mechanism that limits cholinergic stimulus-evoked catecholamine release.

The above lines of evidence, considered as a whole, support the concept that adrenal purinergic signalling represents a functionally relevant component of sympatho-adrenal and cardiovascular autonomic regulation beyond the classical cholinergic control of chromaffin secretion. Although the adrenal medulla functions as a modified sympathetic ganglion [8], the available evidence indicates that ADP exerts predominantly modulatory rather than strong secretagogue effects on chromaffin cell excitability [28,80]. Accordingly, while an adrenal contribution cannot be completely excluded, a substantial participation of adrenal adrenaline release in the cardiovascular responses observed in the present pithed rat model is unlikely to represent a primary mechanism.

6.5 Segregation of the Receptor Subtypes Underlying ADPβS-Induced Cardiovascular Responses

The cardiovascular responses elicited by ADPβS in rats arise from the segregated activation of different P2Y receptor subtypes operating at different peripheral anatomical and functional sites. Evidence obtained from pithed rats and other preparations demonstrates that vasodepressor, vasopressor, and cardioaccelerator responses are not mediated by a single receptor population, but rather reflect a functionally organized division of labour among P2Y receptors located on blood vessels, sympathetic cardiac efferent pathways and perivascular CGRPergic sensory afferents [14,16,22,70].

The vasodepressor responses induced by ADPβS are selectively associated with activation of endothelial $P2Y_1$ receptors, as these responses were blocked by MRS2500, but not by PSB0739 or MRS2211 [22]. The fact that MRS2500 failed to affect either baseline arterial blood pressure [22] or ADPβS-induced cardiac sympatho-inhibition [14], indicates that the latter response is mediated by a completely different (neurogenic) mechanism. Indeed, ADPβS-induced cardiac sympatho-inhibition [14] is mediated by activation of prejunctional $P2Y_{12}$ and $P2Y_{13}$ receptors, as this response was blocked by PSB0739 or MRS2211, but not by MRS2500 [14]. Interestingly, ADPβS-induced inhibition of the vasodepressor sensory CGRPergic outflow is also mediated by $P2Y_1$ receptors, even though these recep-

tors are located on perivascular sensory CGRPergic nerves [16] rather than on vascular endothelium [22].

The above lines of evidence demonstrate a clear functional segregation, operating in parallel, between purinergic systemic vasodilatation at the endothelial level [22], as well as purinergic modulation of cardiac sympathetic activity [14] and neurogenic sensory CGRPergic vasodilatation [16]. Mechanistically, the signalling properties of the $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$ receptor subtypes (see Introduction section) are well aligned with their functional roles. Evidently, this complexity of cardiovascular responses identified with the combined use of ADPβS and selective P2Y receptor antagonists constitutes a robust pharmacological strategy for dissecting specific receptor subtype mechanisms that reveal the hierarchical organization of purinergic signalling pathways modulating the function of vascular and cardiac effectors [14,16,22,70] (see Fig. 1). This provides a coherent framework that links receptor subtype-specific pharmacology to integrated peripheral cardiovascular function.

6.6 Integrated Pharmacological Profile of Cardiovascular P2Y Receptor Subtypes

The pharmacological dissection of ADPβS-induced cardiovascular responses reveals a functionally segregated yet integrated organization of peripheral P2Y receptor signalling. Rather than encoding a single stereotyped cardiovascular outcome, ADP-sensitive P2Y receptors operate as a coordinated modulatory system in which distinct receptor subtypes engage specific neurogenic and autonomic pathways to shape vascular and cardiac function.

Importantly, the coexistence of vasodepressor, vasopressor, and cardioaccelerator responses elicited by ADPβS should not be interpreted as contradictory effects [14,15,22,62]. Instead, these effects reflect the parallel activation of multiple P2Y receptor subtypes operating at distinct peripheral anatomical and functional levels, including perivascular sensory afferents, sympathetic efferent nerve terminals, and vascular tissues. In this regard, previously reported biphasic vascular responses to purinergic stimulation in isolated preparations (primarily involving $P2Y_2$ and $P2Y_6$ receptors) are conceptually consistent with the receptor subtype-phenotype segregation revealed *in vivo* using the pithed rat model, despite involving different receptor populations and experimental contexts [62].

Within this integrated framework, four main pharmacological components of the action of ADPβS at the peripheral level can be identified:

(i) $P2Y_1$ receptors that mediate (via G_q-PLC-Ca^{2+} signalling) a prejunctional inhibition of the vasodepressor sensory CGRPergic outflow of neurogenic origin [16].

(ii) $P2Y_1$ receptors that produce (via G_q-PLC-Ca^{2+} signalling and activation of endothelial NO synthase) endothelium-dependent vasodepressor responses of vascular origin [22].

(iii) $P2Y_{12}$ and $P2Y_{13}$ receptors that exert a sympatho-inhibitory modulation, particularly at the level of the cardioaccelerator pathway, through G_i-mediated inhibition of adenylate cyclase and downstream cAMP-dependent mechanisms [14].

(iv) A secondary vasopressor component mediated by activation of $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$ receptor subtypes, most likely at the level of cardiac left ventricle, along with modulation of peripheral sympathetic vasomotor pathways [22]. It is noteworthy that this vasopressor component can only be observed in pithed rats with restored vascular tone [15,22,62].

Accordingly: (i) the pithed rat model provides a unique experimental context in which these mechanisms can be clearly separated, allowing cardiovascular responses to be unequivocally attributed to specific populations of peripheral P2Y receptors without confounding influences arising from central baroreflex integration [14,16,22,70]; and (ii) P2Y receptors constitute a hierarchically organized peripheral regulatory system capable of differentially modulating sensory vasodilatation and sympathetic cardiovascular output. This integrated framework provides a mechanistic basis for understanding the physiological relevance and pathophysiological implications of purinergic P2Y receptor signalling in cardiovascular disease, which are addressed in the following sections.

7. Functional and Pathophysiological Implications of P2Y Receptor Modulation

The lines of evidence analysed in the previous sections support the view that purinergic signalling, and particularly the activation of P2Y receptors, constitutes a finely tuned regulatory system that integrates sensory, autonomic, and vascular mechanisms involved in cardiovascular control. Rather than acting as a single effector pathway, P2Y receptors operate as functionally segregated yet complementary modulators that shape arterial blood pressure and cardiac function across multiple levels of organization, from peripheral neurovascular junctions to systemic autonomic output [1,13,61]. Moreover, the pithed rat model allows a clear attribution of vasodepressor, vasopressor, and cardiac modulatory effects to specific peripheral mechanisms [22,67,81].

7.1 Vascular-Sensory-Autonomic Integration in Cardiovascular Modulation

One of the most relevant functional implications from the above studies is that the vascular, sensory and sympathetic components of cardiovascular control can be independently modulated by distinct P2Y receptor subtypes. Indeed, activation of $P2Y_1$ receptors preferentially inhibits the vasodepressor sensory CGRPergic outflow [16], while it can also produce potent vasodepressor responses [22]. In contrast, $P2Y_{12}$ and $P2Y_{13}$ receptors selectively inhibit sympathetic efferent pathways, particularly those control-

ling cardiac chronotropic responses [14]. Interestingly, endothelial $P2Y_2$ and $P2Y_6$ receptors contribute to vascular tone modulation in a manner specific to each vascular bed, particularly in coronary arteries [62,82], but these mechanisms are different from the vasodepressor responses [22] or the inhibition of the vasodepressor sensory CGRPergic pathway [16] mediated by $P2Y_1$ receptors.

Collectively, these findings support a model in which P2Y receptors act as nodal points for differential vascular, sensory, and autonomic integration, for example, by allowing rapid local vasodilatation via vascular endothelial mechanisms [22], while restricting excessive sympathetic cardiac activation [1,83,84].

7.2 Implications for Autonomic Balance and Cardiovascular Homeostasis

From a physiological perspective, the differential actions of P2Y receptors described above may represent an intrinsic buffering system designed to counteract the elevated sympathetic tone induced by stress, exercise, and acute cardiovascular challenges. By acting primarily as modulators, P2Y receptors fine-tune autonomic gain and magnitude of the response, rather than producing a complete loss or activation of transmission [33]. This modulatory role positions P2Y receptor signalling as a key contributor to short-term cardiovascular homeostasis [1,61].

7.3 Relevance to Cardiovascular Pathophysiology

Dysregulation of purinergic signalling has been implicated in a variety of cardiovascular disorders, including hypertension, heart failure, endothelial dysfunction, and ischemic heart disease [1,61]. In these conditions, alterations in extracellular nucleotide metabolism, changes in P2Y receptor expression, or impairment of downstream signalling pathways may alter the balance between sensory vasodilatation and sympathetic control [1,61]. Consequently, dysregulation of purinergic signalling is more likely to disrupt autonomic gain and reflex balance, a feature that may underlie its involvement in cardiovascular autonomic disorders [33]. Furthermore, reduced CGRP bioavailability and impaired sensory neurogenic vasodilatation have been reported in hypertension and heart failure, potentially limiting the protective vasodilator influence of $P2Y_1$-receptor-mediated pathways [62].

Conversely, excessive sympathetic activation (partly modulated by G_i-coupled $P2Y_{12}$ and $P2Y_{13}$ receptors [14]) contributes to adverse cardiac remodelling, arrhythmogenesis, and disease progression. The present framework suggests that selective dysfunction of specific P2Y receptor subtypes could shift the autonomic balance toward either excessive vasoconstriction or inadequate cardiac restraint, thereby exacerbating pathological cardiovascular states [12,85].

7.4 Therapeutic Perspectives

The aforementioned differential functions of P2Y receptor subtypes [14,16,22,70] offer potential opportunities for therapeutic intervention in cardiovascular disorders associated with autonomic imbalance and neurovascular dysfunction [1,12,61]. In particular, selective blockade of prejunctional $P2Y_1$ receptors on perivascular sensory nerves may enhance CGRP-dependent sensory vasodilatation and improve peripheral perfusion under pathological conditions characterized by excessive vasoconstriction or impaired vascular function [16,22,82]. Conversely, modulation of prejunctional $P2Y_{12}$ and $P2Y_{13}$ receptors on cardiac sympathetic pathways [14] may contribute to limiting excessive sympathetic cardiac activation without directly interfering with vascular tone [12,52].

Importantly, $P2Y_{12}$ receptor antagonists such as clopidogrel, prasugrel and ticagrelor are already widely used in clinical practice as antiplatelet agents for the prevention of thrombotic cardiovascular events [86,87]. Moreover, emerging evidence suggests that some of these drugs may also exert additional vascular or autonomic effects beyond platelet inhibition [61,88,89,90]. However, the precise physiological and clinical relevance of these actions is not yet completely understood.

On the whole, these lines of evidence support the concept that P2Y receptors represent promising modulatory targets in cardiovascular disease. Nevertheless, further experimental and translational studies will be necessary to determine the therapeutic feasibility, selectivity and safety of targeting peripheral purinergic pathways under pathological conditions.

7.5 Integrative Perspective

Taking everything into account, the findings analysed in this review support a model in which P2Y receptors form an integrated modulatory network that coordinates vascular tone, cardiac output, sensory neurogenic vasodilatation and autonomic modulation of cardiac function. This organization operates independently of central baroreflex control, as demonstrated in the pithed rat model, but mirrors similar principles observed within central autonomic nuclei [1,61].

By linking receptor pharmacology to systemic cardiovascular responses, purinergic signalling emerges as a unifying framework for understanding how local and global mechanisms interact to maintain arterial pressure and cardiac function. These insights provide a strong foundation for future studies aimed at harnessing P2Y receptor pathways in the treatment of cardiovascular and autonomic pathologies.

8. Limitations and Future Directions

8.1 Unresolved Issues/Questions

Despite the progress in the pharmacological characterization of P2Y receptors involved in cardiovascular mod-

ulation, several aspects regarding their physiological and pathological relevance remain unclear. In this context, most of the currently available evidence derives from experimental animal models, particularly reflex-independent *in vivo* preparations and *in vitro* pharmacological studies, whereas direct translational evidence in humans is still limited [9,19]. Among other unresolved issues/questions, the following stand out:

(i) The relative contribution of $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$ receptors under physiological and pathological conditions. For example, studies in healthy pithed rats have demonstrated a functional segregation between the $P2Y_1$ receptors that inhibit the vasodepressor sensory CGRPergic outflow [16] and the $P2Y_{12}$/$P2Y_{13}$ receptors that inhibit the sympathetic cardioaccelerator outflow [14]. However, it remains unknown whether these mechanisms are altered during hypertension, diabetes mellitus, heart failure or ischemic injury. In addition, pathological changes in receptor expression, receptor sensitivity or extracellular nucleotide metabolism may disrupt the balance between sensory and sympathetic cardiovascular modulation [9].

(ii) The precise cellular localization of P2Y receptors within autonomic pathways. Evidence obtained from *in vitro* studies suggests the role of these receptors in postganglionic sympathetic neurons, sensory CGRPergic fibres, vascular endothelium and vascular smooth muscle [13,91]. Nevertheless, the relative contribution of neuronal and non-neuronal receptor populations in intact *in vivo* animal models remains poorly understood.

(iii) The debate on whether the cardiovascular effects attributed to P2Y receptors are primarily determined by the receptor subtype or by the cellular compartment in which they are expressed and activated. Experimental evidence indicates that $P2Y_1$ receptors contribute to sensory afferent signalling in experimental animal models [29], whereas endothelial $P2Y_1$ receptors mediate NO-dependent vasorelaxation in vascular tissues [82]. These observations suggest that the physiological consequences of $P2Y_1$ receptor activation may differ substantially depending on whether signalling occurs in the sensory, endothelial, or autonomic compartments. Consequently, therapeutic strategies targeting P2Y receptors may require selective modulation of the compartment rather than global receptor inhibition.

(iv) The interpretation of studies using ADPβS as a stable ADP analogue. Although ADPβS has proven to be a valuable pharmacological tool in both *in vivo* and *in vitro* experimental models due to its resistance to degradation by ectonucleotidases [19], endogenous ADP signalling is highly dynamic and tightly regulated by ectonucleotidases such as CD39 and CD73 [73]. Therefore, the extent to which ADPβS reproduces the physiological response of endogenous ADP remains uncertain.

(v) The interaction between purinergic signalling and other neuromodulatory systems requires further investigation. Experimental evidence in rodents and *in vitro* neuronal preparations suggests functional interactions between purinergic, adrenergic, serotonergic and CGRPergic pathways during cardiovascular modulation [92,93]. However, the physiological and pathological relevance of these interactions remains poorly defined, particularly in humans.

(vi) While the pithed rat model has proven particularly useful for studying peripheral neurogenic cardiovascular mechanisms *in vivo* [21,67], this preparation eliminates central autonomic reflex integration. Consequently, further studies combining reflex-independent animal models, intact *in vivo* preparations and translational approaches will be necessary to fully integrate peripheral P2Y receptor pharmacology with central autonomic cardiovascular regulation.

8.2 Future Directions for Drug Development

As previously pointed out, the pharmacological profile of the P2Y receptors mediating cardiovascular responses suggests that these receptors may represent potential therapeutic targets for cardiovascular disorders associated with autonomic imbalance and neurovascular dysfunction. However, most of the currently available evidence derives from experimental animal models and *in vitro* studies; therefore, additional translational studies in humans will be required to determine the clinical relevance of these mechanisms.

For example, the ability of G_i-coupled $P2Y_{12}$ and $P2Y_{13}$ receptors to inhibit the sympathetic cardioaccelerator activity is particularly relevant [14]. Hence, in pathological conditions characterized by chronic sympathoexcitation, such as hypertension, heart failure and diabetic autonomic dysfunction, selective blockade of these receptors may help reduce cardiovascular overstimulation without abolishing physiological autonomic modulation. Moreover, from a translational perspective:

(i) $P2Y_{12}$ receptors are already recognized as clinically relevant therapeutic targets given that antagonists such as clopidogrel, prasugrel and ticagrelor are widely used in humans as antiplatelet agents for the prevention of thrombotic cardiovascular events [94]. Nevertheless, most therapeutic strategies have focused primarily on platelet aggregation, whereas the possible contribution of $P2Y_{12}$ receptors to autonomic and neurovascular cardiovascular modulation remains largely unexplored. Consequently, future studies should determine whether currently available $P2Y_{12}$-targeted drugs can also exert beneficial or adverse effects on autonomic cardiovascular modulation.

(ii) $P2Y_{12}$ receptor antagonists, such as ticagrelor, exert vascular effects in humans that cannot be explained exclusively by its antiplatelet activity [95]. These findings support the concept that $P2Y_{12}$ receptors may participate in vascular and autonomic regulation beyond haemostasis. Likewise, experimental studies suggest that $P2Y_{12}$ and $P2Y_{13}$ receptors contribute to autonomic signalling within sympathetic pathways in cardiovascular disease models

[83]. These findings highlight the need for tissue- and subtype-selective ligands capable of discriminating between neuronal, vascular and platelet P2Y receptors.

On the other hand, the fact that $P2Y_1$ receptors participate in sensory CGRPergic [16] and endothelial vasodepressor [22] mechanisms suggests that modulation of $P2Y_1$ receptor signalling could influence vascular resistance and endothelial function during pathological states associated with neurovascular dysfunction or impaired vasodilatation. Nonetheless, since $P2Y_1$ receptors are also involved in platelet activation and vascular signalling, selective therapeutic modulation would require careful evaluation to avoid undesirable haemodynamic or thrombotic effects.

Furthermore, extracellular nucleotide metabolism cannot be overlooked, in which the ectonucleotidases CD39 and CD73 crucially modulate the availability of extracellular ADP and, consequently, the activation of P2Y receptors [73]. Thus, the modulation of extracellular nucleotide metabolism could represent an indirect therapeutic strategy for modulating purinergic cardiovascular signalling. However, the physiological complexity of extracellular purine metabolism may hinder the development of targeted therapeutic interventions.

In summary, the aforementioned perspectives and lines of reasoning position P2Y receptors as promising, though still poorly understood, pharmacological targets in cardiovascular disease. Future translational studies integrating evidence from molecular pharmacology and *in vitro* studies, as well as from experimental animal models and clinical research will be essential to determine the therapeutic feasibility and safety of modulating peripheral purinergic pathways in humans.

8.3 Future Experimental Approaches and Transgenic Animal Models

Based on the aforementioned perspectives and knowledge, future mechanistic studies combining molecular biology, pharmacological (*in vitro* and *in vivo*) and transgenic approaches will be essential for more precisely characterizing the specific cardiovascular functions mediated by P2Y receptor subtypes [9,19]. With these approaches in mind, and considering that ectonucleotidases, such as CD39, modulate local extracellular ADP concentrations and influence vascular adaptation to haemodynamic stimuli [96], forthcoming research should define receptors' localization and function across the sensory, autonomic, endothelial, and vascular compartments. These strategies could help identify viable therapeutic targets for modulating cardiovascular homeostasis under conditions of chronic sympathoexcitation and neurovascular dysfunction.

One important limitation of pharmacological studies is the incomplete selectivity of several currently available P2Y receptor ligands. While antagonists such as MRS2500, PSB0739, and MRS2211 have been valuable experimental tools for identifying the involvement of $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$ receptors, respectively, the possibility of off-target effects or partial receptor overlap cannot be completely excluded, in particular for MRS2211. Indeed, the availability of highly selective ligands for the $P2Y_{13}$ receptor is limited [1,2,8,9,10,11]; this drawback represents a major restriction for the pharmacological characterization of this receptor subtype, especially at high doses [13,74]. Consequently, complementary approaches using genetically modified animal models will be required to validate pharmacological findings obtained *in vivo*.

In this regard, transgenic animal models, particularly receptor knockout and knock-in mice, may provide critical information regarding the specific contribution of individual P2Y receptor subtypes to cardiovascular regulation. Global knockout models lacking $P2Y_1$, $P2Y_{12}$ or $P2Y_{13}$ receptors could help determine whether the cardiovascular phenotypes identified in pharmacological studies truly depend on these receptor populations. Likewise, conditional or tissue-specific knockout models may allow differentiation between neuronal, endothelial, vascular smooth muscle and sensory receptor populations involved in cardiovascular modulation [97,98].

Further studies should also investigate whether P2Y receptors undergo adaptive changes during pathological conditions such as hypertension, diabetes mellitus, heart failure or ischemic injury. Experimental animal models of chronic cardiovascular disease could provide important information on potential alterations in receptor expression, receptor sensitivity or extracellular nucleotide metabolism under conditions of sustained autonomic dysfunction [9,93]. In addition, combining molecular approaches with reflex-independent models such as the pithed rat may help elucidate the relationship between peripheral purinergic signalling and cardiovascular autonomic imbalance [21,67].

Another promising area for future research involves the integration of electrophysiological, molecular and imaging techniques. Experimental studies using calcium imaging, optogenetics or electrophysiological recordings in autonomic neurons and sensory fibres can help define the precise cellular mechanisms by which P2Y receptors modulate the release of neurotransmitters and neuropeptides. Similarly, molecular studies evaluating receptor distribution and signalling pathways in animal tissues and human cardiovascular samples could improve the translational interpretation of current pharmacological findings [74,99].

Undoubtedly, further translational studies in humans will be necessary to determine the clinical relevance of the purinergic cardiovascular modulation identified in experimental models. While current evidence strongly supports the participation of P2Y receptors in neurovascular cardiovascular regulation in animals, the physiological and pathological significance of these mechanisms in humans is not yet completely understood. Therefore, integrating experimental pharmacology, molecular biology, and translational

cardiovascular research will be essential to establishing the therapeutic potential of targeting peripheral purinergic signalling pathways.

8.4 Translational Perspective

The available evidence, considered as a whole: (i) supports the concept that P2Y receptors play a role in cardiovascular modulation through functionally segregated sensory, autonomic, vascular, and endothelial mechanisms; and (ii) provide a mechanistic framework for understanding how peripheral purinergic signalling may contribute to cardiovascular dysfunction under conditions characterized by autonomic imbalance, endothelial dysfunction and neurovascular dysregulation [33,61].

From a translational perspective, the development of subtype- and tissue-selective pharmacological strategies could ultimately allow for the differential modulation of sensory CGRPergic, sympathetic and endothelial P2Y receptor populations without globally interfering with physiological purinergic signalling. In this regard, the integration of experimental pharmacology, molecular biology, transgenic animal models and clinical cardiovascular research will be essential to determine the therapeutic feasibility and safety of targeting peripheral purinergic pathways in humans [61,88,89,90].

9. Conclusions

Altogether, the insights, ideas and lines of evidence considered in this review position P2Y receptors as key integrative elements that coordinate sensory, autonomic and cardiovascular mechanisms across multiple anatomical levels, extending from central autonomic nuclei to peripheral neurovascular and neurocardiac junctions [17,61]. Accordingly: (i) P2Y receptor subtypes do not operate redundantly, but rather exert distinct and complementary cardiovascular functions (e.g., $P2Y_1$ receptors modulate vasodepressor and sensory CGRPergic mechanisms [16,22,70,100], whereas $P2Y_{12}$ and $P2Y_{13}$ receptors inhibit sympathetic cardioaccelerator pathways) [14]; and (ii) the physiological consequences of P2Y receptor activation may depend not only on the receptor subtype involved, but also on the cellular and neuroeffector compartment in which signalling occurs, including sensory, autonomic, endothelial, cardiac and vascular domains.

Additionally, the interpretation of purinergic cardiovascular responses in intact or anaesthetised animals is frequently hampered by supraspinal reflexes and central autonomic integration [22,23]. By eliminating these influences, while preserving peripheral autonomic and sensory pathways [50,67], the pithed rat model has enabled precise pharmacological characterization of peripheral cardiovascular mechanisms mediated by P2Y receptors. In this context, ADPβS has proven to be a valuable experimental tool for identifying P2Y receptor subtype-specific contributions to neurogenic cardiovascular modulation [15,16,22,23,101].

From a systems-level physiological perspective, there exists an integrated purinergic modulatory network capable of fine-tuning cardiovascular homeostasis through the coordinated modulation of sensory vasodilatation, vascular tone, and sympathetic autonomic activity [1,19,61]. Importantly, this organization appears to involve highly localized purinergic microdomains in which extracellular nucleotide metabolism, receptor subtype distribution and compartment-specific signalling collectively determine the final cardiovascular response.

From a translational standpoint, the functional specialization of P2Y receptor subtypes highlights potentially relevant therapeutic opportunities in cardiovascular disorders associated with autonomic imbalance, endothelial dysfunction and neurovascular dysregulation [52,61,82,85]. In particular, selective modulation of sensory $P2Y_1$ receptor pathways or sympathetic $P2Y_{12}$/$P2Y_{13}$ receptor populations could represent useful strategies for modulating peripheral vascular resistance and excessive sympathetic cardiovascular activation. Moreover, the clinical use of $P2Y_{12}$ receptor antagonists raises the possibility that these drugs may exert additional vascular or autonomic effects beyond platelet inhibition [61,88,89,90].

Finally, in the overall picture, the lines of evidence analysed in this review support the concept that P2Y receptors constitute a crucial and previously underestimated component of cardiovascular modulation, both under physiological and pathological conditions. Forthcoming studies integrating targeted pharmacological tools, molecular approaches, transgenic animal models and translational cardiovascular research will be essential to more precisely define the therapeutic potential of targeting peripheral purinergic signalling pathways. While several important issues/questions remain unresolved, these findings define clear directions for future research (e.g., investigating how purinergic activation interfaces with peptidergic vasodilator systems, particularly CGRP, across different vascular territories and physiological conditions) [83,100].

Abbreviations

ADP, adenosine diphosphate; ADPβS, adenosine 5′-O-(β-thio)-diphosphate; AMP, adenosine monophosphate; AP5, (2R)-amino-5-phosphonopentanoic acid; ATP, adenosine triphosphate; BBB, blood-brain barrier; CGRP, calcitonin gene-related peptide; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; CNS, central nervous system; DRG, dorsal root ganglion; G_i, inhibitory G protein alpha subunit; GPCR, G protein-coupled receptor; G_q, stimulatory G protein alpha subunit coupled to phospholipase C; NO, nitric oxide; PLC, phospholipase C; PPADS, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid; UDP, uridine diphosphate; UTP, uridine triphosphate.

Author Contributions

RCSV conceptualized the review article, performed the literature search, analysed and interpreted the literature, prepared the original draft, and designed the figures and table. KAH contributed to the interpretation of the literature, provided scientific expertise on purinergic pharmacology, and critically revised and edited the manuscript. BVC contributed to the literature search, data collection and analysis, and critically revised the manuscript. AMvdB contributed to the interpretation and analysis of the literature, and critically revised and edited the manuscript. CMV conceptualized and supervised the review article, prepared the original draft, contributed to the interpretation of the literature, critically revised and edited the manuscript, and coordinated the project. In addition, all authors have read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

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Conflicts of Interest

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Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used ChatGPT (OpenAI, GPT-5.3 under licence) for language editing and translation purposes in some parts of the manuscript. After using this tool, the authors carefully reviewed and edited the manuscript to ensure accuracy, coherence, and originality, and took full responsibility for the content of the publication. The scientific content, literature review, critical analysis, interpretations, and conclusions were entirely developed by the authors.

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