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Approaches for designing and discovering Purinergic Drugs for Gastrointestinal Diseases

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APPROACHES FOR DESIGNING AND DISCOVERING PURINERGIC DRUGS FOR GASTROINTESTINAL DISEASES

Abstract

Introduction: Purines finely modulate physiological motor, secretory, and sensory functions in the gastrointestinal tract. Their activity is mediated by the purinergic signalling machinery, including receptors and enzymes regulating their synthesis, release and degradation. Several gastrointestinal dysfunctions are characterized by alterations affecting the purinergic system.

Areas covered: the authors provide an overview on the purinergic receptor signalling machinery, the molecules and proteins involved and a summary of medicinal chemistry efforts aimed at developing novel compounds able to modulate the activity of each player involved in this machinery. The involvement of purinergic signalling in gastrointestinal motor, secretory, and sensory functions and dysfunctions, and the potential therapeutic applications of purinergic signalling modulators, are then described.

Expert opinion: A number of preclinical and clinical studies demonstrate that the pharmacological manipulation of purinergic signalling represents a viable way to counteract several gastrointestinal diseases. At present the paucity of purinergic therapies is related to the lack of receptor-subtype-specific agonists and antagonists that are effective *in vivo*. In this regard, the development of novel therapeutic strategies should be focused to include tools able to control the P1 and P2 receptor expression as well as modulators of the breakdown or transport of purines.

Keywords: adenosine; ADP; ATP; gastrointestinal diseases; gut; intestine; purinergic receptors; purinergic signalling.

Article highlights

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- Extracellular purine nucleosides and nucleotides, like adenosine or ATP, modulate a variety of cellular functions through the interaction of membrane proteins called purinergic receptors; the extracellular levels of those nucleosides and nucleotides are regulated by a set of enzymes and transporters.
- Medicinal chemistry efforts developed synthetic modulators for receptors and enzymes involved in the purinergic signalling machinery; standard ligands are available for all these protein players.
- In the gastrointestinal tract, purine nucleosides and nucleotides modulate motor, secretory, and sensory functions and dysfunctions.
- Purinergic signalling alterations may occur due to abnormal receptor expression and/or extracellular nucleoside/nucleotide levels; these factors have a role in both onset and development of gastrointestinal disorders.
- Pharmacological studies showed that the administration of synthetic purinergic receptor/enzyme modulators helps to restore normal motor, secretory, and sensory functions; these data suggest the development of potent and selective purinergic signalling modulators as novel therapeutic tools for gastrointestinal diseases.

This box summarizes key points contained in the article.

List of abbreviations

ATP = adenosine triphosphate

ADP = adenosine diphosphate

AMP = adenosine monophosphate

Ado = adenosine

GI = gastrointestinal

P2XR = P2X receptor

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P2YR = P2Y receptor

NTPDase = nucleoside triphosphate diphosphohydrolase

5'-NT = 5'-nucleotidase

AR = adenosine receptor

ADA = adenosine deaminase

ENT = equilibrative nucleoside transporter

CNT = concentrative nucleoside transporter

ADK = adenosine kinase

oATP = oxidized-ATP

NECA = *N*-ethylcarboxamidoAdo

MECA = *N*-methylcarboxamidoAdo

NBMPR = nitrobenzylthioinosine

CNS = central nervous system

IBD = inflammatory bowel disease

IBS = irritable bowel syndrome

DRG = dorsal root ganglions

TNBS = trinitrobenzenesulfonic acid

CFTR = cystic fibrosis transmembrane conductance regulator

VAMP = vesicle-associated membrane protein

SNAP = synaptosomal-associated protein

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1. Introduction

In the early 1970s, set of pioneering studies performed by Prof. Geoffrey Burnstock, highlighted a relevant role of purine nucleotides/nucleosides in the physiological control of digestive functions, providing evidences that adenosine triphosphate (ATP) and its related nucleotides/nucleosides (i.e., adenosine diphosphate, ADP, adenosine monophosphate, AMP, and adenosine, Ado) operated as transmitters at the intestinal level [1]. Despite the initial hard criticism, a great deal of evidence demonstrated the existence of specific receptors, designated as purinergic receptors and classified as P1 and P2 (for Ado and ATP/ADP, respectively) [1]. Such receptors appeared widely and heterogeneously distributed throughout the gut, thus corroborating their relevant contribution to the regulation of digestive functions [1-3]. In particular, it has been observed that the gastrointestinal (GI) cells are literally soaked into a "biological sea" of functionally active nucleotides and nucleosides, which carry out the critical task of shaping various cellular functions, thus actively participating at the maintenance of gut homeostasis [4].

In this regard, the current body of knowledge highlighted a pivotal role played by the purinergic pathways in the integration of enteric functions, representing an ideal bridge among different structures, such as the neuromuscular or mucosal layer and the immune cells, which strictly collaborate for the preservation of GI health [5]. Indeed, the intestine holds the capability of generating sets of immune-neural responses aimed at triggering stereotypical and specific programs of coordinated mucosal secretion and motor activity, to maintain the enteric functional integrity [6].

In this context, while on the one hand the purinergic pathway takes a pivotal part in orchestrating and integrating the physiological gut functions, on the other hand it is emerging as a critical point in several GI dysfunctions [1,7,8]. Alterations of the purinergic signalling, in terms of receptor populations and/or variations of extracellular nucleotide/nucleoside concentrations, seem to be intimately involved in the onset and development of several gut disorders [2,4]. In particular, it has been widely demonstrated that in the presence of inflammation, several pro-inflammatory cytokines

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such as TNF and INF-γ can induce an alteration in the purinergic receptor expression (i.e. up-regulation of $P2Y_1$, $P2Y_2$, $P2Y_6$, $P2X_3$, $P2X_7$, A_{2A} and A_{2B} receptors or down-regulation of A_3 receptors), along with an increase in the expression and activity of adenosine deaminase (ADA) [9-16].

Based on this background, current research efforts are focused on achieving a more detailed characterization of the various components of purinergic pathways in an integrated manner, with the aim to identify novel molecular targets for the development of innovative pharmacological approaches to manage several digestive disorders.

This review provides a critical appraisal of the available knowledge about the involvement of purinergic pathways in the pathophysiological mechanisms underlying digestive diseases, pointing out the possibility of counteracting such dysfunctions through pharmacological interventions on purinergic molecular targets.

PLEASE INSERT FIGURE 1 HERE

2. Purinergic system: receptors, metabolic pathways and transporters

Purinergic signalling arise from nucleotides released into the extracellular milieu via anion channels, exocytotic pathways, transporters, connexins or pannexins, or in response to biochemical or mechanical/physical stimuli as well as in the presence of adverse events (i.e. membrane damage) [4]. Once released into the extracellular space, ATP, ADP, UTP, UDP, or UDP-glucose engage the P2 receptors, heterogeneously distributed in small and large intestine (see Table 1), participating at the modulation of several cellular responses. The P2 receptors, accordingly to their different intracellular signalling pathways and selectivity toward nucleotides, are currently classified into ionotropic P2X (P2X1–7) and metabotropic P2Y ($P2Y_{1,2,4,6,11–14}$) receptors (P2XRs and P2YRs, respectively) [17]. While P2XRs are ligand-gated ion channels, permeable to Na^+, K^+, and Ca^{2+} cations, and

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endogenously activated only by extracellular ATP [18,19], P2YRs are G protein-coupled receptors (GPCRs) whose endogenous ligands are ATP, ADP, UTP, UDP, and UDP-glucose, depending on the P2YR subtype considered [20]. ATP and/or ADP undergo a quick conversion into AMP operated by CD39 (ecto-nucleoside triphosphate diphosphohydrolase 1, E-NTPDase1), and then, via CD73 (ecto-5'-nucleotidase or 5'-NT), AMP is dephosphorylated into Ado [21,22]. The biological actions of Ado, the most important metabolite deriving from the ATP degradation, are mediated by GPCRs named adenosine receptors (ARs) and currently distinguished into four subtypes: A_1, A_{2A}, A_{2B} and A_3 ARs. The A_1 and A_3 ARs are coupled to G_i, G_q and G_o proteins, while the A_{2A} and A_{2B} ARs activate adenylyl cyclase via G_s or G_{olf}. Of note, the engagement of A_{2B}AR can trigger also phospholipase C via G_q [23]. UTP is hydrolysed as well into UDP; both molecules (and the glycosylated derivative UDP-glucose) act as P2YR agonists with different degrees of potencies at the various P2YR subtypes. Fig. 1 presents a schematic overview of the purinergic signalling machinery.

It has been widely demonstrated that the majority of purinergic processes, beyond to be strictly related to the expression of purinergic receptors, are finely tuned by few dominant enzymes. These proteins are actively involved in shaping the composition of the purinergic signalling, based on the tissue metabolic conditions [24-26]. Beyond the CD39/CD73 enzyme axis, critically acting as "immunological switches" able to shift an ATP-driven pro-inflammatory immune cell activity toward an anti-inflammatory state mediated by Ado [22,27], other enzymes and transporters operate to calibrate the purinergic signalling. In particular, ADA, a key enzyme involved in the degradative pathway for Ado to inosine, represents another critical checkpoint in the regulation of extracellular Ado levels and, consequently, in the control of receptor stimulation either under physiological or pathological conditions [25,28]. In this regard, several lines of evidence reported that a chronic increase of Ado can be harmful to host tissues, leading to the development of tissue injury [29,30]; therefore, ADA exerts a pivotal role in counteracting excessive extracellular Ado production, thus avoiding such potential toxicity [30]. However, pathological events such ischemia are associated with

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a chronic increase in ADA activity that can irreversibly eliminate adenylates from the metabolite pool, with consequent impairment of tissues energetics [31]. A study performed in post-reperfused patients with myocardial infarction, showed an increase in ADA activity in erythrocytes along with a reduction of adenylates and Ado [32]. In the same study, the authors also observed that the reduction of Ado lead to enhanced production of free radicals, which may be the major contributor to reperfusion injury [32]. Accordingly, the blockade of ADA with selective antagonists attenuate ischemic injury and improve post-ischemic recovery through enhancement of endogenous Ado levels and preserving ATP tissue levels [31,33].

In parallel, under physiological conditions, the levels of purines are finely tuned also by the activity of the nucleoside transporters [34]. Nowadays, these transporters are classified as: *a)* equilibrative nucleoside transporters (ENTs), designated as ENT1, ENT2, ENT3 and ENT4, which transport nucleosides across cell membranes in either directions, based on concentration gradients; *b)* concentrative nucleoside transporters (CNTs), classified in CNT1, CNT2 and CNT3, promoting the intracellular influx of nucleosides against their concentration gradient, using the sodium ion gradient across cellular membranes as source of energy [35]. Once transported intracellularly, Ado gets phosphorylated to AMP by the intracellular adenosine kinase (ADK) enzyme, which controls the poly-phosphorylation of Ado to ATP. Intracellular Ado may also be converted to inosine by the intracellular ADA [26].

PLEASE INSERT TABLE 1 HERE

3. Medicinal chemistry of the purinergic system: reference ligands and pharmacological probes

Since the '80s, medicinal chemistry efforts were made to develop compounds able to potently and/or selectively stimulate or inhibit receptors or enzymes involved in the purinergic signalling system. Various compounds were provided to the community as pharmacological probes to evaluate the

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physio-pathological roles of specific receptor/enzymes or as candidates for preclinical and clinical evaluation.

PLEASE INSERT FIGURE 2 HERE

3.1. P2X receptor ligands

Agonists of the P2XRs were developed based on the structure of the endogenous ligand ATP, modified at the triphosphate chain, the purine moiety, or the ribose ring [18,50,51]. The low chemical stability of ATP was at the basis of the development of αβ-meATP and ATPγS (Fig. 2, ATPγS active also at P2YRs), or βγ-meATP (Fig. 2), compounds endowed with agonist activity at the P2XRs and less susceptible to degradation by ectonucleotidases. Modifications of the purine moiety or the ribose ring led to compounds like 2-meSATP (Fig. 2) and BzATP [18], compounds active also at the P2YRs. Inhibitors of the P2XRs were obtained as ATP competitive antagonists or as non-competitive inhibitors. Some of these derivatives were developed again as ATP derivatives, modified at the sugar ring to obtain i.e. oxidized-ATP (oATP, irreversible antagonist of the P2X7 receptor) or the potent P2X1/P2X3 inhibitor TNP-ATP (Fig.2) [18,50]. An ATP-competitive inhibitor of the P2X3 receptor was also obtained even if not as ATP derivative, A-317491 (Fig. 2) [50]. Further classes of P2XRs inhibitors were obtained based on various structural classes like suramin-like analogues [18] or anthraquinones (i.e. the P2X2 selective antagonists PSB-10211 and PSB-1011) [52]. Some derivatives behave as inhibitors of both P2XRs and P2YRs, like suramin or PPADS (Fig. 2). A significant number of P2X7 inhibitors were developed and reported due to the great interest for this P2XR subtype as possible therapeutic target [53,54]. Among these compounds, AZD9056 (Fig. 2) was clinically tested for the treatment of moderately to severely active Crohn's Disease, showing to improve symptoms in patients [55]. Compounds able to inhibit the P2X4R at sub-micromolar (5-

BDBD [56], NP-1815-PX [57], and BX430 [58]) or micromolar (PSB-12062 [59]) concentrations were also reported.

3.2. P2Y receptor ligands

Agonists of P2YRs were developed based on the endogenous ligands similarly to the P2XRs. Reference P2YR agonists are chemically stable analogues of ATP, ADP, UTP, and UDP, or compounds obtained by modifying the purine or pyrimidine base or the sugar ring of these nucleotides. Among these molecules are the above cited ATPγS, 2-meSATP (Fig. 2) and BzATP, and compounds like 2-meSADP and 2-thioUTP (Fig. 2) [20,60,61]. Modifications of endogenous nucleotide ligands led also to the development of P2YR antagonists. In this category are cangrelor and ticagrelor (Fig. 2), selective $P2Y_{12}$ inhibitors available in therapy as platelet anti-aggregatory agents. Further P2YR inhibitors were developed by inserting phosphate groups in both the 3' and 5' positions of Ado to obtain the so-called bisphosphate derivatives. Further modifications of the purine core and/or the ribose ring led to compounds with improved affinity or selectivity (i.e. MRS 2179, MRS2279, MRS 2500, selective $P2Y_1$ antagonists; Fig. 2). Irreversible inhibitors of the P2YRs were also obtained. These compounds need an enzymatic transformation in the liver to become active metabolites and to irreversibly bind the target [62-64]. Among these derivatives are clopidogrel, ticlopidine, and prasugrel (Fig. 2), selective $P2Y_{12}$ inhibitors available in therapy as platelet anti-aggregatory tools. Recently, 2-(phenoxypyridine)-3-phenylureas were developed as $P2Y_1$ inhibitors. Among these molecules, compound BPTU (Fig. 2) showed to inhibit the receptor with sub-micromolar potency. Its allosterical mechanism of action was elucidated by the aid of X-ray crystallography [65,66].

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3.3. Adenosine receptor ligands

A relevant number of potent and subtype selective AR ligands have been developed to date [67-71]. A key modification of the endogenous agonist Ado was made at the 5'-position of the sugar with the insertion of an alkylcarboxamido group, to obtain the so-called NECA (*N*-ethylcarboxamidoAdo, Fig. 3) or MECA (*N*-methylcarboxamidoAdo). NECA is a non-selective standard AR agonist. Generally, the AR agonists are Ado or NECA/MECA derivatives, presenting various substituents in the 2- or N^6-position of the purine core and/or additional modifications at the ribose ring. In the N^6-position of the nucleoside, the A_1AR agonists often present a cycloalkyl group (i.e. CPA, CCPA, CHA, Fig. 3), while the A_3AR agonists present small alkyl groups or an iodobenzyl substituent (IB-MECA or Cl-IB-MECA, Fig. 3) and the A_{2A}AR agonists generally present an unsubstituted 6-amine. At the 2-position, the A_1AR agonists often are unsubstituted or present small groups, while the A_{2A}AR agonists generally bear large substituents with a mix of polar and non-polar functional groups (i.e. ATL-146e or CGS 21680, Fig. 3). Among these compounds is regadenoson, clinically used as coronary vasodilator. The most selective A_3AR agonists may present a 2-halogen atom or 2-arylalkynyl substituents. In the last years, non-nucleoside agonists of the ARs were reported (i.e. capadenoson, Fig. 3), generally endowed with A_1AR selectivity [72], even if selective A_{2A}AR and A_{2B}AR (i.e. BAY 60-6583, Fig. 3, the only one selective A_{2B}AR agonist currently available) agonists were also obtained. Antagonists of the ARs have been obtained based on heterocyclic scaffolds. Several of these derivatives are xanthine-based compounds, like DPCPX or 8-CPT (A_1AR antagonists, Fig. 3), DMPX (A_{2A}AR antagonist, Fig. 3) and analogues, istradefylline (A_{2A}AR antagonist clinically approved for Parkinson's Disease, Fig. 3), and PSB 603, MRS 1754, or 8-SPT (A_{2B}AR antagonists, Fig. 3), compounds frequently used as pharmacological probes. Non-xanthine-based AR antagonists were developed by using various mono-, bi-, and tri-cyclic scaffolds, obtaining potent and subtype selective compounds (i.e. ZM241385, MRS 1220, and MRS 1334, Fig. 3) [67-71].

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3.4. Enzyme and transporter ligands

Medicinal chemistry efforts were made also to develop compounds able to modulate the activity of enzymes and transporters tuning the levels of extracellular nucleotides and nucleosides.

NTPDase (CD39) enzymes hydrolyse di- and tri-phosphate purine/pyrimidine nucleotides to obtain the corresponding mononucleotide analogues. Inhibitors of these enzymes were initially developed as purine nucleotides with modifications in the 8-, 2-, or N^6-position of the purine core (i.e. ARL 67156, Fig. 4), and/or with the phosphate chain composed by a various number of phosphate groups or modified to get chemically stable analogues (i.e. ARL 67156, Fig. 4) [73,74]. NTPDase inhibitory activity was observed also for compounds acting on P2 receptors, like suramin or PPADS (Fig. 2). Anthraquinone-based NTPDase inhibitors (i.e. PSB 069 and PSB 06126, Fig. 4) were recently developed and reported [73,74].

5'-NT (CD73) enzymes hydrolyse AMP to Ado. Inhibitory activity against these proteins was observed for the endogenous compound ADP. An ADP stable analogue (αβ-meADP, Fig. 4) was hence developed and is used as standard inhibitor [73,74]. Modifications in the N^6-position of this compound led to the development of potent 5'-NT inhibitors [75]. Combining modifications in the 2- and N^6-position led to compound AB680 (Fig. 4), a picomolar inhibitor of 5'-NT with no residual activity on NTPDase [76]. Currently, this compound is in clinical evaluation for the treatment of gastrointestinal malignancies [76]. 5'-NT inhibitory activity was observed also with non-nucleotidic structures, based on anthraquinone or flavonoid scaffolds [73].

Modulators of the nucleoside transporters (both ENTs and CNTs) activity were developed based on purine or pyrimidine nucleoside structure. Among these compounds is the ENT1 inhibitor nitrobenzylthioinosine (NBMPR, Fig. 4) [77]. Another purine nucleoside derivative is 5-iodotubercidin (Fig. 4), a potent ADK inhibitor able also to inhibit nucleosides transporters. Modification of uridine led to the recent development of the compound MeThPmR (Fig. 4), inhibitor

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of CNTs as well as its 2'-deoxy analogue [78]. Non-nucleoside inhibitors (Fig. 4) of nucleoside transporters are dilazep, dipyridamole, and lidoflazine, compounds based on various scaffolds.

Enzymes able to modify Ado (like ADA and ADK) directly or indirectly modulate the extracellular concentration of this nucleoside. Ado is modified to inosine by ADA, and this degradation occurs also intracellularly. Inhibitors of ADA have been developed based on nucleoside or non-nucleoside scaffolds [25]. Among the nucleoside-based ADA inhibitors are 2'-deoxycoformycin (or pentostatin, Fig. 4) and cladribine (or leustatin, Fig. 4), approved by FDA for the treatment of hairy cell leukaemia. Coformycin analogues were developed also as inhibitors of ADA and adenosine 5'-monophosphate deaminase (AMPDA) [79]. Further Ado analogues are 1-deazaadenosine and 8-azaadenosine. Classical non-nucleoside inhibitors of ADA are EHNA (a purine derivative with a long 9-substituent; Fig. 4) and the imidazole-based compound FR221647 (Fig. 4) [25].

Intracellular Ado gets phosphorylated to ATP by ADK [26,80]. Inhibitors of ADK were initially developed as Ado analogues, modified in the purine core (i.e. 5-iodotubercidin, Fig. 4) or in the sugar ring (i.e. 5'-amino-5'-deoxyadenosine). The combination of these modifications led to the development of low-nanomolar or picomolar ADK inhibitors (i.e. GP-515 or A-134974, Fig. 4); additional insertion of substituents in the exocyclic amine group of these nucleosides led to further derivatives with anti-ADK activity. Non-nucleoside ADK inhibitors were also developed, based on heterocyclic cores like pyridopyrimidine or alkynylpyrimidine. An example of these compounds is ABT-702 (Fig. 4), endowed with low nanomolar inhibitory potency [26,80].

PLEASE INSERT FIGURE 4 HERE

4. The purinergic pathways in intestinal disorders associated with motor, secretory and sensory dysfunctions: pathophysiological and pharmacological aspects

Intestinal disorders are an umbrella term that groups several pathological conditions, arising from unsettled integrations of signals from the central nervous system (CNS), the enteric neuromuscular/mucosal compartment and/or the immune system, leading to the onset and development of digestive disorders, including secretive/reabsorptive, motor and sensorial alterations [4]. Nowadays, several features characterizing such intestinal diseases are still scarcely characterized and the available therapeutic tools appear insufficiently satisfactory, or allow only a scarce symptom relief [4]. Over the years, a number of evidences pointed out a critical role of purinergic system in shaping various enteric functions, revealing also a marked alteration in the purinergic machinery in the presence of several intestinal diseases (see Table 2), suggesting an involvement in the pathophysiology of gut disorders and indicating novel putative therapeutic targets [4,81].

4.1. Visceral pain

Visceral pain is a hallmark feature of several GI diseases such as inflammatory bowel diseases (IBDs), irritable bowel syndrome (IBS), intestinal ischemia, diverticular disease and intestinal obstruction.

Visceral hypersensitivity, less characterized than other pain syndromes, refers to a decreased pain threshold following nociceptor activation, or to an exaggerated response to the painful stimulus [82]. The persistence of inflammatory mediators as well as an abnormal visceral stimulation activate and sensitize the primary afferent nerves and recruit previously silent nociceptors, leading to an increase in pain sensitivity (peripheral sensitization). This amplified sensory transmission activates intracellular signalling cascades within spinal dorsal horn neurons, leading to an amplified central neuronal activity (central sensitization), which intensifies the effects of signals coming from the affected viscera [82,83]. Over the last years, research efforts have been focused on the therapeutic potential of endogenous mediators released during GI dysfunctions and actively involved in pain perception. In this regard, purinergic receptors have been proposed to be involved in the

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rearrangement of enteric sensory pathways. Indeed, several reviews have exhaustively described the anti-nociceptive effects following the pharmacological modulation of such receptors [84-88]. Several authors demonstrated that the activation of P2 receptors in dorsal root ganglions (DRG) and spinal cord dorsal horns, via endogenous ATP, modulates both the nociceptor excitability and the transduction of acute algogenic stimuli [89,90]. In particular, Wirkner et al. showed that homomeric P2X3 and heteromeric P2X2/P2X3 receptors mediate the most of pro-nociceptive responses [91], whereas $P2Y_1$ and $P2Y_2$ receptors are more involved in the algogenic transmission [92-96]. In support to this view, a recent study demonstrated that the selective stimulation of P2YRs sensitizes visceral neurons and directly excites both rodent and human colonic nociceptors, suggesting a P2Y-dependent mechanism in the generation of visceral pain during GI diseases [97]. However, several reports demonstrated an anti-nociceptive effect mediated by ATP, via $P2Y_{12}$ and $P2Y_{13}$ receptor activation in sensory neurons, and by UTP or UDP, through the stimulation of spinal $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors [98,99]. Therefore, based on current evidence, future investigations are needed to clarify the contribution of P2YRs in visceral pain.

Wynn et al. showed that endogenous ATP, released following an abnormal stretch of the gut wall, induces visceral pain through the activation of P2X3 and P2X2/P2X3 receptors expressed on nociceptive neurons [100]. In line to this view, a more recent study confirmed the contribution of these receptor subtypes, expressed in DRG neurons, in colonic hypersensitivity [101]. Indeed, the systemic administration of non-selective P2XRs antagonists, suramin or PPADS, showed to reduce the abdominal contractions in dose-dependent manner in a mouse model of visceral pain induced by intraperitoneal injection of acetic acid [102]. In this study, the authors also showed that the P2X3 antagonist TNP-ATP was more active than suramin and PPADS in reducing acetic acid-induced pain, with an efficacy comparable to morphine [102]. Likewise, the systemic administration of the selective P2X3 antagonist A-317491 significantly reduced the hyperalgesia associated with chronic inflammation in rats [103]. By contrast, other authors observed discrepant findings on the analgesic

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activity associated with P2X3 receptor blockade [104]. In particular, in several rat models of acute nociception using different noxious stimuli, A-317491 was ineffective in reducing visceromotor responses after colonic distension and inflammation-induced visceral hyperalgesia [104]. Interestingly, pre-clinical evidence have also highlighted an involvement of the purinergic signalling in the modulation of mechanosensory transduction in experimental colitis [100]. In this regard, Wynn et al., observed an increment of ATP release, after colorectal distension, in a rat model of trinitrobenzenesulfonic acid (TNBS)-induced colitis, along with a marked expression of P2X3 receptors in DRG neurons, particularly in those containing calcitonin gene-related peptide [100]. This evidence suggests that this receptor subtype is deeply involved in the mechanosensory transduction during intestinal inflammation [100]. In the same study, the authors also observed that the treatment of rat colorectal preparations with a non-selective A_{2B}AR antagonist 8-SPT showed a smaller effect on the inflamed colon, as compared with normal preparations [100]. These data suggest that Ado plays a minor role in the modulation of mechanosensory transduction during bowel inflammation probably due to less efficient enzymatic breakdown of ATP into Ado, with consequent scarce recruitment of ARs in the presence of colitis [100]. In this setting, a more recent study investigated the effect of the selective P2X3 antagonist A-317491 on visceral sensitivity under physiological conditions, during acute colitis and in the post-inflammatory phase of colitis. The authors showed that P2X3 receptors are not involved in colonic mechanosensitivity under physiological conditions, but interestingly they modulate visceral hypersensitivity during acute TNBS-colitis and in the post-inflammatory phase of colitis [105].

The involvement of purinergic system in the modulation of visceral pain has also been investigated in IBS, a functional GI disorders characterized by abdominal pain or discomfort, bloating, constipation or diarrhea. In this regard, Galligan exhaustively described the role of P2XRs in the transmission of abdominal pain occurring in different IBS models [106]. In this context, Xu et al. investigated the role of P2X3 receptors in the pathogenesis of visceral hypersensitivity associated

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with IBS [107]. In this study, the authors used a rat model of chronic visceral hypersensitivity induced by colonic injection of acetic acid in neonatal rats that results in persistent sensory dysfunction in adults without evident morphologic or inflammatory alterations [107]. In this model, chronic functional visceral hyperalgesia was associated with a significant increase in P2X3 receptors in colon-specific sensory neurons and an enhancement of algogenic ATP-mediated actions via this receptor subtype [107]. More recently, other authors paid the attention on the involvement of P2X7 receptors in the development of visceral hypersensitivity in a mice model of post-infectious IBS induced by *Trichinella spiralis* [108]. The authors demonstrated that P2X7 receptors play a pivotal role in intestinal inflammation and represent a trigger for the development of visceral hypersensitivity associated with post-infectious GI dysfunction [108].

At present, the evidence regarding the participation of endogenous Ado in the pathophysiology and modulation of visceral pain are conflicting, since opposite excitatory or inhibitory actions of Ado, mediated by A_1 or A_{2A} ARs, have been described [87,109]. With regard for A_1AR stimulation, several authors showed an inhibitory effect on pain transmission in rodents, both at pre-synaptic level, through the inhibition of pain-associated neurotransmitter release (i.e., substance P, calcitonin gene-related peptide and glutamate), and at post-synaptic level, through membrane cell hyperpolarization [87,110]. For what it concerns the A_{2A}AR, despite previous studies reported a scarce contribution of this receptor subtype in spinal pain transmission [111], current data demonstrated that the activation of A_{2A}AR trigger the transmission of nociceptive input into the spinal cord [112,113]. Indeed, A_{2A}AR knock-out mice or mice treated with the selective A_{2A}AR antagonist, ZM241385, showed a reduced sensitivity to nociception [112,113]. Interestingly, despite evidence supporting the anti-nociceptive effects of A_{2A}AR antagonists, other authors observed a scarce reduction of algogenic inputs in inflamed A_{2A}AR knockout animals [114]. A plausible explanation of opposite effects of A_1 and A_{2A} ARs on pain transmission depends to a different interaction of these receptor subtypes, expressed on the same nerve terminals, with the different sources of Ado, which are strictly related to the

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underlying pathophysiological condition [115]. However, despite interesting, this hypothesis needs further experimental evidence.

The involvement of Ado to the modulation of visceral pain has fostered the pharmacological research towards the identification of novel analgesics targeting this system. Some authors paid the attention on the potential anti-nociceptive effects of A_1AR agonists or A_{2A}AR antagonists, which have displayed promising efficacy in the reduction of mechanical hyperalgesia [116,117]. Interestingly, other authors observed that the administration of drugs able to increase endogenous Ado levels, such as ADK or ADA inhibitors, significantly reduced the neuronal responses to noxious stimulations at spinal level in several rodent pain models [118-120]. Currently, a limited number of experimental studies have focused the attention to the possible involvement of A_{2B} or A_3 ARs in pain transmission. For instance, Chen et al. showed analgesic effects after treatment with A_3AR agonists in a mouse model of neuropathic pain following chronic constriction injury of the sciatic nerve [121].

Taken together, current data highlight a pro-nociceptive activity of ATP, mediated by P2XRs and P2YRs, while ADP exert both a pro-nociceptive (via $P2Y_1$ receptors) and an anti-nociceptive (via $P2Y_{12}$ and $P2Y_{13}$ receptors) activity, depending on the engagement of receptor subtypes. In this context, adenosinergic signalling modulate an anti-nociceptive effect by the activation of A_1ARs, expressed on peripheral sensory and spinal cord neurons, and a pro-algogenic activity via A_{2A}AR activation. Of note, current knowledge on the impact of local distribution and activity of synthetic/catabolic enzymes and transporters in the pathophysiology of pain transmission are limited. In this regard, Vongtau et al., beyond to explore the expression and distribution of nucleotidases in DRG and spinal cord of mice, demonstrated that these enzymes act as key regulator of nociceptive transmission mediated by P2XRs and P2YRs [122]. Overall, current data reported that purinergic receptors are widely expressed in nociceptive circuits and exert opposite actions, depending on the receptor subtypes activated. However, a better understanding on how the modulation of nucleotides/nucleosides concentrations, by the purinergic receptors, controls the pain transmission

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could drive the development of innovative therapies for a more specific management of different pain conditions.

4.2. Diarrhea

Diarrhea is an intestinal disorder characterized by abnormal frequency and fluidity of fecal evacuations arising from an imbalance between absorption and secretion of electrolytes as well as altered bowel movements [123]. This symptom is common to a wide variety of GI diseases and represents an important public health problem, mainly in underdeveloped regions of the world [123]. The intestinal epithelium represents the main physical barrier between the external environment and the internal compartment, preventing the passage of pathogenic microorganisms [124]. Pathogenic microbiota and their products impair the enteric epithelial cell functions, altering key intracellular signal pathways and changing the epithelial cell-cell interactions [125]. These effects induce an inflammatory response and an influx of activated neutrophils into the epithelium. Infiltrating neutrophils deliver abnormal signals to the epithelial cells and stimulate an exaggerated response, leading epithelial injury and consequently secretory diarrhea [126]. In this regard, Crane et al. provided new insight regarding the molecular mechanisms underlying the onset of diarrhea by *Escherichia coli* [127]. The authors reported that *Escherichia coli* induce a significant release of ATP from dying intestinal epithelial cells triggering a prolonged chloride (Cl^-) secretory response with consequent water diarrhea [127]. The secretory alteration associated with the bacterial infection was completely reversed by A_{2B}AR antagonists, 8-SPT and MRS1754, suggesting a key role of Ado in the pathogenesis of *Escherichia coli*-induced Cl^- hypersecretion through the activation of apical A_{2B}ARs [127]. Of note, in a subsequent study the authors demonstrated that during the bacterial infection the release of ATP was not entirely ascribable to host cell killing, but in part by an increased activity of cystic fibrosis transmembrane conductance regulator (CFTR) [128].

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Under enteric bacterial infection, ATP exerts a diarrheagenic action by its conversion into Ado, thus suggesting a critical role of CD73. Crane et al. reported that *Escherichia coli* is able to induce the activation of phosphatidylinositol-specific phospholipase C, which cleave CD73 from its glycosylphosphatidylinositol lipid anchor obtaining a soluble-free form [129]. The soluble form of CD73, released into the intestinal lumen, becomes more accessible by its substrate, thus participating to the secretory alterations associated with *Escherichia coli* infection [129]. An interest study, conducted by Crane and Shulgina, investigated the role of Ado on bacterial proliferation under *Escherichia coli* infection [130]. The authors showed that Ado, beyond stimulating bacterial growth, changes the expression of several important virulence genes such as bundle-forming pilus structural gene *(bfpA)*, coding for a protein required for *Escherichia coli* auto-aggregation and adhesion to epithelial cells [130]. Based on this data, ADA, a catabolic enzyme, should not be viewed only as a marker of infection, as previously claimed by several authors [131,132], but rather as a host defence factor able to reduce the Ado accumulation in the intestinal lumen, thus counteracting the intra-luminal proliferation of pathogens [130].

The presence of noxious agents including bacteria, viruses and toxins in the intestinal lumen induces the migration of inflammatory cells such as platelets, polymorphonuclear leukocytes, monocytes and lymphocytes towards the lumen. Once in the luminal compartment, the inflammatory cells release several mediators, including purines, responsible of isotonic fluid transport into the lumen [133]. In this context, neutrophils represent a significant source of Ado. Indeed, several studies reported that under inflammatory, ischemic or infectious conditions these cells release a significant amount of AMP, which, following its conversion into Ado through CD73 express on the intestinal mucosa, elicits a massive intestinal Cl^- secretion [129,134-139]. Likewise, in inflamed intestine, platelets release large quantities of ATP, which is metabolized into Ado by CD39-CD73 enzymes expressed on the apical membrane of the intestinal epithelial cells [140]. Ado liberated in this manner is made available to $A_{2B}AR$ to activate electrogenic Cl^- secretion, thus favouring the elimination of bacteria

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and bacterial products under inflammatory conditions [140]. In support to this view, other authors reported that Ado, arising from CD73 over-activity, preferentially target A_{2B}ARs, over-expressed under adverse conditions [127,141,142], inducing a massive movement of isotonic fluids into the intestinal lumen [140,141,143,144].

Interestingly, several studies demonstrated that A_{2B}AR is actively involved also in interplays between immune cells and epithelial layer. In this regard, Sitaraman et al. observed that the stimulation of A_{2B}ARs, expressed in intestinal epithelial cell line T84, induces the release of IL-6, a proinflammatory cytokine involved in the control of lymphocyte differentiation and neutrophil degranulation [145]. Under resting conditions, this receptor subtype is mainly localized at intracellular level, preventing the inappropriate stimulation induced by Ado [144]. Upon agonist stimulation, A_{2B}ARs are recruited on the apical membrane, through soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE) proteins, vesicle-associated membrane protein (VAMP)-2 and synaptosomal-associated protein (SNAP)-23, resulting in a electrogenic Cl^- secretion and IL-6 release [144].

Taken together, current evidence suggest that the purinergic system takes a part in the onset and progression of diarrhea. In particular, CD73, which is extremely sensitive to variations of AMP levels, promote bacterial clearance under inflammatory conditions. Thus, the interaction between CD73 and A_{2B}AR is crucial in the regulation of the complex cross-talk between luminal microorganisms and epithelial cells. In this context, the endogenous Ado, arising from CD73 over-activity, promote the translocation of A_{2B}AR from the intracellular site to the apical membrane, with consequent Cl^- secretion and IL-6 release. Indeed, pioneering studies reported promising ameliorating effects in experimental models of diarrhea after treatment with CD73 inhibitors or selective A_{2B}AR antagonists [129,136,143,146,147]. Therefore, based on current evidence, the pharmacological modulation of Ado system could represent a useful strategy for the therapeutic management of diarrhea. However, intensive work is needed to verify these beneficial actions in the clinical setting.

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4.3. Intestinal ischemia

Intestinal ischemia is a serious clinical problem characterized by a reduction or interruption of blood flow within the superior mesenteric artery or vein, resulting in structural and functional bowel alterations, including absorptive/secretory dysfunctions and motor disorders [158,159]. Intermittent intestinal ischemia has been shown to potentiate reperfusion injury compared to continuous ischemia, triggering a marked ROS production and release of inflammatory mediators with subsequent destruction of vascular integrity and tissue edema [160].

An increasing number of studies have reported a critical role of purinergic system in the pathophysiology of intestinal ischemia. In this regard, several authors paid their attention on the involvement of purinergic enzymes in the development of vascular injury. For instance, Guckelberger et al. investigated the role of CD39, highly expressed in vascular endothelial cells under normal conditions, in intestinal ischemia-reperfusion injury [150]. The authors observed that mice lacking CD39 showed increase in vascular permeability, platelet activation, and mortality rate following induction of intestinal ischemic injury, suggesting that this enzyme is deeply involved in the preservation of blood vessel homeostasis [150]. Interestingly, the treatment with exogenous CD39 or Ado abrogated the increased vascular permeability and intestinal alterations, observed in both wild-type and mice lacking CD39 [150]. Other authors examined the contribution of CD73, enzyme responsible for extracellular Ado production, in the intestinal ischemia-reperfusion injury [151]. Hart et al. demonstrated that CD73 plays an anti-inflammatory role during GI ischemia-reperfusion injury through the increase in extracellular Ado. Indeed, genetic deficiency of CD73 or its pharmacological inhibition results in excessive injury during acute GI ischemia, whereas the treatment with soluble CD73 induces a significant amelioration of tissue damage [151]. Several lines of evidence have

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highlighted an increase in Ado concentration during tissue hypoxia or ischemia [152]. In particular, Morote-Garcia et al. observed that under normal condition the Ado uptake is rapid from the apical surface of epithelia and only minimal Ado transport occurs at the basolateral surface. By contrast, during intestinal hypoxia, extracellular Ado concentrations markedly increase, causing a directed Ado transport from the luminal surface towards the intracellular space through the repression of ENT2-dependent Ado transport [152]. These data highlight an adaptive response to hypoxia aimed to attenuate hypoxia-associated inflammation of mucosal through an increment of Ado signalling. This study is consistent with previous studies, which reported the ameliorative effects of Ado during intestinal ischemia. For instance, Grisham et al. showed that the intra-arterial administration of Ado reduced neutrophil activation and attenuated leukocyte adherence to the venular endothelium in a feline model of intestinal ischemia-reperfusion [161]. Likewise, other authors observed that the increment of endogenous Ado levels, through the pharmacological blockade of nucleoside transporters, attenuated the detrimental events associated with the intestinal ischemia [148]. These positive effects of Ado could be partly ascribed to direct vasodilator effects of this nucleoside. The involvement of Ado in attenuating tissue inflammation and injury during ischemia through the recruitment of A_{2B}AR was summarized in an exhaustive review [162]. However, data on possible contribution of other ARs in this setting are currently lacking.

At present, the information about the contribution of purinergic pathways in enteric motor dysfunctions associated with intestinal ischemia are negligible. For instance, Kadowaki and colleagues investigated the involvement of A_1AR in a rat model of the experimental ischemia/reperfusion-induced motility disorder [149]. They observed that the colonic propulsion was significantly slowed following ischemia/reperfusion, whereas the treatment with selective A_1AR antagonists, FK352 or DPCPX, significantly restored propulsive motility [149]. These data suggest that endogenous Ado can exercise a sustained and potent inhibitory effect on colonic propulsion in both normal conditions and pathophysiological conditions. A more recent study, conducted by

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Paulino et al. paid the attention on the contribution of P2X2 receptors in the enteric dysmotility associated with ischemia. In this study, the authors showed a significant reduction P2X2 receptor in both the cytoplasm and surface membranes of the myenteric and submucosal neurons following ischemia/reperfusion of the rat ileum [153]. In addition, a marked reduction in the density of P2X2 receptors on nitrergic and cholinergic nerves along with a decrease size of myenteric and submucosal neurons, was observed [153].

Taken together, despite the encouraging results, further studies are required to elucidate the contribution of purinergic system in the intestinal injuries and motor disturbances associated with ischemic conditions.

4.4. Other diseases

At present, exhaustive reviews well described the role played by the purinergic receptors in the modulation of intestinal motility under physiological and pathological conditions [1,4,7,163]. In particular, these reviews depict the $P2Y_1$ receptor as a pivotal receptor subtype responsible for inhibitory control of gastrointestinal motility in both rodents and human [44,164,165]. Indeed, $P2Y_1$ knock-out mice showed a completely absence of purinergic relaxation [166]. In support to this view, a study highlighted that treatment with MRS2179, MRS2279 and MRS2500, selective $P2Y_1$ antagonists, inhibited the purinergic relaxant responses in both rat and human colonic preparations, thus corroborating the relevant contribution of this receptor subtype in the purinergic inhibitory neurotransmission [164,167]. Considering the enteric motor dysfunction in the setting of bowel inflammation, Strong et al. provided interesting findings about the participation of $P2Y_1$ receptors in the regulation of colonic neuromuscular activity in a murine model of TNBS-induced colitis [168]. The authors reported a marked decrease in the fecal pellet output and a reduction of propulsive motility in circular muscle cells of the TNBS-inflamed guinea pig distal colon [168]. In particular,

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they observed that the purinergic component, mediated by $P2Y_1$ receptors, was selectively affected, thus contributing to dysmotility in the inflamed colon [168].

For what it concerns other intestinal pathological conditions a limited number of studies described the involvement of purinergic system in the pathophysiology of Hirschsprung's disease and post-operative ileus. With regard for Hirschsprung's disease, characterized by absence of ganglia in the distal colon, Zagorodnyuk et al. investigated the excitatory purinoceptor-mediated contraction in colonic longitudinal smooth muscle preparations from these patients. The authors observed that the contractile responses, mediated by P2YRs, were higher than those recorded from preparations of normal colon [169]. Subsequently, several studies investigated the purinergic receptor expression in colonic tissues from patients with Hirschsprung's disease. For instance, Facer et al. reported a significant reduction of P2X3 receptors [154]. Likewise, O'Donnell and Puri observed a lack of $P2Y_1$ and $P2Y_2$ receptor expression in the colon of these patients [155]. The absence of these receptors suggests an unsettled ATP neurotransmission, which might contribute to the condition of persistent contraction occurring in the aganglionic gut of these patients. Overall, current evidence demonstrate that the purinergic system is involved in the pathophysiology of Hirschsprung's disease, although data on the functional significance of colonic P2 receptors are lacking.

For what it concerns post-operative ileus, few data are currently available about the involvement of purinergic pathways in the development of such disorder. Post-operative ileus is a frequent adverse consequence of abdominal surgical procedures that leads to discomfort, morbidity and prolonged hospital stay [170]. Such disorder is characterized by altered patterns of motor activity throughout the GI tract. In this setting, it has been reported an increase in P2YRs, on smooth muscle, and in ATP production, thus contributing to delayed colonic transit [156]. Other authors examined the contribution of A_1AR to the alterations of colonic propulsion in an experimental model of post-operative ileus [157]. In this study, the authors observed an improvement of propulsive motility in rats treated with the selective A_1AR antagonist FK352, suggesting that Ado is involved in the

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pathogenesis of post-operative ileus via A_1AR activation. More recently, a set of adenine-based A_1AR antagonists were tested at a mouse ileum tissue preparation, showing to counteract the CCPA-induced inhibition of rhythmic spontaneous contractions, with a potency in good correlation with the A_1AR binding affinity [171].

Future investigations are needed to develop pharmacological interventions on the Ado system to counteract the underlying pathogenic mechanisms such as neuromuscular inflammation and initiation of neural reflex, and hence to prevent or attenuate post-operative ileus.

Conclusions

The purinergic signalling system is a complex machinery, where various membrane receptors intracellularly transfer a signal received by extracellular nucleosides and nucleotides, whose concentrations are finely tuned by a harmonised work by a heterogeneous set of enzymes. This integrated purinergic network regulates the GI homeostasis functions by coordinating neuromuscular or mucosal structures and the enteric immune cells. Changes in the receptor expression or abnormal activity of enzymes regulating nucleotides/nucleosides extracellular levels take to alterations of this signalling network that may have a key role in the onset and development of gut motor, secretory, and sensory dysfunctions. The activation of P2 or P1 receptors often take to opposite effects, suggesting that the use of receptor agonists or antagonists or enzyme inhibitors may modulate the nucleotide/nucleoside balance, with beneficial effects. Medicinal chemistry efforts led to the synthesis of pharmacological probes that have been used to interpret the role of specific purinergic receptors or enzymes in pathological conditions of the GI tract. Even if further investigations are needed to clearly depict the role of the purinergic signalling players, the development of compounds with suitable target selectivity and pharmacokinetic profile represents a promising strategy for the treatment of GI disorders.

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A number of evidences depict a high level of complexity about the purinergic system, pointing out the need for a deeper knowledge about the purinergic receptors, both as individual units as well as components of intricate purinergic networks, relevantly involved in the modulation of enteric functions. In this regard, the novel multidisciplinary approach, focused toward a holistic evaluation of the regulatory actions exerted by purinergic system in the gastrointestinal tract need to be implemented in the future. This novel approach will allow to identify innovative opportunities for pharmacological interventions against a number of digestive disorders.

Nowadays, several lines of pre-clinical data revealed the possibility of beneficial effects arising from the pharmacological modulation of purinergic pathways in several digestive pathological conditions, such as functional disorders, visceral pain, diarrhea, ischemia. However, a number of issues regarding the regulatory role of digestive functions by the purinergic system are still pending and deserve further investigations. A deeper comprehension about the complexity of the purinergic pathways controlling the gut functions requires a multidisciplinary approach, including biochemical and pharmacological studies, in vitro and in vivo functional assays, aimed clarify the actual role of the purinergic mediators at level of the whole organism, as well as at characterizing the pathological consequences associated with alterations of purinergic signalling in various intestinal disorders. Subsequently, a detailed characterization of the purinergic metabolic/receptor networks, in the onset and development of digestive dysfunctions, could be critical to drive the development of innovative drugs potentially useful for the therapeutic management of digestive disorders.

In this regard, the actions exerted by purines on enteric glial cells represent, in our opinion, an intriguing issue deserving further investigation. The enteric glia is gaining increasing attention due to its pivotal role in the gut homeostasis. In this respect Gulbransen and Sharkey [172-174], provided increasing evidences about the involvement of purinergic signalling in the coordination of interactions among enteric glia and neurons. In particular, the assessment of mechanisms involved in the regulation of purinergic pathways in enteric neuron-to-glia and glia-to-glia interplay represent an

area of exciting interest for future investigations. What is more is that these might evolve into the characterization of the mechanisms underlying enteric motor dysfunctions.

Therefore, we can be optimistic that novel purinergic ligands with greater specificity and affinity will offer a promising approach to treating gastrointestinal diseases. In particular, future efforts should be focused toward the synthesis of highly selective tools, in order to target the receptor subtypes mainly involved in the pathophysiology of specific gut diseases (i.e. P2X3, P2X4, A_{2A} and A_3 ARs), in order to curb the adverse effects. Of note, the microRNA, is interesting field of research in the purinergic pharmacology, which is attracting a lot of attention. The miRNA are small noncoding RNAs pivotally involved in the regulation of multiple target genes through sequence-specific hybridization to the 3′-untranslated region of messenger RNAs [34]. Recently, an interesting review by Ferrari et al. [175] highlighted the modulatory role exerted by several miRNAs in regulating the expression of several proteins involved in the purinergic network. For these reasons, the pharmacological modulation of purinergic miRNAs represents another viable way to manage digestive disorders. In addition, an alternative approach could be represented by purinergic regulating agents, such as drugs acting on ADA, ADK or AMP-activated protein kinase, which are currently under active investigation as promising therapeutical approaches to the treatment of several pathological conditions.

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List of Tables and Figures

Table 1. Distribution and function of P2 receptors in the small and large intestine of rodents and human

Distribution		P2 receptor subtypes	Function	References
Small intestine	*Mucosa*	Mouse: $P2Y_1$, $P2X_2$, $P2X_3$ Rat: $P2Y_2$, $P2X_7$ Human: $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2X_7$	Mouse: • $P2Y_{1\text{-}2\text{-}4}$: ↓ contractile activity • $P2Y_6$, neuronal P2X: ↑ contractile activity • $P2X_3$: ↑ peristaltic activity • $P2Y_{2\text{-}4}$: ↑ chloride and potassium secretion • $P2Y_2$: ↓ sodium absorption	Mouse: [36-39]
	Submucosal plexus	Mouse: $P2Y_1$, $P2Y_4$, $P2X_2$, $P2X_3$, $P2X_5$ Rat: $P2X_1$, $P2X_2$, $P2X_3$, $P2X_6$, $P2X_7$ Human: $P2Y_1$, $P2Y_6$, $P2X_1$, $P2X_2$, $P2X_3$	Rat: • P2Y: ↓ contractile activity • $P2Y_1$: ↓ acetylcholine release • P2X: ↑ acetylcholine release • $P2Y_{1\text{-}2\text{-}4}$: ↑ chloride and potassium secretion • $P2X_{1\text{-}3}$: ↑ chloride secretion • $P2Y_1$: ↑ sodium secretion	Rat: [36,40-42]
	Circular muscle	Mouse: $P2Y_1$, $P2Y_2$, $P2X_2$, $P2X_3$ Rat: $P2Y_1$, $P2X_2$ Human: $P2Y_1$, $P2Y_4$, $P2Y_6$		
	Myenteric plexus	Mouse: $P2Y_1$, $P2Y_4$, $P2X_1$, $P2X_2$, $P2X_3$, $P2X_5$ Rat: $P2Y_{14}$, $P2X_1$, $P2X_2$, $P2X_3$, $P2X_6$, $P2X_7$ Human: $P2Y_1$, $P2Y_6$, $P2X_3$	Human: • P2Y: ↓ contractile activity • $P2Y_{2\text{-}4\text{-}6}$: ↑ chloride secretion	Human: [43-49]
	Longitudinal muscle	Mouse: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2X_2$, $P2X_3$ Rat: $P2Y_1$, $P2X_2$		

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		Human: $P2Y_1$, $P2Y_6$		
Large intestine	*Mucosa*	Mouse: $P2Y_1$, $P2Y_2$, $P2X_2$, $P2X_3$	Mouse: • P2Y: ↓ contractile activity • Muscular $P2X_2$: ↑ contractile activity • $P2Y_2$, $P2Y_4$: ↑ chloride and potassium secretion $P2Y_2$: ↓ sodium absorption	Mouse: [36-39]
		Rat: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$, $P2X_2$, $P2X_3$, $P2X_5$, $P2X_6$		
		Human: $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2X_7$		
	Submucosal plexus	Mouse: $P2X_2$, $P2X_3$		
		Rat: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{14}$, $P2X_1$, $P2X_2$, $P2X_3$, $P2X_6$, $P2X_7$		
		Human: $P2Y_1$, $P2X_1$, $P2X_2$, $P2X_3$	Rat: • P2Y: ↓ contractile activity • $P2Y_{1\text{-}2\text{-}4}$: ↑ chloride and potassium secretion • $P2X_1$, $P2X_3$: ↑ chloride secretion • $P2Y_1$: ↑ sodium secretion	Rat: [36,40-42]
	Circular muscle	Mouse: $P2Y_1$, $P2X_1$, $P2X_2$, $P2X_3$		
		Rat: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2X_1$		
		Human: $P2Y_1$, $P2Y_4$		
	Myenteric plexus	Mouse: $P2Y_1$, $P2X_2$, $P2X_3$		
		Rat: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{14}$, $P2X_1$, $P2X_2$, $P2X_3$, $P2X_6$, $P2X_7$	Human: • P2Y: ↓ contractile activity • $P2Y_{2\text{-}4\text{-}6}$: ↑ chloride secretion	Human: [43-49]
		Human: $P2Y_1$, $P2X_3$		
	Longitudinal muscle	Mouse: $P2Y_1$, $P2X_3$		
		Rat: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2X_1$		
		Human: $P2Y_1$		

Abbreviations: ↑, increase; ↓, decrease; n.a, not available.

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Table 2. Pathophysiological role of purinergic pathways in intestinal disorders associated with motor, secretory and sensory dysfunctions

Intestinal disorders	**Purinergic pathways**	**Ref.**
Visceral pain	↑ ATP release A_{2A}AR, P2X2, P2X3 and $P2Y_1$ mediate pro-nociceptive responses ↑ P2X3 expression in DGR neurons P2X7 mediate visceral pain in post-infectious GI dysfunction A_1AR, $P2Y_{12}$ and $P2Y_{13}$ mediate anti-nociceptive effect	[87,91-96,100,108,110,112,113]
Diarrhea	↑ ATP release resulting in a diarrheagenic action ↑ soluble form of CD73 and adenosine concentration Ado stimulates bacterial growth and ↑ expression of virulence genes as well as induce Cl^- secretion and IL-6 release via A_{2B}AR	[127,129,130,140,146,147]
Intestinal ischemia	Mice lacking CD39 develop vascular injury CD73 exerts an anti-inflammatory effect, through ↑ extracellular adenosine, attenuating the tissue injury during ischemia A_1AR stimulation ↓ colonic propulsion following I/R ↓ P2X2 expression following I/R	[148-153]
Hirschsprung's disease	↓ P2X3 expression and a lack of $P2Y_1$ and $P2Y_2$ contribute to a persistent contraction	[154,155]
Post-operative ileus	↑ P2Y expression and ATP release contribute to delayed colonic transit A_1AR stimulation ↓ colonic propulsion	[156,157]

Abbreviations: ↑, increase; ↓, decrease; ATP, adenosine triphosphate; CD73, ecto-5'-nucleotidase; Cl^-, chloride; DRG, dorsal root ganglion; GI, gastrointestinal; IL-6, interleukin 6; I/R, ischemia/reperfusion; n.a, not available

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Figure 1. Schematic description of the purinergic signalling system (ATP = adenosine triphosphate; ADP = adenosine diphosphate; AMP = adenosine monophosphate; Ado = adenosine; UTP = uridine triphosphate; UDP = uridine diphosphate; UDP-glucose = uridine diphosphate glucose; ADA = adenosine deaminase; ADK = adenosine kinase; NT = nucleoside transporter; 5'-NT = 5'-nucleotidase; NTPDase = nucleoside triphosphate diphosphohydrolase).

Figure 2. Examples of ligands of P2X and P2Y receptors.

Figure 3. Examples of adenosine receptors reference ligands and/or pharmacological probes (NECA is an unselective AR ligand).

Figure 4. Examples of reference ligands of transporters (NT = nucleoside transporter) and enzymes (ADA = adenosine deaminase; ADK = adenosine kinase; 5'-NT = 5'-nucleotidase; NTPDase = nucleoside triphosphate diphosphohydrolase) involved in purinergic signalling.

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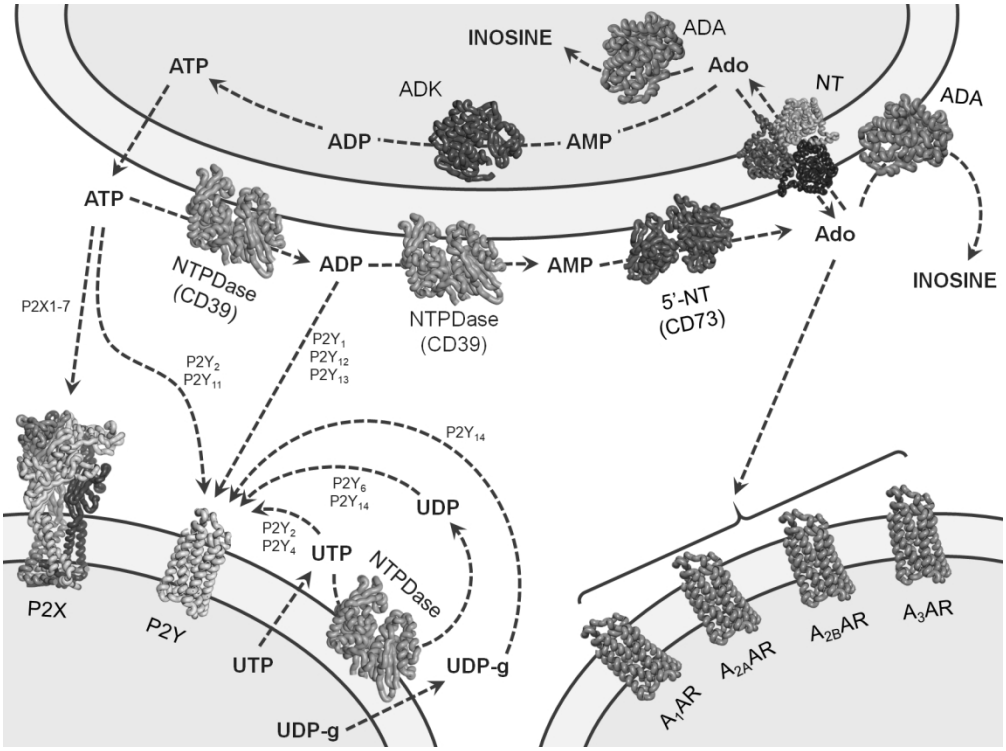

Figure 1. Schematic description of the purinergic signalling system (ATP = adenosine triphosphate; ADP = adenosine diphosphate; AMP = adenosine monophosphate; Ado = adenosine; UTP = uridine triphosphate; UDP = uridine diphosphate; UDP-glucose = uridine diphosphate glucose; ADA = adenosine deaminase; ADK = adenosine kinase; NT = nucleoside transporter; 5'-NT = 5'-nucleotidase; NTPDase = nucleoside triphosphate diphosphohydrolase).

151x112mm (300 x 300 DPI)

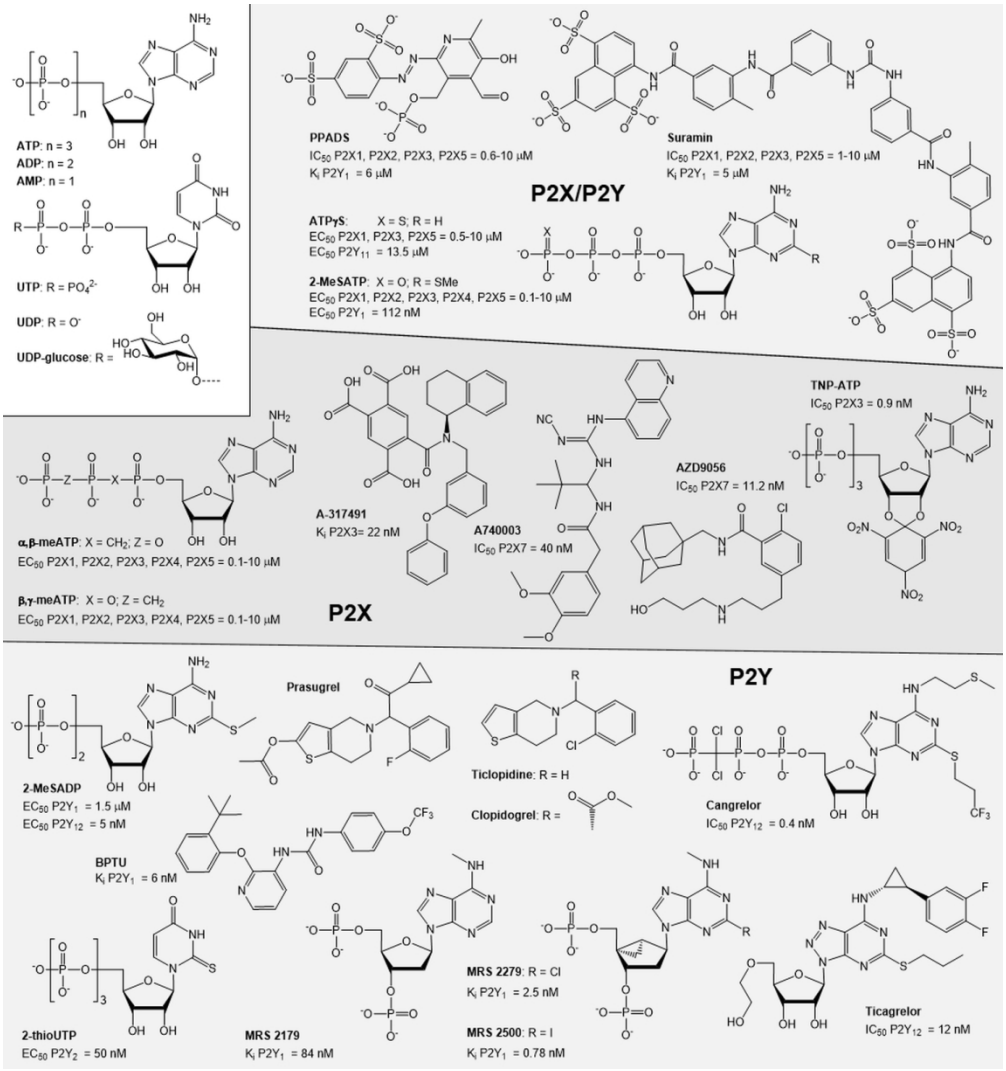

Figure 2. Examples of ligands of P2X and P2Y receptors.

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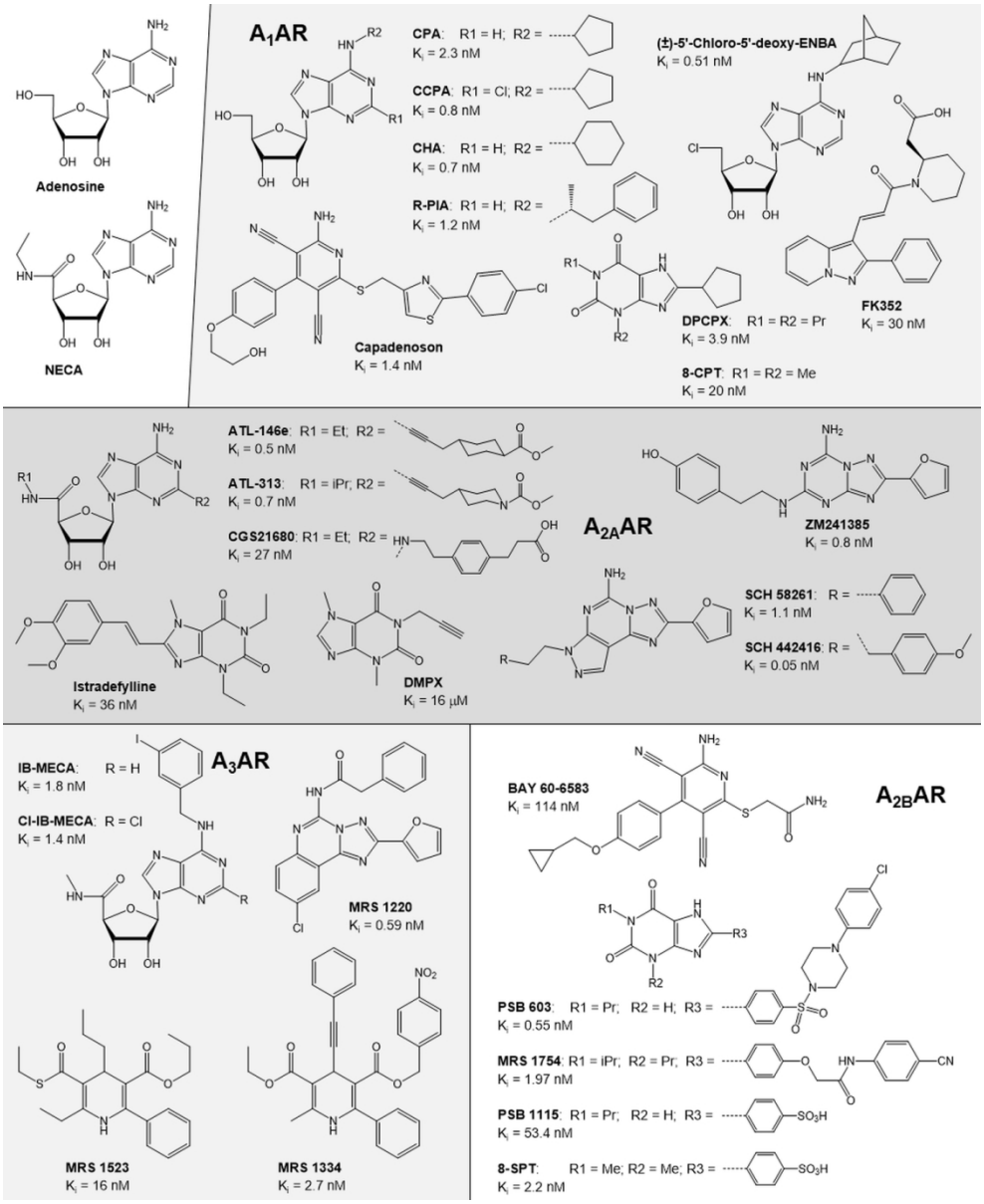

Figure 3. Examples of adenosine receptors reference ligands and/or pharmacological probes (NECA is an unselective AR ligand).

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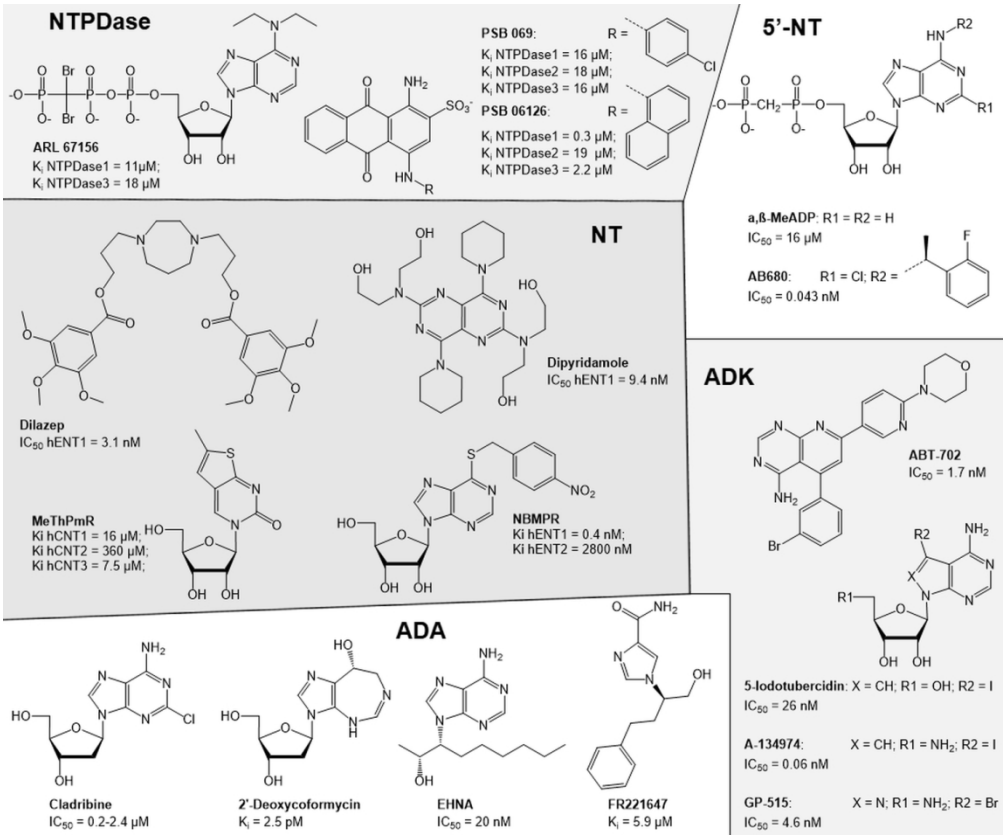

Figure 4. Examples of reference ligands of transporters (NT = nucleoside transporter) and enzymes (ADA = adenosine deaminase; ADK = adenosine kinase; 5'-NT = 5'-nucleotidase; NTPDase = nucleoside triphosphate diphosphohydrolase) involved in purinergic signalling.

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