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# Regulation of enteric functions by adenosine: Pathophysiological and pharmacological implications

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*Abbreviations:*
AMP, adenosine monophosphate
ATP, adenosine triphosphate
APC, antigen-presenting cell
cAMP, cyclic adenosine monophosphate
CD39, ecto-apyrase
CD73, ecto-5′-nucleotidase
CNT, concentrative nucleoside transporter
DSS, dextran sodium sulphate
EC, enterochromaffin cell
ENS, enteric nervous system
ENT, equilibrative nucleoside transporter
5-HT, 5-hydroxytryptamine
IBD, inflammatory bowel disease
IB-MECA, *N*(6)-(3-iodobenzyl)-adenosine-5′-*N*-methyluronamide
ICC, interstitial cell of Cajal
IFN-γ, interferon-γ
IL-1, interleukin-1
IL-1β, interleukin-1β
IL-6, interleukin-6
IL-8, interleukin-8
IL-10, interleukin-10
IL-12, interleukin-12
IPAN, intrinsic primary afferent neuron
ROS, reactive oxygen species
Th, T-helper cell

ABSTRACT

The wide distribution of ATP and adenosine receptors as well as enzymes for purine metabolism in different gut regions suggests a complex role for these mediators in the regulation of gastrointestinal functions. Studies in rodents have shown a significant involvement of adenosine in the control of intestinal secretion, motility and sensation, via activation of $A_1$, $A_{2A}$, $A_{2B}$ or $A_3$ purinergic receptors, as well as the participation of ATP in the regulation of enteric functions, through the recruitment of P2X and P2Y receptors. Increasing interest is being focused on the involvement of ATP and adenosine in the pathophysiology of intestinal disorders, with particular regard for inflammatory bowel diseases (IBDs), intestinal ischemia, post-operative ileus and related dysfunctions, such as gut dysmotility, diarrhoea and abdominal discomfort/pain. Current knowledge suggests that adenosine contributes to the modulation of enteric immune and inflammatory responses, leading to anti-inflammatory actions. There is evidence supporting a role of adenosine in the alterations of enteric motor and secretory activity associated with bowel inflammation. In particular, several studies have highlighted the importance of adenosine in diarrhoea, since this nucleoside participates actively in the cross-talk between immune and epithelial cells in the presence of diarrhoeogenic stimuli. In addition, adenosine exerts complex regulatory actions on pain transmission at peripheral and spinal sites. The present review illustrates current information on the role played by adenosine in the regulation of enteric functions, under normal or pathological conditions, and discusses pharmacological interventions on adenosine pathways as novel therapeutic options for the management of gut disorders and related abdominal symptoms.



**Contents**



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## 1. Introduction

The earliest experimental observation describing the biological activity of adenosine in the digestive system can be dated back to 1949, when it was demonstrated that adenine derivatives induced a significant inhibition of the spontaneous contractile activity of ileum in rabbits, hamsters and guinea-pigs (Ewing et al., 1949). This study was followed by other pioneering investigations which examined the effects of exogenous adenosine on intestinal motility (Mihich et al., 1954; Stafford, 1966), but it was in the early 1970s that more specific studies led to the identification of the purinergic system, and described its significant impact on the physiology of enteric neurotransmission. In particular, based on findings from experiments designed to assess the role of previously identified non-adrenergic, non-cholinergic (NANC) neurotransmitters (Burnstock et al., 1966; Ambache & Freeman, 1968), Burnstock et al. (1970) proposed the novel concept that adenosine triphosphate (ATP) and related nucleotides/nucleosides (adenosine diphosphate, ADP; adenosine monophosphate, AMP; adenosine) act as transmitters involved in NANC-mediated relaxing responses of smooth muscle in the gastrointestinal tract and bladder. Two years later, the term "purinergic" was coined and the purinergic neurotransmission hypothesis was put forward (Burnstock, 1972). This concept initially met considerable resistance and skepticism in the scientific community, used to regarding the purine system only as an ubiquitous biochemical source of energy, but subsequently it met wide acceptance and became a cornerstone in the physiology of gastrointestinal tract.

Implicit in the purinergic hypothesis was the existence of purinergic receptors, which were first characterized in 1976 (Burnstock, 1976; Spedding & Weetman, 1976). A step forward was taken in 1978 when, in a seminal review, Burnstock proposed a basis for distinguishing two types of purinoceptors, named P1 and P2, which were preferentially activated by adenosine and ATP, respectively (Burnstock, 1978). About at the same time, pharmacological studies allowed the distinction of two P1 receptor subtypes, based on their ability to inhibit ($A_1$ receptor) or stimulate ($A_2$ receptor) intracellular cAMP accumulation (Van Calker et al., 1979; Londos et al., 1980). In 1985, Burnstock and Kennedy proposed pharmacological criteria for discriminating two types of ATP P2 receptors (P2X and P2Y) (Burnstock and Kennedy, 1985). Subsequently, on the basis of studies on transduction mechanisms and cloning of nucleotide receptors, it was established that P2X are ligand-gated ion channel receptors, whereas P2Y belong to the G-protein-coupled receptor family (Abbracchio & Burnstock, 1994). Several ATP receptor subtypes have been then identified on myenteric and submucosal neurons, where they control the synaptic neurotransmission and contribute to the neuromodulation of gut functions (Burnstock, 2008).

The above observations fostered strong interest in the role played by adenosine in the modulation of enteric functions. It was initially demonstrated that adenosine contributes to the inhibitory regulation of intestinal motility through the reduction of acetylcholine release from myenteric nerves (Vizi & Knoll, 1976; Kazić & Milosavljević, 1976), as well as via activation of purinergic receptors on smooth muscle cells (Ally & Nakatsu, 1976; McKenzie et al., 1977). In the same period, the presence of enzymes involved in adenosine metabolism was documented in the intestinal mucosa (Kolassa et al., 1977; Harms & Stirling, 1977), and these findings, together with the subsequent identification of adenosine receptors in the intestinal epithelium (Dobbins et al., 1984; Barrett et al., 1989) and submucosal plexus (Barajas-Lopez et al., 1991; Barajas-Lopez, 1993), paved the way to the characterization of the role played by adenosine in the control of bowel fluid and electrolyte transport. Other investigations, indicating an involvement of adenosine in the regulation of gut sensory functions, then suggested potential therapeutic applications of drugs acting on adenosine receptors in the management of functional digestive disorders associated with abdominal pain, such as irritable bowel syndrome (IBS) (Geiger et al., 1984; De Lander & Hopkins, 1987; Takaki et al., 1993; Bueno et al., 1997).

The concept of purinergic signalling has broadened through the years to include its regulatory actions on the immune system (Sitkovsky & Lukashev, 2005; Sitkovsky & Ohta, 2005). Initial demonstrations concerning the ability of extracellular adenosine to modulate immune processes go back to 1970s (Giblett, 1976; Seegmiller et al., 1977; Allison et al., 1977). Since then, detailed studies have indicated adenosine as a prominent player in the physiological mechanisms deputed to down-regulate activated immune cells and protect tissues from inflammatory damage via specific receptor subtypes expressed on immune/inflammatory cell populations (lymphocytes, neutrophils, monocytes, macrophages and dendritic cells) (Ohta & Sitkovsky, 2001; Fortin et al., 2006; Desrosiers et al., 2007). The involvement of adenosine pathways in the anti-inflammatory and immunomodulating effects exerted by drugs employed in the medical management of chronic inflammatory diseases (i.e. methotrexate, salicylates) has also become apparent (Amann & Peskar, 2002; Montesinos et al., 2007). These observations have stimulated the research of novel drugs suitable for treatment of intestinal inflammatory disorders through the pharmacological modulation of adenosine pathways (Siegmund et al., 2001; Odashima et al., 2005; Guzman et al., 2006; Cavalcante et al., 2006; Antonioli et al., 2007). At present, some of these compounds are

being tested also in preclinical models of non-digestive diseases (asthma, chronic obstructive pulmonary disease, diabetes) with encouraging results (Fozard et al., 2002; Mustafa et al., 2007; Van den Berge et al., 2007; Németh et al., 2007), while others have already entered the phase of clinical development for treatment of rheumatoid arthritis (Silverman et al., 2008).

Thus, after a troubled start, adenosine is now a rapidly expanding field, even though several aspects of its functions at gastrointestinal level need to be clarified. The present review is intended to illustrate and discuss current information on the role played by adenosine pathways in the regulation of enteric motor, secretory, sensory and immune functions, under normal conditions as well as in the presence of functional and inflammatory gut disorders. Special attention has been paid to the implications of adenosine in the pathophysiology of gut dysfunctions (dysmotility, diarrhoea, visceral pain) and to the possibility of modulating such disturbances by pharmacological interventions on molecular targets within the adenosine network (receptors, enzymes, transporters).

### *1.1. Adenosine receptors, metabolic pathways and transporters*

The biological actions of adenosine are mediated by G-protein-coupled receptors currently distinguished into four subtypes: $A_1$, $A_{2A}$, $A_{2B}$ and $A_3$ (Klotz, 2000; Fredholm et al., 2000). Since the classification of adenosine receptors has been exhaustively addressed in a number of previous papers (Linden, 1991; Fredholm et al., 1996, 1997; Ralevic & Burnstock, 1998; Olah & Stiles, 2000; Fredholm et al., 2000, 2001), readers are referred to these reviews for detailed information. The ability of adenosine to regulate several biological functions is strictly related to its extracellular concentration. The levels of adenosine at its receptors are determined by a variety of mechanisms, which include intracellular and extracellular adenosine biosynthesis, as well as cellular adenosine release, reuptake and metabolism (Noji et al., 2004). These processes are intertwined, subjected to highly dynamical regulation and linked in a complex manner to the energy balance of tissues (Deussen, 2000). There is also evidence to suggest that extracellular adenosine levels can vary significantly in response to several pathological conditions (Cronstein, 1995; Latini & Pedata, 2001; Sitkovsky & Ohta, 2005).

Under physiological conditions, adenosine is formed mainly at the intracellular level from *S*-adenosylhomocysteine by *S*-adenosylhomocysteine hydrolase, and transported across cell membranes by nucleoside transporters, which play a key role in the control of extracellular adenosine concentrations (Cabrita et al., 2002). These transporters are classified into two categories according to their molecular and functional characteristics (Noji et al., 2004): 1) equilibrative nucleoside transporters (ENTs), which carry nucleosides across cell membranes in either direction, depending on concentration gradients; they include four subtypes, designated as ENT1, ENT2, ENT3 and ENT4 (Baldwin et al., 2004); 2) concentrative nucleoside transporters (CNTs), subdivided in CNT1, CNT2 and CNT3, which promote the intracellular influx of nucleosides against their concentration gradient, using the sodium ion gradient across cellular membranes as a source of energy (Gray et al., 2004). After intracellular reuptake, adenosine undergoes rapid phosphorylation to AMP by adenosine kinase, or deamination to inosine by adenosine deaminase. These pathways ensure the maintenance of low intracellular adenosine concentrations through a strict enzymatic control (Noji et al., 2004) (Fig. 1A). Another relevant source of extracellular adenosine is represented by ATP physiologically released from nerve endings, immune cells and smooth muscle cells (Haskò et al., 2005; Burnstock, 2007).

Adverse conditions, including hypoxia or inflammation, are associated with increased intracellular and extracellular dephosphorylation

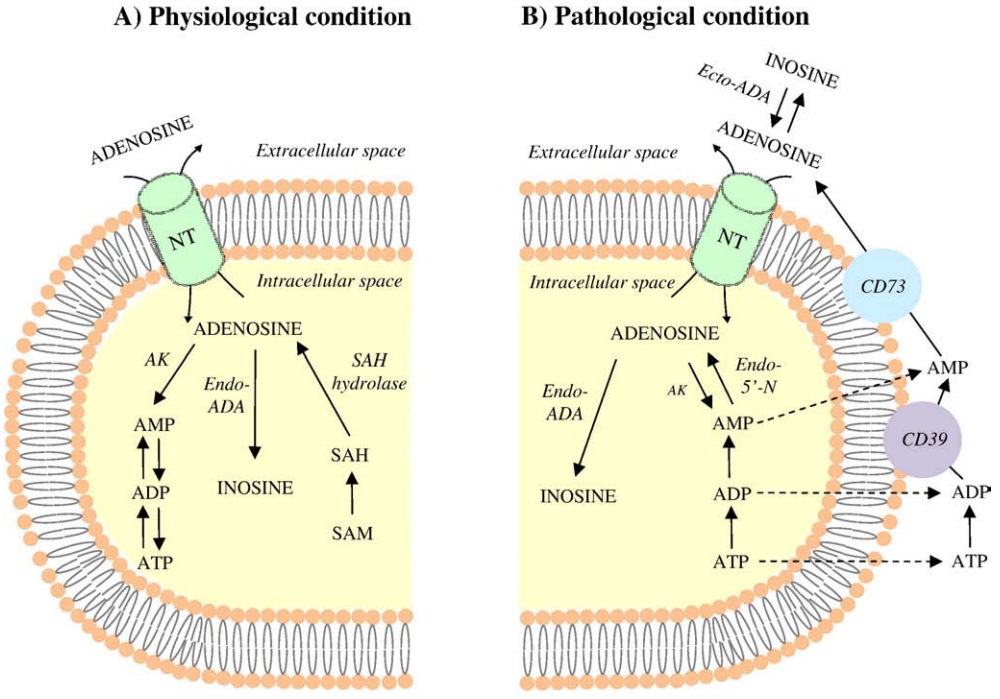


**Fig. 1.** Schematic diagram illustrating the biosynthesis and catabolism of adenosine under physiological or pathological conditions. (**A**) Under physiological conditions, adenosine is formed mainly at intracellular level from *S*-adenosylhomocysteine (SAH) by *S*-adenosylhomocysteine hydrolase (SAH hydrolase), and transported across cell membranes by nucleoside transporters (NT). After intracellular reuptake, adenosine undergoes rapid phosphorylation to adenosine monophosphate (AMP) by adenosine kinase (AK), or deamination to inosine by adenosine deaminase (ADA). (**B**) Pathological conditions are associated with increased intracellular and extracellular dephosphorylation of adenosine triphosphate (ATP) through ecto-apyrase (CD39) and subsequently by ecto-5′-nucleotidase (CD73) and endo-5′-nucleotidase (endo-5′-N), which, in parallel with the suppression of adenosine kinase (AK) activity, lead to an increase in adenosine levels. In the extracellular environment, adenosine concentrations are controlled by ecto-adenosine deaminase (ecto-ADA), which catalyzes its conversion into inosine. ADP: adenosine diphosphate; Endo-ADA: endo-adenosine deaminase; NT: nucleoside transporter; SAM: *S*-adenosylmethionine.

of ATP to adenosine through ecto-apyrase (also named CD39) and 5′-nucleotidase enzymes (endo-5′-nucleotidase and ecto-5′-nucleotidase) in parallel with the suppression of adenosine kinase activity (Deussen, 2000). Recent evidence indicates that during inflammatory insults, ecto-5′-nucleotidase, designated also as CD73, represents a critical check point for the control of adenosine production, deputed to preserve tissue integrity (Narravula et al., 2000; Niemelä et al., 2004; Colgan et al., 2006). Indeed, increments of adenosine levels have been shown to result in readjustments of the energy supply-to-demand ratio via augmentation of organ blood flow by vasodilatation, and protection of inflamed tissues by down-regulation of the immune response (Niemelä et al., 2004; Sitkovsky & Ohta, 2005) (Fig. 1B). Under pathological conditions associated with impairment of energy supply to cells, such as hypoxia or ischemia, a relevant contribution to adenosine production is given also by adenylate kinase, since this enzyme acts on ADP to generate ATP plus AMP, the latter being a substrate for adenosine formation through dephosphorylation (Hardie, 2003; Yegutkin, 2008).

Adenosine concentrations in the extracellular compartment are controlled by ecto-enzymes catalysing its conversion into inosine (Haskò & Cronstein, 2004), and ultimately to the stable end product uric acid (Haskò et al., 2004). Currently known ecto-enzymes include adenosine deaminase and adenosine kinase. Adenosine deaminase, regarded mainly as a cytosolic enzyme (endo-adenosine deaminase), can be expressed also on the external membrane surface of several immune and non-immune cells (ecto-adenosine deaminase) (Cristalli et al., 2001; Latini & Pedata, 2001).

### *1.2. Intestinal localization of adenosine receptors, enzymes and transporters*

Two studies have examined the expression and localization of adenosine receptor subtypes in the human gastrointestinal tract (Puffinbarger et al., 1995; Christofi et al., 2001), through reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemical analysis. These investigations have demonstrated a wide distribution of adenosine receptors in the neuromuscular compartment and mucosa/submucosal layer of both small and large intestine. In addition, among the enzymes involved in adenosine metabolism, adenosine deaminase has been found in various compartments of the human intestinal wall, whereas the expression of CD73, ENTs and CNTs has been investigated only at the mucosal level. Current information on the expression and compartmental localization of adenosine receptors, enzymes and transporters in the human intestine are summarized in Table 1. With regard to rodents, most of data on the distribution of adenosine receptors in rat gastrointestinal tract are based on studies designed to identify mRNA without further characterization of cellular localization, and they support the expression of the four adenosine receptors in both small and large intestine, as summarized in Table 2. Such information is lacking for mice and guinea-pigs, but several pharmacological studies have demonstrated that adenosine receptors and enzymes are functionally active in the digestive system of these rodent species (Table 2). Functional studies which have identified the presence of adenosine receptors and enzymes in various parts of the gut, both in humans and rodents, are discussed in Section 2.1.

The expression of adenosine deaminase in both small and large intestine of rat and mouse has been demonstrated by immunohistochemistry, which revealed a predominant localization in the mucosal layer (Dinjens et al., 1989). The enzyme CD73 has been found in the small intestine of rat and guinea-pig as well as in rat and mouse colon (Nitahara et al., 1995; Karhausen et al., 2004; Giron et al., 2008). A faint expression of equilibrative nucleoside transporters (ENT1, ENT2 and ENT3) has been shown in the small and large intestine of rat and mouse, while a marked expression of CNT1 and CNT2 has been detected in the small intestine, and to a lesser extent in the colon, of both species. In the same study, a scarce or null presence of CNT3 has been found both in the small and large intestine (Lu et al., 2004) (Table 2).

## 2. Adenosine receptors and enteric functions

Intestinal functions result from an integrated regulatory interplay between the enteric nervous system (ENS), smooth muscle and the mucosal/immune system, aimed at maintaining a homeostatic status and ensuring adaptative responses in the presence of pathological conditions (Bueno, 2000; Wood, 2004). This complex network is regulated by various mediators, and there is compelling evidence indicating adenosine as one of the most important modulating agent (Antonioli et al., 2008). A functional characterization of adenosine receptors in the digestive tract has been performed mostly in animal models, while studies on the role of adenosine pathways in the human gut are lacking. There is also significant evidence supporting the involvement of ATP in the regulation of intestinal secretory and motor activity. For instance, Hu et al. (2003) observed that sinaptically released ATP acts at $P2Y_1$ receptors, located on submucosal secretomotor neurons, to stimulate electrolyte and water secretion in guinea-pig small intestine. Moreover, $P2Y_1$ receptors mediate inhibitory effects of ATP on enteric motor neurons in both human and rodent gut (Wood, 2006).

### *2.1. Myenteric plexus and smooth muscle*

Several lines of evidence highlight a prominent role of adenosine in the complex interactions between enteric neurons and smooth muscle, with fine tuning operated by this nucleoside on motility,

**Table 1**
Intestinal localization of adenosine receptors, enzymes and transporters in human

| Intestinal region | $A_1$ | $A_{2A}$ | $A_{2B}$ | $A_3$ | Adenosine deaminase | CD73 | ENTs | CTNs |
|---|---|---|---|---|---|---|---|---|
| *Small intestine* | | | | | | | | |
| Mucosa | −[a] (PRT) | +[a] (PRT) | +[a,b] (PRT) | +[a] (PRT) | +[c] (PH) | +[d] (PRT) | +[e] (mRNA) | +[f] (PRT) |
| Submucosal plexus | −[a] (PRT) | +[a] (PRT) | +[a] (PRT) | +[a] (PRT) | n.a. | n.a. | n.a. | n.a. |
| Circular muscle | +[a] (PRT) | −[a] (PRT) | n.a. | n.a. | +[g] (PRT) | n.a. | n.a. | n.a. |
| Myenteric plexus | +[a] (PRT) | +[a] (PRT) | +[a] (PRT) | +[a] (PRT) | +[g] (PRT) | n.a. | n.a. | n.a. |
| Longitudinal muscle | +[a] (PRT) | +[a] (PRT) | −[a] (PRT) | +[a] (PRT) | +[g] (PRT) | n.a. | n.a. | n.a. |
| *Large intestine* | | | | | | | | |
| Mucosa | +[a] (PRT) | −[a] (PRT) | +[a,b] (PRT) | n.a. | +[g] (PRT) | +[d] (PRT)* | +[e] (mRNA) | +[f] (PRT) |
| Submucosal plexus | +[a] (PRT) | +[a] (PRT) | +[a] (PRT) | n.a. | +[g] (PRT) | n.a. | n.a. | n.a. |
| Circular muscle | +[a] (PRT) | +[a] (PRT) | +[a] (PRT) | n.a. | n.a. | n.a. | n.a. | n.a. |
| Myenteric plexus | +[a] (PRT) | +[a] (PRT) | +[a] (PRT) | n.a. | +[g] (PRT) | n.a. | n.a. | n.a. |
| Longitudinal muscle | +[a] (PRT) | −[a] (PRT) | −[a] (PRT) | n.a. | +[g] (PRT) | n.a. | n.a. | n.a. |

+: present; −: absent; n.a.: data not available.
Abbreviation: CD73: ecto-5′-nucleotidase; ENTs: equilibrative nucleoside transporters; CNTs: concentrative nucleoside transporters; PH: pharmacological study; PRT: protein (immunohistochemistry).
References: [a]Christofi et al. (2001); [b]Puffinbarger et al. (1995); [c]Namiot et al. (1992); [d]Strohmeier et al. (1997), [e]Podgorska et al. (2005); [f]Govindarajan et al. (2007); [g]Sakamoto et al. (1993).

**Table 2**
Intestinal localization of adenosine receptors, enzymes and transporters in rat

| Intestinal region | $A_1$ | $A_{2A}$ | $A_{2B}$ | $A_3$ | Adenosine deaminase | CD73 | ENTs | CTNs |
|---|---|---|---|---|---|---|---|---|
| *Small intestine* | | | | | | | | |
| Mucosa | +[a] (PH) | n.a. | n.a. | +[g] (mRNA) | +[i,l] (PRT, PH) | n.a. | +[o] (mRNA) | +[o] (mRNA) |
| Submucosal plexus | +[a] (PH) | n.a. | n.a. | +[g] (mRNA) | +[i,l] (PRT, PH) | n.a. | +[o] (mRNA) | +[o] (mRNA) |
| Circular muscle | +[a] (PH) | n.a. | n.a. | +[g] (mRNA) | +[i,l] (PRT, PH) | +[m] (PRT) | +[o] (mRNA) | +[o] (mRNA) |
| Myenteric plexus | +[a,b] (PH) | +[b] (PH) | n.a. | +[g] (mRNA) | +[i,l] (PRT, PH) | +[m] (PRT) | +[o] (mRNA) | +[o] (mRNA) |
| Longitudinal muscle | +[a] (PH) | n.a. | n.a. | +[g] (mRNA) | +[i,l] (PRT, PH) | +[m] (PRT) | +[o] (mRNA) | +[o] (mRNA) |
| *Large intestine* | | | | | | | | |
| Mucosa | +[c] (mRNA) | n.a. | +[f] (PRT) | +[g] (mRNA) | +[i,l] (PRT, PH) | n.a. | +[o] (mRNA) | +[o] (mRNA) |
| Submucosal plexus | +[c] (mRNA) | n.a. | +[g] (mRNA) | +[g] (mRNA) | +[i,l] (PRT, PH) | n.a. | +[o] (mRNA) | +[o] (mRNA) |
| Circular muscle | +[c] (mRNA) | +[e] (mRNA) | +[g] (mRNA) | +[g] (mRNA) | +[i,l] (PRT, PH) | +[n] (mRNA) | +[o] (mRNA) | +[o] (mRNA) |
| Myenteric plexus | +[c] (mRNA) | +[e] (mRNA, PH) | +[g] (mRNA) | +[g] (mRNA) | +[i,l] (PRT, PH) | +[n] (mRNA) | +[o] (mRNA) | +[o] (mRNA) |
| Longitudinal muscle | +[c,d] (mRNA) | +[e] (mRNA) | +[g,h] (mRNA, PH) | +[g] (mRNA) | +[i,l] (PRT, PH) | +[n] (mRNA) | +[o] (mRNA) | +[o] (mRNA) |

+: present; −: absent; n.a.: data not available.
Abbreviations: CD73: ecto-5′-nucleotidase; ENTs: equilibrative nucleoside transporters; CNTs: concentrative nucleoside transporters; PH: pharmacological study; PRT: protein (immunohistochemistry).
References: [a]Coupar (1999); [b]Duarte-Araujo et al. (2004a,b); [c]Kadowaki et al. (2000b); [d]Peachy et al. (1994); [e]Antonioli et al. (2006); [f]Puffinbarger et al. (1995); [g]Dixon et al. (1996); [h]Fozard et al. (2003); [i]Dinjens et al. (1989); [l]Obata & Yamanaka (1998); [m]Giron et al. (2008); [n]Blandizzi et al. unpublished observation; [o]Lu et al. (2004).

peristaltic reflex and transit in the small and large intestine of rats, mice and guinea-pigs.

In the small intestine, exogenous adenosine or related compounds were found to inhibit cholinergic or tachykininergic transmission in guinea-pigs (Gustafsson et al., 1978; Hayashi et al., 1978; Gustafsson, 1984; Hayashi et al., 1985; Palmer et al., 1987; Broad et al., 1992; Moneta et al., 1997), rats and mice (Nichols & Hourani, 1997; Coupar, 1999; De Man et al., 2003; Duarte-Araujo et al., 2004a; Zizzo et al., 2006) via a prejunctional action on neurotransmitter release, with a significant reduction of intestinal propulsion (Shinozuka et al., 1985a; Broad et al., 1992; Coupar & Hancock, 1994; Tomaru et al., 1994; Hancock & Coupar, 1995; Suzuki et al., 1995; Storr et al., 2002). With the identification of $A_1$ receptors, most attention was focused on their functional characterization. Christofi and Wood (1993, 1994) demonstrated an involvement of this receptor in the inhibition of excitability in myenteric neurons through electrophysiological studies. Moreover, the activation of $A_1$ receptors was shown to mediate the inhibitory action of adenosine on excitatory cholinergic (Shinozuka et al., 1985b; Tomaru et al., 1995; Nitahara et al., 1995; Coupar, 1999; Storr et al., 2002; Lee et al., 2001; Duarte-Araujo et al., 2004a; Zizzo et al., 2006) or tachykininergic transmission (Christofi et al., 1990; Broad et al., 1992) of motor neurons innervating circular and longitudinal smooth muscle. In particular, this inhibitory control was ascribed to the ability of $A_1$ receptors to reduce the availability of intraneuronal calcium, via inhibition of N-type $Ca^{2+}$ channels (Shinozuka et al., 1985c; Barajas-Lopez et al., 1996; Lee & Parsons, 2000). The roles of $A_2$ and $A_3$ receptors in the control of neuromuscular functions in small bowel are much less clear. Conflicting evidence has been obtained on $A_{2A}$ receptors, which have been suggested to reduce the cholinergic motor responses in guinea-pigs and rats (Gustafsson et al., 1985; Storr et al., 2002), while, according to other reports, this receptor seems to facilitate acetylcholine release in the same species (Tomaru et al., 1995; Duarte-Araujo et al., 2004a,b).

There is currently scarce information on the regulatory activity of adenosine in rodent large intestine. An inhibitory effect mediated by $A_1$ receptors has been observed in colonic preparations from guinea-pigs (Kadowaki et al., 2000a), rats (Blandizzi et al., unpublished observation) and mice (Zizzo et al., 2006). These findings are supported by in vivo experiments demonstrating a significant increase in the colonic propulsion of animals treated with DPCPX, a selective $A_1$ receptor antagonist (Kadowaki et al., 2000b). Other experiences have demonstrated a participation of adenosine in the regulation of colonic motor activity through a facilitatory control mediated by $A_{2A}$ (Antonioli et al., 2006) or $A_{2B}$ receptors (Zizzo et al., 2006) located on inhibitory nitrergic nerves in rats and mice, respectively.

In addition to the control on myenteric nerves, adenosine has been shown to exert direct effects on intestinal smooth muscle cells. Exogenously applied adenosine analogs induced relaxant responses in muscle cells isolated from small intestine and contracted with cholecystokynin-8 as well as in colonic tissues pre-contracted with carbachol (Satchell & Burnstock, 1975; Brown & Burnstock, 1981; Burnstock et al., 1984; Murthy et al., 1995). These relaxant responses have been ascribed to a decrease in intracellular calcium concentration as a consequence of both enhanced sequestration in cellular deposits and extracellular extrusion (Frischknecht & Ferrero, 1984, 1985). Bailey and Hourani (1992) have provided evidence that $A_2$ receptors can relax or prevent contractions of intestinal smooth muscle, and subsequent studies have proposed that this inhibitory effect is driven by $A_{2B}$ receptors both in the small and large intestine (Murthy et al., 1995; Kadowaki et al., 2000a; Fozard et al., 2003).

At present, few data are available on the changes of intestinal neuromotor functions resulting from the pharmacological modulation of enzyme pathways involved in the regulation of extracellular adenosine concentration. Recently, Duarte-Araujo et al. (2004a) observed that, under normal conditions, the control of synaptic adenosine levels in the myenteric plexus is driven by extracellular deamination and nucleoside transport, followed by rephosphorylation into AMP via adenosine kinase. In the absence of pathological challenges, the catalytic activity of CD73 appears to participate marginally in the regulation of extracellular adenosine concentration, thus supporting previous data indicating a scarce involvement of this enzyme in the physiological control of extracellular adenosine metabolism (Katsuragi et al., 1993; Nitahara et al., 1995).

Although a wide distribution of adenosine receptors has been documented in the human gastrointestinal tract (see Table 1), studies focused on their functional characterization are lacking. However, we have recently obtained evidence that adenosine, acting at both muscular and neuronal sites, through $A_1$ and $A_{2A}$ receptors respectively, contributes significantly to the inhibitory control of human colonic motility (Fornai et al., in press). Likewise, there is currently a lack of information concerning the enzymatic control of extracellular adenosine levels in the neuromuscular compartment of human gut.

A considerable body of evidence has revealed that interstitial cells of Cajal (ICC), distributed in specific locations within the tunica muscularis of digestive tract, participate actively in the regulation of gastrointestinal motor functions, acting as electrical pacemakers and modulators of enteric neurotransmission as well as transducing inputs from enteric motor nerves to the smooth muscle syncytium (Mazzone & Farrugia, 2007; Sarna, 2008). Furuzono et al. (2005) have evaluated the contribution of purinoceptors (in particular ATP receptors) to the mechanisms underlying spontaneous rhytmicity of gastrointestinal motility. These experiments showed the involvement of ATP receptors in the modulation of ICC-pacemaker activity in murine ileum. However,

the authors concluded that a variety of purinoceptors are likely to control this spontaneous electrical activity in a complex manner. This proposal raises questions concerning the possible participation of adenosine receptors in the regulation of ICC activity under normal conditions, as well as the implication of adenosine pathways in altered ICC networks associated with the occurrence of pathological conditions (for instance, achalasia, bowel inflammation and slow transit constipation).

### 2.2. Secretory reflexes

The main physiological task of enteric secretion is the continuous hydration of luminal contents, to ensure an appropriate mixing and absorption of nutrients, and an effective protection against potentially harmful pathogens or enterotoxins (Xue et al., 2007). The intestinal secretory reflexes arise from complex interactions between excitatory and inhibitory neurotransmitters, released by both ENS and extrinsic primary afferent neurons (EPANs), in conjunction with a variety of modulatory messengers released from epithelial, endocrine and immune cells (Cooke, 2000). Within this signalling network, adenosine contributes to the autocrine and paracrine regulation of gastrointestinal secretion and blood flow under physiological conditions (Fig. 2), and it is also responsible for the occurrence of appropriate motor and secretory responses in the presence of pathological stimuli, to facilitate a rapid removal of noxious contents through diarrhoea (Roman & Fitz, 1999).

#### 2.2.1. Intestinal epithelium

The intestinal epithelium plays a critical role in the control of secretory/absorptive processes, and it represents the fulcrum of a permanent interaction between the immune system and commensal flora, deputed to the maintenance of tissue homoeostasis (Magalhaes et al., 2007). The net fluid movements across the mucosal surface towards the intestinal lumen are strictly regulated by the activity of crypt cells through the modulation of chloride ($Cl^-$) flux and sodium ($Na^+$) secretion (Xue et al., 2007). The activity of this physical barrier is continuously tuned by several molecular factors produced within the gut lumen, the epithelium itself or the underlying lamina propria (Mowat, 2003).

The regulatory actions of adenosine and purinergic receptors on the mucosal activity of small intestine have been scarcely investigated. Some authors showed that the $A_2$ agonist NECA was able to inhibit jejunum and ileum secretion in rats treated with prostaglandin-$E_2$ or vasoactive intestinal peptide, and suggested that this inhibitory effect could be mediated by $A_{2B}$ receptors (Coupar & Hancock, 1994; Hancock & Coupar, 1995). Ghanem et al. (2005) then examined the role of adenosine in the control of jejunal $Cl^-$ secretion in $A_1$ or $A_{2A}$ knockout mice. They observed a significant reduction of adenosine-induced $Cl^-$ secretion in $A_1$ knockout mice, while this effect was unaffected in jejunal tissues from control or $A_{2A}$-knockout mice. Furthermore, in jejunal specimens from control mice, the pro-secretory effect of adenosine was abolished in the presence of DPCPX ($A_1$ receptor antagonist), thus confirming that

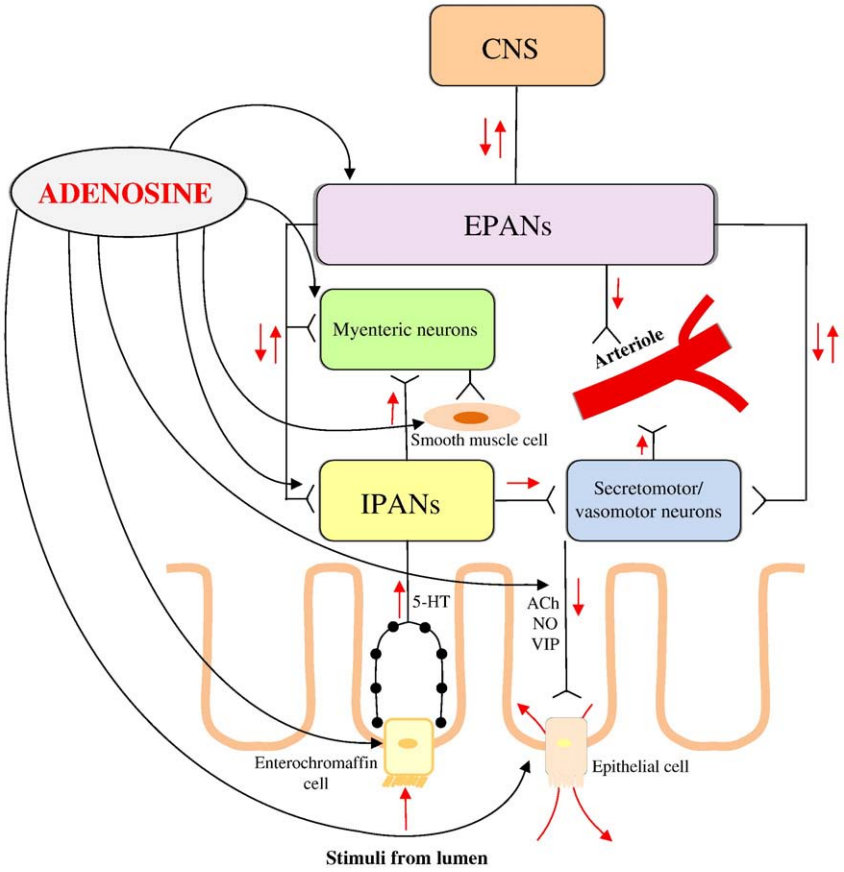


**Fig. 2.** Schematic diagram showing the suggested sites of adenosine action on intestinal neuronal circuitries involved in the interplay between central nervous system (CNS) and the enteric neuro-motor pathways and effectors. Intrinsic primary afferent neurons (IPANs) receive stimulatory signals (mechanical or chemical) from the gut lumen and activate a network of motor, secretory/absorptive and vasomotor reflexes through the excitation of motor and secretomotor/vasomotor neurons of myenteric and submucosal plexus. Extrinsic primary afferent neurons (EPANs) receive signals from intramural neuronal circuitries and transfer these inputs to the CNS. Thus, the local activity of intramural neuronal circuitries can be modulated by CNS through efferent nerve pathways in response to EPANs. This complex neuronal network is regulated by adenosine, which operates a fine tuning of intestinal motility, peristaltic reflex and transit through the modulation of IPANs, myenteric neurons and smooth muscle. Adenosine also contributes to the complex autocrine and paracrine regulation of intestinal secretion and enteric blood flow via interaction with EPANs, secretomotor/vasomotor neurons, epithelial and enterochromaffin cells. Detailed explanations about the role of adenosine in the regulation of intramural and extramural enteric reflexes are provided in Section 2. ACh: acetylcholine; 5-HT: 5-hydroxytryptamine; NO: nitric oxide; VIP: vasoactive intestinal peptide.

$A_1$ receptors play a role in the regulation of gut secretory functions. Very limited evidence supports the involvement of adenosine in the secretory activity of human small intestinal epithelium. In this respect, Christofi et al. (2001) have described a participation of adenosine receptors in the regulation of jejunal secretion in humans, but further studies are warranted to elucidate the differential role of adenosine receptor subtypes in the control of absorptive/secretory activity in the human gut.

Most of the available data on the regulatory actions of adenosine on intestinal epithelium have been obtained in colon (Roman & Fitz, 1999; Bucheimer & Linden, 2004). Experiments performed on the human colonic epithelial cell line T84 demonstrated that adenosine and related receptor agonists induced a sustained increase in $Cl^-$ secretion through the activation of $A_2$ receptors (Barrett et al., 1989). Subsequent molecular analysis showed a marked expression of $A_{2B}$ receptors at apical and basolateral sites of this cell line (Strohmeier et al., 1995; Christofi et al., 2001). Functional experiments indicated a direct control of these receptors on colonic $Cl^-$ secretion via intracellular increase in cAMP (Barrett et al., 1990), leading to activation of the cystic fibrosis transmembrane conductance regulator (CFTR) $Cl^-$ channel (Stutts et al., 1995; Strohmeier et al., 1995) (Fig. 3A). It has been speculated for many years that, beside the "classical" cAMP-dependent pathway, the excitatory control of $A_{2B}$ receptors on $Cl^-$ secretion may depend on an alternative intracellular signalling, and several authors have claimed an involvement of phospholipase $A_2$ ($PLA_2$) in parallel with the activation of adenylyl cyclase (Barrett & Bigby, 1993; Bouritius et al., 1999) (Fig. 3A). This hypothesis has received support from the demonstration that the increase in $Cl^-$ secretion, stimulated by application of the agonist NECA on colonic cells, was significantly counteracted by pre-incubation with $PLA_2$ or diglyceride lipase inhibitors (Barrett & Bigby, 1993). Nevertheless, the

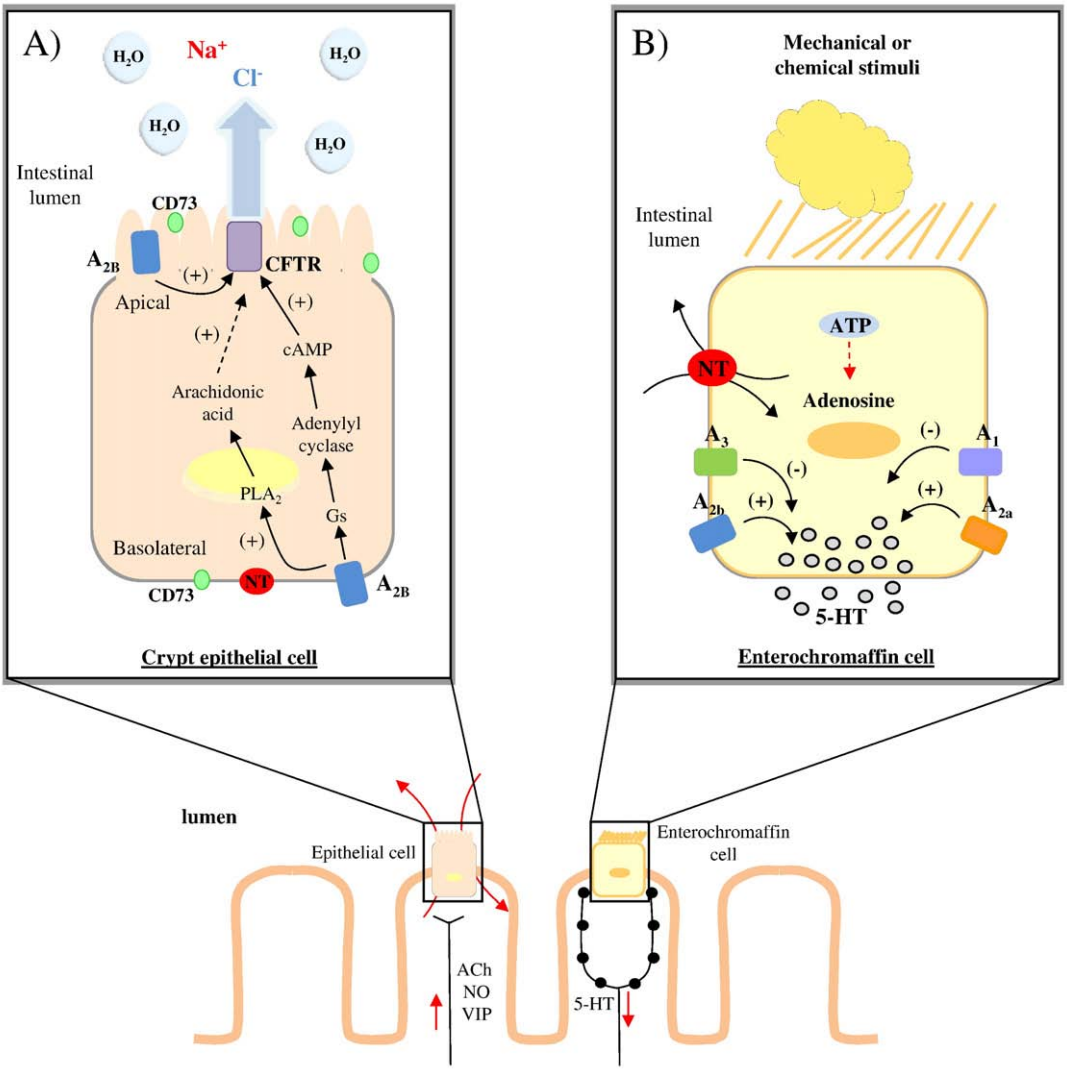


**Fig. 3.** Schematic representation showing the role of adenosine pathways in the regulation of crypt epithelial cell (**A**) and enterochromaffin cell activity (**B**). (**A**) In human crypt epithelial cells, adenosine stimulates a sustained increase in $Cl^-$ secretion through the activation of $A_{2B}$ receptors, expressed at both apical and basolateral sites. $A_{2B}$ receptors mediate a direct control of adenosine on $Cl^-$ secretion via intracellular increase in cyclic adenosine monophosphate (cAMP), which leads to activation of the cystic fibrosis transmembrane conductance regulator (CFTR) $Cl^-$ channel. Phospholipase $A_2$ ($PLA_2$) seems to serve as alternative intracellular signalling in the excitatory control of $A_{2B}$ receptors on $Cl^-$ secretion. The enzyme ecto-5′-nucleotidase (CD73), predominantly expressed on the apical side of intestinal epithelium, represents a critical link in the mutual interaction between the immune system and enteric mucosa. This enzyme, through a rapid conversion of luminal AMP into adenosine, is able to modulate the secretory reflex under both physiological and pathological conditions. (**B**) Studies performed on the BON cell line, which display an apparent EC phenotype, suggest that enterochromaffin cells express $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$ receptors. Basal 5-hydroxytryptamine (5-HT) release from enterochromaffin cells is under tonic control by adenosine. The stimulation of enterochromaffin cells induces intracellular conversion of ATP into adenosine, which then modulates the activity of these cells predominantly via excitatory $A_{2A}$ and $A_{2B}$ receptors or inhibitory $A_1$ or $A_3$ receptors. The concentration of extracellular adenosine represents the critical determinant for the differential activation of adenosine receptors on enterochromaffin cells, and nucleoside transporters (NT) play a pivotal role in the control of endogenous adenosine levels. ATP: adenosine triphosphate; Gs: stimulatory G-protein.

exact mechanisms through which phospholipid-derived mediators contribute to the activity of $A_{2B}$ receptors in this setting require better clarification.

Adenosine receptors have been localized in the apical domain of secretory epithelial cells, where they are exposed to variable concentrations of endogenous adenosine in the extracellular environment (Roman & Fitz, 1999). At this level, the enzymatic pathways involved in the regulation of adenosine concentration are pivotal to the preservation of enteric homeostasis. In particular, the enzyme CD73 represents a critical link in the mutual interaction between the immune system and enteric mucosa (Strohmeier et al., 1997). This enzyme, which is abundantly expressed on the apical side of intestinal epithelium, appears to be extremely sensitive to variations of AMP released in the gut lumen by neutrophils as discussed in detail below (see Section 3.4).

Under normal or pathological conditions, substantial amounts of adenosine, produced by cellular metabolism or released from immune cells, may accumulate in the extracellular biophase of secretory intestinal crypts. However, under normal conditions the intestinal epithelium is likely to be endowed with a control mechanism that dampens its secretory responsiveness to locally released adenosine. In the basolateral membrane of T84 cells, Tally et al. (1996) demonstrated the presence of an efficient nucleoside transporter system, which is involved in the maintenance of extracellular adenosine levels below the pro-secretory threshold, thus preventing the activation of $Cl^-$ secretion (and hence the onset of diarrhoea). Subsequently, these authors characterized the kinetics of adenosine transport across the apical and basolateral surfaces in the same cell model. In this setting, the uptake of adenosine across the apical membrane was not saturable, but consistent with a passive diffusion. Moreover, the study revealed the presence of a $Na^+$-independent facilitated adenosine carrier system at the basolateral level (Mun et al., 1998). Another limiting step to avoid excessive extracellular adenosine levels is represented by adenosine deaminase. However, few studies have addressed the role of adenosine deaminase in the regulation of mucosal purine concentration, although several lines of evidence indicate a wide expression of this catabolic enzyme in animal and human intestinal mucosa (see Tables 1 and 2). Of note, findings in cultured cells may not reflect the actual in situ effects of adenosine on native cells, since differences in the receptor populations may occur. For these reasons, studies performed on integrated models are needed to obtain more conclusive information concerning the role of adenosine in the regulation of enteric secretory functions.

### 2.2.2. Submucosal/vasomotor plexus

The submucosal plexus ensures a precise and complex regulation of gut epithelial functions and blood flow through a complex network of reflexes (Vanner & McNaughton, 2004). This activity is initiated by the mechanical/chemical stimulation of mucosal sensory cells (enterochromaffin cells, ECs) which results in the activation of intrinsic primary afferent neurons (IPANs) deputed to convey nervous inputs to submucosal ganglia. IPANs represent the afferent arms of intramural reflexes which control the activity of the submucosal secretomotor/vasomotor plexus, thus participating in the regulation of bowel movements, transfer of water and electrolytes across the mucosal epithelium and blood flow within the gut wall (Cooke, 2000). The following sections provide an appraisal of the role played by adenosine in the control of various components subserving these gut reflexes.

*2.2.2.1. Enterochromaffin cells.* ECs, sparsely distributed in intestinal crypts, are responsible for the initiation of motor and secretory neural reflex programs in the gut (Kim et al., 2001a). These endocrine cells are equipped with mechano- and chemosensitive elements, which allow them to detect changes in intestinal luminal pressure or content composition and respond with the release of 5-hydroxytryptamine (5-HT). This mediator then acts as a paracrine messenger affecting the function of epithelial cells, or as a neurocrine mediator influencing the activity of sensory neurons (Gershon & Tack, 2007). The scattered distribution of ECs has slowed progress in isolating a pure population of these cells. At present, the BON cell line displays an apparent enterochromaffin phenotype and appears to be an appropriate model for investigating sensory transduction mechanisms and signalling pathways involved in the regulation of 5-HT release from ECs (Kim et al., 2001b). Molecular analysis of BON cells has revealed the presence of all four adenosine receptors, suggesting a complex pattern of excitatory and inhibitory modulation of 5-HT release by the adenosine pathway (Christofi et al., 2001; Christofi et al., 2004). Christofi et al. (2004) demonstrated that basal 5-HT release from BON cells is under tonic control by adenosine, predominantly via cAMP-dependent excitatory $A_{2A}$ or $A_{2B}$ receptors or $Ca^{2+}$-dependent inhibitory $A_1$ or $A_3$ receptors (Fig. 3B). Moreover, these authors indicated the concentration of extracellular adenosine as a critical determinant for the differential activation of adenosine receptors on ECs, highlighting the importance of transporters in the control of endogenous adenosine levels. Indeed, the mechanical stimulation of ECs produced an increment of extracellular adenosine levels, which led to the activation of $A_1$ and $A_3$ receptors, resulting into a physiological brake of 5-HT release (Fig. 3B). It remains to be demonstrated whether this pattern of receptor expression and functional regulation, as highlighted in BON cells, occurs also in native ECs.

*2.2.2.2. Intrinsic primary afferent neurons.* IPANs are intramural sensory neurons which can detect signals arising from the transduction of mechanical and chemical stimuli, that reflect changes in the tension of gut wall and chemical nature of gut luminal contents. IPANs make synaptic contacts with interneurons and enteric motor neurons to form the circuitries of intrinsic motor, secretory and vasomotor reflexes (Fig. 2) (Clerc & Furness, 2004). IPANs may function also as nociceptors, since their activation by noxious stimuli triggers protective responses. Moreover, changes in the properties of these neurons can occur following inflammation, contributing to persistent post-inflammatory bowel dysfunctions (Sharkey & Mawe, 2002). The influence of adenosine pathways on IPAN activity has been exhaustively reviewed by Christofi (2001). Overall, endogenous adenosine exerts an inhibitory control on gut IPANs under both normal and pathological conditions. A large body of experimental evidence suggests that such modulation is mainly ascribable to the activation of pre-junctional or somatic $A_1$ and $A_3$ receptors, which mediate a condition of membrane hyperpolarization with a decrease in the sensitivity of IPANs to excitatory stimuli. By contrast, the ability of adenosine to increase excitability in a minority of IPANs through activation of $A_{2A}$ receptors has been reported (Christofi, 2001). No data are currently available concerning the role of $A_{2B}$ receptors in the control of IPAN activity.

*2.2.2.3. Secretomotor and vasomotor neurons.* Secretomotor/vasomotor neurons are excitatory neurons deputed to control the functions of intestinal crypts and submucosal arterioles (Vanner & Macnaughton, 2004). The main findings supporting the regulatory actions of adenosine on the activity of these nerves have been obtained in experiments on guinea-pig small intestine. Barajas-Lopez et al. (1991) provided the first demonstration of the ability of adenosine and its analogs to act on secretomotor neurons. In particular, these authors observed that adenosine exerts a depolarizing effect on secretomotor nerve fibres by acting at $A_2$ receptors, and an inhibitory effect on acetylcholine release via pre-junctional $A_1$ receptors. Subsequently, they demonstrated that $A_2$-mediated depolarization was related to a significant reduction of membrane potassium conductance (Barajas-Lopez, 1993), whereas $A_1$-mediated inhibition of cholinergic transmission could be ascribed to the inhibition of voltage-activated calcium currents in submucosal neurons (Barajas-Lopez et al., 1996). The inhibitory effect of adenosine was also observed in colonic submucosal nerves of guinea-pigs. In this neuronal population, immunohistochemical analysis demonstrated a significant expression of $A_1$ receptors. Functional experiments revealed an inhibitory effect mediated by these receptors, suggesting a relevant role of endogenous adenosine as a physiological brake in the modulation

of reflex-evoked chloride secretion (Cooke et al., 1999). Recently, Wunderlich et al. (2008) have provided the first demonstration regarding the implication of adenosine in the control of synaptic transmission in submucosal plexus isolated from human jejunum. In this study, the authors focused their attention on the functional characterization of $A_3$ receptors, demonstrating their involvement in the modulation of distension reflexes, as well as in the inhibition of synaptic cholinergic transmission. In the same study, the authors observed a purinergic modulation of synaptic transmission occurring through activation of $P2Y_1$ receptors, which may be important in the control of mucosal and distension reflexes (Wunderlich et al., 2008). The possible involvement of other adenosine receptors in the regulation of human submucosal plexus activity has not yet been examined. Advances in knowledge in this area could unravel targets for the development of novel therapies against secretory/absorptive disturbances associated with inflammatory and infectious diseases.

### 2.3. Extrinsic primary afferent neurons

The extrinsic sensory innervation of the gastrointestinal system provides an important link for the detection of noxious or non-noxious events by the central nervous system (CNS), thus allowing a central control of critical enteric functions and enabling the conscious awareness of intestinal tract status (Berthoud et al., 2004). Several mediators influence the sensitivity of visceral afferents, interacting with specific receptors expressed on cell bodies of sensory neurons, in the dorsal root or nodose ganglion, or their endings in the gut wall (Bueno et al., 1997). In this regard, adenosine receptors, probably $A_1$ and $A_2$, have been found in mesenteric nerves innervating the rat jejunum, where they facilitate the excitation of peripheral sensory terminals (Brunsden & Grundy, 1999; Kirkup et al., 1998). Further excitation could be produced by endogenous adenosine release secondary to contraction of intestinal smooth muscle (Kirkup et al., 2001). In vitro experiments, performed on mesenteric afferents isolated from rat jejunum, indicate that adenosine may activate and/or sensitize gastrointestinal nociceptive fibres, and that this effect may occur under pathological conditions (ischemia, inflammation), when extracellular concentrations of adenosine are likely to be elevated (Brunsden & Grundy, 1999). Since extracellular adenosine levels could increase as a consequence of ATP breakdown by ubiquitous ecto-nucleotidases, it is also conceivable that the modulation of afferent nerves may result from a synergistic action of adenosine and ATP, the latter being recognized as an important initiator and modulator of nociceptive sensitivity (Kirkup et al., 2001). Overall, these observations could lead to a better understanding of the mechanisms regulating visceral pain transmissions, hence opening the way to the identification of novel and targeted pharmacological interventions for visceral pain management.

### 2.4. Immune system

Increasing evidence highlights the existence of close interactions between ENS and enteric immune cells deputed to establishing a first line of defense against foreign invasion (Collins, 1996; Bueno, 2000). Several immune/inflammatory cell types are present in continuously varying numbers in the intestinal mucosa, lamina propria and smooth muscle layers, where they establish close anatomic proximity with the neuronal elements of ENS, vagal nerve fibres and spinal sensory nerves. These cells are actively involved in adaptive motor and secretory changes, as well as in the regulation of sensory fibres, under normal or pathological conditions (Bueno, 2000). In this complex framework, adenosine and its receptors appear to contribute to the regulation of immune and inflammatory responses in order to provide tissue protection (Sitkovsky & Lukashev, 2005; Sitkovsky & Ohta, 2005). In particular, detailed studies have indicated adenosine as a prominent player deputed to down-regulate activated immune cells and protect tissues from inflammatory injury via receptors expressed on immune cell populations (lymphocytes, neutrophils, monocytes, macrophages, dendritic cells and mast cells) (Haskò & Cronstein, 2004; Haskò et al., 2007).

Cytotoxic T lymphocytes and T-helper (Th) cells are endowed with a marked expression of $A_{2A}$, $A_{2B}$ and $A_3$ receptors, whereas $A_1$ receptors are scarce or absent (Lukashev et al., 2003; Gessi et al., 2005). A significant up-regulation of $A_{2B}$ receptors has been observed following activation of human peripheral $CD4^+$ and $CD8^+$ T cells (Lappas et al., 2005). Analogously, evidence of a rapid up-regulation was reported by Gessi et al. (2004) for $A_3$ receptors upon activation of human T lymphocytes. Recent findings have shown a dual opposite action of adenosine on T cell proliferation through $A_{2A}$ (stimulatory) and $A_3$ (inhibitory) receptors (Takahashi et al., 2007). However, the scarcity of data regarding the expression and function of $A_3$ receptors in lymphocytes warrants additional investigations, particularly to verify whether experimental findings can be translated to the human gut immune system.

Adenosine can suppress the expression of intercellular adhesion molecule-1 (ICAM-1) in lymphocytes from enteric Peyer's patches (Johnston et al., 2005). Based on this mechanism, extracellular adenosine may inhibit ongoing accumulation of lymphocytes at inflammatory sites by limiting their adhesion and migration into extravascular sites (Yang et al., 2005). Adenosine receptors appear to be involved in cytokine production in activated Th cells. Indeed, $A_{2A}$ receptors are predominantly expressed in human cytokine-producing T cells and the stimulation of Th cells with anti-CD3 monoclonal antibodies was shown to evoke a rapid up-regulation of these receptors with subsequent decrease in the release of interferon-γ (IFN-γ) (Lappas et al., 2005). More recently, Deaglio et al. (2007) investigated the role played by CD39 and CD73 in the control of lymphocyte activity. Their results indicate that the co-expression of these enzymes on cell surface distinguishes $CD4^+$, $CD25^+$ and $Foxp3^+$ T regulatory cells from other T cells, suggesting that both enzymes are suitable cell surface markers. Moreover, Deaglio et al. (2007) have shown that these ecto-enzymes convert extracellular nucleotides into pericellular adenosine, which then induces immune suppression through stimulation of $A_{2A}$ receptors on activated T effector cells, indicating a relevant role of CD39 and CD73 in the modulating functions of T regulatory cells.

Adenosine is abundantly released from neutrophils following their activation, and contributes actively to the regulation of these cells during inflammatory responses (Linden, 2006). Neutrophils express both $A_1$ and $A_{2A}$ receptors, and their functions appear to be modulated by variations in extracellular adenosine levels. In particular, stimulation of $A_1$ receptors induces up-regulation of the neutrophil adhesion receptor Mac-1 and increased expression of complement receptors responsible for enhanced adhesion of neutrophils to vascular endothelium (Bours et al., 2006). By contrast, the activation of $A_{2A}$ and $A_{2B}$ receptors, via adenosine produced by CD39 and CD73 during inflammation, inhibits the adhesion of neutrophils to endothelial cells (Eltzschig et al., 2004). Molecular studies have demonstrated the expression of $A_3$ receptors on human neutrophils, but their functional characterization is still lacking (Gessi et al., 2002). Adenosine exerts a protective action on host tissues through modulation of neutrophil bactericidal functions. A dual regulatory influence of adenosine on phagocytosis has been reported, since the activation of $A_1$ receptors enhances this process, while the stimulation of $A_{2A}$ receptors causes a marked reduction of phagocytic activity (Zalavary & Bengtsson, 1998). There is also evidence that adenosine can differentially regulate the generation of reactive oxygen species (ROS) with stimulatory or inhibitory actions depending on its predominant levels at different receptor sites. Indeed, adenosine promotes the production of ROS from activated neutrophils through recruitment of $A_1$ receptors, whereas it down-regulates ROS generation via $A_{2A}$ receptors (Sun et al., 2007). Moreover, adenosine exerts a strict control over the release of microbicidal agents from neutrophils via interaction with $A_{2A}$ and $A_3$ receptors (Theron et al., 2002).

Monocytes and macrophages express all four adenosine receptors, and recent studies have demonstrated significant variations in their

expression and function during the maturation process: the expression of $A_1$, $A_{2A}$ and $A_3$ receptors appears to be low in quiescent monocytes, while their density increases during differentiation into macrophages (Thiele et al., 2004). The patterns of purinergic receptor expression are sensitive to the release of cytokines in response to inflammatory stimuli. In particular, $A_{2A}$ receptors undergo up-regulation in human monocytes stimulated by interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), while treatment with IFN-γ significantly reduces their expression (Khoa et al., 2001). A regulatory role of adenosine in the recruitment of monocytes at inflammation sites, through $A_{2A}$ and $A_{2B}$ receptor-mediated inhibition of cell adhesion molecule expression on endothelium, has been also described (Delikouras et al., 2003).

Several studies have investigated the effects of adenosine on cytokine production in monocytes and macrophages. Current evidence indicates that the production of IL-12, TNF-α, IL-6, macrophage inflammatory protein (MIP)-1α and nitric oxide (NO) is negatively affected by adenosine through activation of $A_{2A}$, $A_{2B}$ and $A_3$ receptors (Haskò et al., 2000; Ohta & Sitkovsky, 2001; Haskò et al., 2007). In addition, extracellular adenosine stimulates the release of the anti-inflammatory cytokine IL-10 by monocytes and macrophages via $A_{2A}$ and $A_{2B}$ receptors, thus promoting the termination of inflammatory responses (Khoa et al., 2001; Nèmeth et al., 2005).

Dendritic cells belong to a specialized system of antigen-presenting cells (APCs), which play an important role in the stimulation of the immune response, participating in antigen interception, processing, and inducing the activation of specific lymphocyte-effector mechanisms (Haskò & Cronstein, 2004). Adenosine has been shown to modulate dendritic cell functions by interaction with specific receptors, the expression and function of which is strictly related to the maturation status of these immune cells (Haskò & Cronstein, 2004). Immature human dendritic cells express predominantly $A_1$ and $A_3$ receptors, which mediate chemotaxis via an increase in intracellular calcium, thus allowing the recruitment of dendritic cells into inflammation sites. Activated mature dendritic cells are endowed mainly with $A_{2A}$ receptors, which confer sensitivity to the anti-inflammatory effects of adenosine, with a significant reduction of cytokine production and compensatory effects against chronic cell activation, responsible for tissue damage (Schnurr et al., 2004). Moreover, Pacheco et al. (2005) have demonstrated the presence of $A_{2B}$ receptors in both immature and mature dendritic cells, where they act as adenosine deaminase anchoring proteins. Adenosine deaminase and $A_{2B}$ receptor form a molecular complex that interacts with CD26 expressed on T cells and evokes a co-stimulatory signal on IFN-γ and TNF-α production. Dickenson et al. (2003) performed studies on the murine dendritic cell line XS-106, demonstrating that $A_{2A}$ and $A_3$ receptors inhibit the release of TNF-α, and proposed these cells as useful model to evaluate the role of adenosine in the regulation of dendritic cell functions. Since most investigations, designed to examine the effects of adenosine on APC functions, have been performed on single cell lines or a single source of APCs, additional studies, conducted on different cell models, might help to better determine the role played by adenosine in these immune cells.

Mast cells are generally recognized as crucial players in allergic reactions and important initiators of innate immune responses. They can also have a role in the pathogenesis of inflammatory processes and pain sensation (Thacker et al., 2007). Interestingly, mast cells might be the exception to the general 'rule', according to which adenosine promotes immunosuppression and tissue protection, since this nucleoside acts as a potent stimulator of mast-cell function. In particular, adenosine, mainly through occupancy of $A_{2B}$ and $A_3$ receptors, stimulates the degranulation of mast cells, which then release histamine, 5-HT, chemokines and proteases (Haskò & Cronstein, 2004). There is also evidence that histamine, released from mast cells in response to adenosine, elicits inhibitory effects, via interaction with macrophage $H_2$ histamine receptors, on TNF-α biosynthesis (Smith et al., 2002). Nevertheless, the pro-inflammatory actions resulting from direct adenosine-induced mast cell stimulation seem to overcome the anti-inflammatory response promoted by adenosine via the histamine-mediated negative feedback on mast cell activation (Haskò & Cronstein, 2004).

## 3. Pathophysiological role and pharmacological modulation of adenosine in gut disorders associated with motor, secretory and sensory dysfunctions

The term "gut disorders" covers a wide spectrum of pathological conditions, affecting any part of the gastrointestinal tract, originating from heterogeneous etiopathogenetic factors. Gut disorders are characterized by a variable combination of chronic or recurrent symptoms, resulting from dysregulation of visceral functions (motor, secretory and immune), often influenced by afferent sensations and associated with emotional factors and stress (Ouyang & Locke, 2007). Increasing progress has been made in understanding the pathogenesis of gut dysfunctions, with the identification of novel therapeutic options based on pathophysiological rationales. However, despite important advances, several of the primary features of human intestinal diseases remain unexplained, and the available pharmacological tools are often either ineffective or allow only partial symptom remission. Since adenosine is deeply involved in the regulation of enteric functions, a detailed characterization of the roles played by this pathway during adverse intestinal conditions could pave the way to development of targeted drugs potentially useful for the management of gut disorders.

### 3.1. Inflammatory bowel diseases

Ulcerative colitis and Crohn's disease, collectively known as inflammatory bowel diseases (IBDs), are severe and debilitating disorders with a growing incidence in both developing and advanced countries (Hanauer & Present, 2003). Both diseases are characterized by recurrent and serious inflammation of the enteric mucosa at different levels of the gastrointestinal tract, and are associated with significant alterations of gastrointestinal motor, secretory and sensory functions, as a consequence of structural and/or functional changes in the enteric nervous system (Lomax et al., 2005; De Schepper et al., 2008). Although the precise etiologies leading to onset of these chronic inflammatory conditions remain unclear, increasing evidence supports the concept that abdominal symptoms in patients with IBD depend on amplified interactions between the immune system and enteric neural circuitries (Kucharzik et al., 2006). Multiple humoral factors, released in response to tissue injury or exogenous pathogens, participate in the regulation of this complex immune/inflammatory network to promote primary defensive immune responses, control their course and ensure proper resolution of concomitant inflammatory reactions (Baumgart & Carding, 2007).

IBDs are thought to arise from an abnormal response of the enteric immune system against components of the intestinal flora wrongly recognized as pathogens by dendritic cells, which enter a maturation program promoting the differentiation of naïve T cells into effector T cells (Th1, Th2 and Th3) and natural killers (Neuman, 2007; Baumgart & Carding, 2007). This activation of T lymphocytes and macrophages is followed by a massive release of several pro-inflammatory cytokines, including IL-1, IL-6 and TNF-α, which stimulate the secretion of chemotactic cytokines, such as IL-8 and monocyte chemoattractant protein-1, responsible for the recruitment of leukocytes into inflamed mucosa (Huibregtse et al., 2007). Chronically activated macrophages and neutrophils then generate excessive amounts of ROS, which can exceed the defending capacity of the enteric antioxidant system, promoting intestinal oxidative injury (Torres & Rios, 2008). The increased and continuous exposure of neurons to inflammatory mediators is thought to contribute not only to tissue injury, but also to alterations in the contractile and secretory activity of digestive tract (Lomax et al., 2006).

An active role of extracellular ATP and its receptors in the alterations occurring during intestinal inflammation has been suggested (Burnstock, 2008). In a recent study on humans and rodents, Grbic et al. (2008) observed that intestinal inflammation was associated with up-regulation of $P2Y_2$ and $P2Y_6$ receptor expression at the level of intestinal epithelium, thus supporting a possible involvement of P2Y receptors in the pathophysiology of gut inflammation. When considering adenosine, this nucleoside is known to regulate both immune and enteric functions, and therefore research efforts are attempting to unravel its roles in the pathophysiology of bowel inflammation and related gut dysfunctions, as well as to evaluate possible therapeutic implications of its pharmacological modulation.

*3.1.1. Adenosine and intestinal inflammation*

Inflammation is characterized by a complex set of interactions among soluble factors and immune/inflammatory cells, arising from traumatic, infectious, post-ischemic, toxic or autoimmune injury, aimed at destroying pathogenic agents and preserving tissue homeostasis (Nathan, 2002). A defective control of the activity of this complex network leads unavoidably to unacceptable levels of collateral injuries towards normal tissues (Torres & Rios, 2008). As a consequence, the host attempts to check these uncontrolled reactions and to protect vital organ functions through activation of several mechanisms, and compelling evidence indicates a significant involvement of adenosine in the mitigation of abnormal inflammatory responses (Sitkovsky & Ohta, 2005).

Under inflammatory conditions, purinergic signalling pathways are subjected to dynamic changes in the expression and/or function of ectoenzymes and adenosine receptors, with variations of extracellular adenosine levels and receptor activation, in order to finely tune inflammatory/immune responses, with consequent limitation of inflammatory tissue injury (Bours et al., 2006). Inflammatory processes are associated with a significant increase in CD73 expression and activity which leads to a rapid conversion of ATP into adenosine (Noji et al., 2004; Louis et al., 2008). Concomitantly, the accumulating extracellular adenosine is rapidly metabolised to inosine by adenosine deaminase, and a close positive correlation has been established between the severity of inflammation and the local increase in adenosine deaminase expression and activity (Conlon & Law, 2004; Cavalcante et al., 2006; Desrosiers et al., 2007).

Changes in purinergic receptor expression appear to contribute to the modulation of gut inflammatory responses. Sundaram et al. (2003) observed a significant up-regulation of $A_1$ receptors in the presence of intestinal inflammation in rabbit. Subsequently, in a mice model of *Escherichia coli* peritonitis, it was demonstrated that the expression of $A_1$ receptors in peritoneal leukocytes reaches its maximum level after 12 h from pathogen inoculation and returns to baseline within 24 h. This study also evaluated the time-dependence of $A_{2A}$ receptor expression, revealing a peak at 24 h, in concomitance with the maximum of adenosine concentration in peritoneal fluid (Rogachev et al., 2006). The same group has recently demonstrated that the stimulation of $A_1$ receptors results in a sequential induction of $A_{2A}$ receptor expression which then reverses the inflammatory response associated with *E. coli*-induced peritonitis (Nakav et al., 2008). These findings point to the intriguing concept that a dominance of immunostimulatory $A_1$ receptors occurs during the early stage of inflammation, while at a later stage the control is predominantly by $A_{2A}$ receptors with the purpose of mitigating the magnitude of inflammatory response. In line with this proposal, a marked up-regulation of high-affinity $A_{2A}$ receptors has been observed in various models of inflammation, including colitis (Antonioli et al., 2006; Fortin et al., 2006; Rogachev et al., 2006). Moreover, an inhibitory interplay occurs between pro-inflammatory cytokines (IL-1β and TNF-α), which enhance the expression of $A_{2A}$ receptors, and the $A_{2A}$-receptor pathway, which reduces the activity of immune and inflammatory cells, and such mutual interactions have been demonstrated in models of bowel inflammation (Odashima et al., 2005; Morello et al., 2006; Naganuma et al., 2006; Haskò & Pacher, 2008). Low affinity $A_{2B}$ receptors also appear to be up-regulated during pathologic conditions, and are thought to participate in the inhibitory modulation of inflammatory responses (Sitkovsky & Ohta, 2005; Kolachala et al., 2005; Sitkovsky et al., 2008).

There is conflicting evidence regarding the role of $A_3$ receptors in the pathophysiology of inflammation, as recently discussed by Gessi et al. (2008). In particular, a pro-inflammatory action, driven by up-regulation and activation of $A_3$ receptors, has been observed in lung tissue and eosinophils from patients with airway inflammation (Walker et al., 1997). Analogously, Spruntulis and Broadley (2001) observed that $A_3$ receptor activation induced a rapid inflammatory cell influx into the lungs of sensitized guinea-pigs. By contrast, Mabley et al. (2003) reported a significant reduction of inflammatory cell activity following $A_3$ receptor activation in two murine models of colitis. More recently, Guzman et al. (2006) demonstrated an increase in $A_3$ receptor expression in intestinal tissues from rats with experimental colitis, and an active role has been proposed for this receptor in the modulation of inflammatory response.

*3.1.2. Pharmacological modulation of adenosine to control intestinal inflammation*

As discussed above, adenosine is able to mediate marked anti-inflammatory actions in a variety of organ systems, including the gastrointestinal tract, through inhibition of Th1 cytokine production (i.e. IL-6, IL-12, TNF-α and IFN-γ) and down-regulation of neutrophil activity (Siegmund et al., 2001; Naganuma et al., 2006; Antonioli et al., 2007), as well as by promoting Th3-cell development, with a subsequent increase in the production of the anti-inflammatory cytokine IL-10 (Kuno et al., 2006; Nalos et al., 2006; Csóka et al., 2007).

An involvement of adenosine pathways has been demonstrated in the anti-inflammatory effects exerted by drugs employed in the clinical management of inflammatory diseases, including IBDs. Cronstein et al. provided initial evidence supporting the ability of methotrexate and sulfasalazine to promote the release of adenosine from a variety of cells and tissues subjected to adverse conditions (Cronstein et al., 1991, 1993, 1994, 1999; Gadangi et al., 1996). Subsequently, Morabito et al. (1998) demonstrated that the activity of CD73 was of primary importance for the increase in extracellular adenosine elicited by methotrexate or sulfasalazine, indicating this enzyme as a critical mediator for the anti-inflammatory effects of both drugs. At present, increasing interest is being paid to the development of drugs that, through direct stimulation of adenosine receptors (in particular $A_{2A}$ and $A_3$) or by an increase in local adenosine levels, could represent promising therapeutic options for treatment of IBDs (Akkari et al., 2006).

A large body of evidence, demonstrating the prominent role of $A_{2A}$ receptors in the anti-inflammatory actions of adenosine, has channelled pharmacological research towards the synthesis of $A_{2A}$ selective agonists and their testing in models of intestinal inflammation. Odashima et al. (2005) investigated the potential anti-inflammatory effect of ATL-146e, a selective $A_{2A}$ receptor agonist, on acute and chronic colitis induced by formalin-immune complex in rabbits, and in a model of spontaneous ileitis in SAMP1/YitFc mice (characterized by age-related impairment of immune functions, including T-dependent antibody response, due to a decrease in Th cell function; Matsumoto et al., 1998). In this study, the activation of $A_{2A}$ receptors was associated with significant improvement of inflammation in the intestinal mucosa, with a reduction of pro-inflammatory cytokine levels (TNF-α, IFN-γ and IL-4) and leukocyte infiltration. Similarly, beneficial effects of ATL-146e were observed in a murine model of enteritis induced by *Clostridium difficile* toxin-A (Cavalcante et al., 2006). Naganuma et al. (2006), in a study designed to unravel the mechanisms through which $A_{2A}$ receptors mediate the anti-inflammatory actions of adenosine, observed that $A_{2A}$ activation promotes a destabilization of mRNA coding for pro-inflammatory cytokines, while sparing the anti-inflammatory

activity exerted by IL-10 and transforming growth factor (TGF)-β. These marked changes in the balance between pro-inflammatory and anti-inflammatory cytokines released by Th cells appear to be responsible for the blunting activity of $A_{2A}$ receptors on the inflammatory process. In contrast with this view, Selmeczy et al. (2007) recently reported that the selective $A_{2A}$ receptor agonist CGS 21680 was ineffective in ameliorating various inflammatory parameters (MIP-1α, MIP-2, IFN-γ, IL-1β, IL-12 and TNF-α) in a model of colitis induced by dextran sodium sulphate (DSS). Overall, the role of $A_{2A}$ receptors in the pathophysiology of intestinal inflammation remains a matter of debate, and further investigation is required to elucidate the potential therapeutic significance of $A_{2A}$ agonists in the treatment of IBD.

Preliminary observations suggest $A_{2B}$ receptors as potential targets for the management of bowel inflammation. A recent study has demonstrated an active involvement of intestinal epithelial $A_{2B}$ receptors in the pro-inflammatory activity of adenosine in gut tissues, and has shown that oral administration of the selective $A_{2B}$ antagonist ATL-801 to mice with DSS-induced colitis as well as IL-10 knockout mice can evoke a significant improvement of histological and clinical scores of inflammation, resulting in a beneficial interference with disease progression (Kolachala et al., 2008).

$A_3$ receptors are also emerging as possible targets for treatment of bowel inflammation (Gessi et al., 2008). In this respect, IB-MECA, an $A_3$ receptor agonist, was shown to ameliorate both DSS-induced intestinal inflammation in mice and spontaneous colitis in IL-10 deficient animals. In particular, IB-MECA markedly decreased colonic levels of pro-inflammatory cytokines (IL-1, IL-6 and IL-12), and reduced the local production of MIP-1α and MIP-2, with a powerful down-regulation of leukocyte trafficking in both models of bowel inflammation (Mabley et al., 2003). Based on these findings, Guzman et al. (2006) were prompted to evaluate the effects of IB-MECA on gene dysregulation and tissue injury in a rat model of colitis induced by 2,4,6-trinitrobenzene sulphonic acid (TNBS). In this study, high density oligonucleotide microarray analysis revealed a remarkable reduction of membrane transporter expression ($H^+/K^+$ ATPase, $Na^+/K^+$ ATPase, $Ca^{2+}$ ATPase), mitogen activated protein kinases, and channel genes ($Na^+$, $Cl^-$) as well as up-regulation of chemokine, heat shock protein and cytokine/inflammatory genes (such as IL-1β and small inducible cytokine A2) in inflamed colonic tissues. In this setting, treatment with the $A_3$ receptor agonist prevented the induction of various cytokine/chemokine/inflammatory genes, and promoted a marked suppression of ROS production with a significant amelioration of intestinal injury.

An alternative strategy to the direct pharmacological modulation of adenosine receptors is represented by the elevation of endogenous adenosine concentrations, through blockade of pivotal catabolic enzymes. The inhibition of adenosine kinase has been investigated by Siegmund et al. (2001) in mice with DSS-induced colitis as a potential target to promote adenosine accumulation and down-regulation of local inflammatory responses. These authors highlighted the therapeutic potential of GP515, a selective adenosine kinase inhibitor, in reducing colonic levels of IFN-γ, and demonstrated its inhibitory effect on CD69 expression, one of the earliest cell surface antigens induced in activated T cells, natural killers and neutrophils. Several authors have reported a significant increase in adenosine deaminase expression and activity in inflamed tissues, including intestinal ones, and tight association of reduced adenosine production with chronicization of inflammatory conditions (Nakamachi et al., 2003; Cavalcante et al., 2006; Desrosiers et al., 2007). Moving from these observations, the effects of adenosine deaminase inhibition have been examined by our group in a rat model of experimental colitis induced by 2,4-dinitrobenzenesulphonic acid (DNBS). In particular, the study was aimed at evaluating the effects of a novel adenosine deaminase blocker, 4-amino-2-(2-hydroxy-1-decyl)pyrazole[3,4-*d*] pyrimidine (APP, endowed with aminopyrazolic structure) in comparison with the reference adenosine deaminase inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine hydrochloride (EHNA, a purine derivative). Results indicated that the blockade of adenosine conversion into inosine, promoted by inhibition of adenosine deaminase, was able to protect the colonic tissues from inflammatory injury, inducing a significant reduction of TNF-α release, neutrophil infiltration and ROS production. Moreover, we demonstrated that APP counteracted intestinal inflammation with greater potency than EHNA, and the difference in the anti-inflammatory effects of these compounds reflects their respective potencies in inhibiting the activity of adenosine deaminase in vitro (Antonioli et al., 2007). More recently, Brown et al. (2008) evaluated the potential therapeutic benefits of pentostatin, an adenosine deaminase inhibitor, in a model of colitis induced by administration of piroxicam for two weeks to IL-10 knockout mice, focusing their attention on the responses of immune cells. These authors observed a marked reduction of effector T cell expansion in the inflamed colon and mesenteric lymph nodes, followed by a decrease in pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, IFN-γ and chemokine C-X-C motif ligand 10), whereas the levels of anti-inflammatory factors (FoxP3 and TGF-β) remained unchanged. Therefore, the ameliorative effects resulting from inhibition of adenosine deaminase in this model of colitis were ascribed to the ability of spared adenosine to restore an adequate balance between pro- and anti-inflammatory mediators.

Cellular adenosine uptake is another key event regulating bioactive extracellular adenosine levels, and therefore the inhibition of nucleoside transporters could be a valuable approach to enhance the anti-inflammatory actions of adenosine (Noji et al., 2004). In this regard, a recent in vitro study compared the immunomodulating effects of dipyridamole (inhibitor of ENTs) with those of methotrexate, and evaluated the effects of these drugs on TNF-α and IL-10 release from intestinal mononuclear cells, obtained from patients with Crohn's disease or healthy controls and stimulated with LPS and phytoemagglutinin. The results showed a similar suppression of TNF-α levels in cells treated with both drugs, whereas dipyridamole was more effective than methotrexate in increasing IL-10 levels (Poturoglu et al., 2007). The knowledge gained from in vitro studies warrants the expansion of research in in vivo models, to obtain more convincing evidence on the immunomodulatory actions of adenosine and to foster the development of novel therapeutic strategies for intestinal inflammation. Currently available data regarding the effects of drugs active on adenosine pathways in experimental models of bowel inflammation are summarized in Table 3.

#### *3.1.3. Implications of adenosine in gut neuromuscular dysfunctions associated with intestinal inflammation*

Intestinal motor dysfunctions are a prominent cause of digestive symptoms in patients affected by IBDs (Lomax et al., 2005). Indeed, intestinal inflammation is associated with complex structural and functional rearrangements of both ENS (Sanovic et al., 1999; Schneider et al., 2001; Neunlist et al., 2003) and smooth muscle cells (Wells & Blennerhassett, 2004), which contribute to the generation of severe disturbances in gut motor and secretory functions.

An involvement of adenosine in the pathophysiology of enteric dysmotility associated with intestinal inflammation has been hypothesized, but supporting data are still scanty. In a model of chronic intestinal inflammation induced by *Schistosoma mansoni*, the physiological control of $A_1$ receptors on small intestine motility was no longer evident (De Man et al., 2003). The mechanism proposed for this loss of control calls into play a desensitisation of $A_1$ receptors, caused by their prolonged exposure to high concentrations of adenosine released as a consequence of the infection. Recently, a pivotal role in the inhibitory control of colonic motor activity has been proposed for $A_{2A}$ receptors in the presence of intestinal inflammation. This involvement is supported by experimental observations showing an increase in $A_{2A}$ receptor expression in the neuromuscular compartment of inflamed colon, followed by

**Table 3**
Summary of pharmacological effects of drugs active on adenosine pathways in experimental models of bowel inflammation

| Species | Experimental model | Pharmacological treatments | Effects | References |
|---|---|---|---|---|
| Mouse | Spontaneous ileitis in SAMP1/YitFc mice | ATL-146e ($A_{2A}$ receptor agonist) | ↓MPO, TNF-α, IFN-γ, IL-4 | Odashima et al., 2005 |
| | Ileitis induced by *Clostridium difficile* toxin-A | ATL-146e | ↓MPO, TNF-α, adenosine deaminase activity | Cavalcante et al., 2006 |
| | DSS colitis | IB-MECA ($A_3$ receptor agonist) | ↓IL-1, Il-6, IL-12, MIP-1α, MIP-2, MDA, MPO | Mabley et al., 2003 |
| | DSS colitis | GP515 (adenosine kinase inhibitor) | ↓IFN-γ, CD69 expression | Siegmund et al., 2001 |
| | DSS colitis | CGS 21680 ($A_{2A}$ receptor agonist) | No variations of MIP-1α, MIP-2, IFN-γ, IL-1/β, IL-12 and TNF-α | Selmeczy et al., 2007 |
| | DSS colitis | ATL-801 ($A_{2B}$ receptor agonist) | ↓MPO, IL-6, MIP-2 | Kolachala et al., 2008 |
| | IL-10 knockout colitis | ATL-801 | ↓IL-6, keratinocyte-derived chemokine | Kolachala et al., 2008 |
| | IL-10 knockout colitis | IB-MECA | ↓MIP-1α, MIP-2, MPO, MDA, IL-1, IL-6 | Mabley et al., 2003 |
| | IL-10 knockout colitis | Pentostatin (adenosine deaminase inhibitor) | ↓IL-1β, IL-6, TNF-α, IFN-γ, chemokine C-X-C motif ligand 10 no variations of FoxP3 and TGF-β | Brown et al., 2008 |
| Rat | TNBS colitis | IB-MECA | ↓Chemokines, cytokines, complement, SOD2, heme oxygenase, iNOS, NCAM 1, ICAM 1, selectin, IL-1β, Jun B protooncogene, | Guzman et al., 2006 |
| | DNBS colitis | APP (adenosine deaminase inhibitor) | ↓IL-6, TNF-α, MDA, MPO | Antonioli et al., 2007 |
| Rabbit | Formalin-immune complex colitis | ATL-146e | ↓MPO, chronic inflammatory index | Odashima et al., 2005 |
| Human | Mononuclear cells from patients with Crohn's disease | Dipyridamole (ENT inhibitor) | ↓TNF-α ↑Il-10 | Poturoglu et al., 2007 |

↑ = increase; ↓ = decrease.
Abbreviations: DNBS: 2,4-dinitrobenzenesulfonic acid; DSS: dextran sulfate sodium; ENT: equilibrative nucleoside transporter; iNOS: inducible nitric oxide synthase; INF-γ: interferon-γ; IL-1: interleukin-1; IL-4: interleukin-4; IL-6: interleukin-6; IL-10: interleukin-10; IL-12: interleukin-12; ICAM: intercellular adhesion molecule; MIP-1α: macrophage inflammatory protein-1α; MIP-2: macrophage inflammatory protein-2α; MDA: malondialdehyde; MPO: myeloperoxidase; NCAM 1: neural cell adhesion molecule 1; SOD2: superoxide dismutase 2; TNBS: 2, 4, 6-trinitrobenzenesulfonic acid; TNF-α: tumor necrosis factor-α.

demonstration of an enhanced inhibitory modulation by this receptor on excitatory cholinergic nerves via a facilitatory control on nitrergic nerve pathways (Antonioli et al., 2006).

The paucity of data on the involvement of adenosine in gut dysmotility associated with intestinal inflammation should encourage novel studies designed to define the exact pathophysiological meaning of purinergic pathways in these disorders.

### 3.2. Intestinal ischemia

Intestinal ischemia results from a deficiency in arterial blood supply or venous drainage in enteric tissues, which leads to marked structural and functional alterations of intestinal physiology, with consequent development of motility disorders and absorptive/secretory dysfunctions (Ballabeni et al., 2002). This disturbance is more common in the elderly and appears to be associated with a variety of pathological conditions such as abdominal angina, mesenteric embolus, abdominal aortic aneurysm, IBD or organ transplantation (Burns & Brandt, 2003). The symptoms and signs of bowel ischemic injury are relatively non-specific (abdominal cramps, lower abdominal pain), and the diagnosis requires a high level of clinical suspicion to resolve the cause of reduced blood perfusion promptly and limit further clinical complications (Frishman et al., 2008). Although restoration of normal blood flow is essential to prevent irreversible tissue injury, it is well known that reperfusion per se may result in a local and systemic inflammatory response (Eltzschig & Collard, 2004). Ischemia–reperfusion injury is characterized by ROS production, complement activation, leukocyte–endothelial cell adhesion, platelet–leukocyte aggregation, increased microvascular permeability and impaired endothelium-dependent relaxation, which can evolve toward multiorgan dysfunction or death (Carden & Granger, 2000).

The identification of a close interplay between hypoxia and adenosine (Eltzschig et al., 2006; Morote-Garcia et al., 2008; Eckle et al., 2008) has prompted investigations into the mechanisms by which purinergic pathways contribute to the pathophysiology of ischemic disease. A further important aspect explored by some authors includes the involvement of adenosine in the regulation of immunological events subsequent to reduced blood flow. Grisham et al. (1989) initially found that adenosine reduced neutrophils activation, with subsequent decrease in superoxide and hydrogen peroxide levels, and attenuated leukocyte adherence to venular endothelium in a feline model of intestinal ischemia–reperfusion. Kaminski and Proctor (1992) then observed that the pharmacological blockade of adenosine uptake by dipyridamole counteracted the detrimental events associated with intestinal ischemia, and proposed that, besides modulating the immune system, adenosine could exert its beneficial activity through a direct vasodilator effect with an improvement of oxygen/supply demand. Guckelberger et al. (2004) have highlighted the important role played by CD39 (a key enzyme involved in adenosine formation from ATP) in the pathogenesis of vascular injury during bowel ischemia. These authors showed that CD39 participates in the homeostasis of blood vessels, blocking ATP-dependent platelet aggregation and counteracting the activation of endothelial cells. They then evaluated the role of CD39 in the pathogenesis of intestinal ischemia–reperfusion injury in CD39-knockout mice. In this model, the induction of bowel injury was associated with a marked increase in vascular permeability and platelet activation and, in animals lacking CD39, the mortality rate was significantly higher in comparison with wild type animals. Moreover, treatment of CD39-knockout mice with adenosine or CD39 supplementation reversed these alterations, suggesting that adenosine is pivotal to the maintenance of vascular integrity. Recently, Hart et al. (2008) have obtained evidence supporting a critical role of CD73 in the generation of endogenous adenosine and modulation of tissue injury in a mouse model of intestinal ischemia. In particular, they showed that the pharmacological inhibition of CD73 by α,β-methylene-adenosine diphosphate (AOPCP) or its gene deletion was associated with a worsening of histological injury in hypoxic colon together with a marked increase in IL-1, IL-6 and neutrophil infiltration. Conversely, treatment of animals with soluble CD73 attenuated tissue damage, suggesting supplementation with this enzyme as a strategy to ameliorate the detrimental consequences of intestinal ischemia–reperfusion.

Information concerning adenosine involvement on intestinal motor dysfunctions in the presence of intestinal ischemia is scarce. Kadowaki

et al. (2000b) showed that endogenous adenosine can interfere with the excitatory pathways driving colonic peristalsis via $A_1$ receptors, and provided evidence that treatment with DPCPX or FK352 restored the propulsive motility in a rat model of intestinal ischemia–reperfusion. At present, data on the implication of other adenosine receptors in this bowel disorder are lacking, and therefore studies in this field are urgently necessary since they might suggest innovative therapeutic strategies to prevent or limit ischemic-induced injury of human gut.

### 3.3. Post-operative ileus

Post-operative ileus represents the most common adverse consequence of abdominal surgical procedures, and it is responsible for an increased morbidity rate and prolonged hospital stay, weighing considerably upon annual health care costs (Greenwood-Van Meerveld, 2007). Surgical manipulation of abdominal viscera is the prominent cause of this bowel disorder, and an increasing body of evidence ascribes the pathophysiology of this syndrome to a post-surgical inflammatory response targeting the intestinal neuromuscular layer and an abnormal activation of sensory/motor enteric reflexes, with the occurrence of abdominal bloating, vomiting and lack of defecation (Maron & Fry, 2008). In addition to inflammatory and neurogenic factors, drugs used during the perioperative period (anesthetics, anticholinergics/antispasmodics, opioids) seem to further amplify the severity and duration of post-operative gut dysfunction (Kehlet & Holte, 2001).

Despite investigational efforts, the role of various gastrointestinal hormones, neurotransmitters and other humoral factors in the onset of post-operative ileus remains unclear. However, it is recognized that intestinal manipulation evokes a complex network of inflammatory responses within the muscularis externa of gut wall, including the release of NO, ROS, prostanoids and a variety of pro-inflammatory cytokines, which can alter, directly or indirectly (i.e., by triggering sensory afferent neurons), the regulation of enteric motility (De Giorgio & Barbara, 2008). Recently, The et al. (2008) suggested mast cell activation as the first pathophysiological step triggering the inflammatory response in the post-operative ileus in humans.

When considering adenosine, very few data are available on the involvement of this autacoid in the development of post-operative ileus. One study has examined the participation of adenosine to colonic dysmotility in an experimental model of post-operative ileus (Kadowaki et al., 2003). These authors focused their attention on the contribution of $A_1$ receptors to the alteration of colonic propulsion in rats treated with pentobarbital or subjected to intestinal manipulation. A significant improvement of propulsive motility was observed after intravenous administration of the selective $A_1$ antagonist FK352 in both experimental models, suggesting that adenosine is implicated in the pathogenesis of post-operative ileus via $A_1$ receptor activation. This preliminary observation should foster further investigations, which might provide a solid basis on which to develop pharmacological interventions on adenosine pathways to counteract the relevant pathogenetic mechanisms (i.e. mast cell activation, neuromuscular inflammation and initiation of neural reflex), and hence to prevent or attenuate post-operative ileus.

### 3.4. Diarrhoea

Diarrhoea represents one of the major worldwide health problems, with an enormous toll in terms of morbidity, loss of work productivity, and consumption of medical resources (Ahlquist & Camilleri, 2005). This disorder is characterized by frequent loose or liquid bowel movements, commonly caused by bacteria, parasites, viral infections or food intolerance (i.e. lactose). It can also represent a symptom of more complex diseases such as IBDs, intestinal ischemia, celiac disease or functional bowel disorders, contributing significantly to the feeling of discomfort and impaired quality of life in patients (Shah & Hanauer, 2007; Sellin, 2007). The pathophysiological mechanisms underlying the loss of intestinal fluids during diarrhoea have been widely discussed and investigated. In the last three decades it has become increasingly evident that alterations in the epithelial transport of ions and water are major causes of intestinal fluid loss, although alterated gut motility may also contribute (Lundgren, 2002; Field, 2003). Moreover, abnormal activation of inflammatory cells, with particular regard for mast cells and neutrophils, may promote altered patterns of intestinal secretion via the release of mediators which overstimulate epithelial $Cl^-$ transport (Madara et al., 1991; Madara et al., 1992; Traynor-Kaplan & Barrett, 1993). Madara et al. (1993) demonstrated that neutrophils migrate across intestinal epithelia in response to noxious stimuli and release large amounts of AMP (either directly or following breakdown of released ATP), which then act on epithelial cells to stimulate $Cl^-$ secretion. Subsequent studies suggested that neutrophil-derived AMP is rapidly converted into adenosine by CD73 located on the apical membrane of intestinal epithelial cells. Adenosine thus appears to be the ultimate mediator in the signalling cascade linking the enteric immune system to the intestinal epithelium, acting as a paracrine mediator that contributes to secretory diarrhoea (Madara et al., 1993; Resnick et al., 1993) (Fig. 4). In this network, the CD73 enzyme, which is extremely sensitive to variations of AMP levels, functions as a fine sensory system able to detect adverse conditions in the gut lumen and to trigger a defensive overstimulation of mucosal hydration. Consistently with this concept, increments of CD73 expression and activity have been observed in tissues subjected to inflammatory, ischemic or infectious stimuli, suggesting an involvement of this enzyme in the pathophysiology of gut mucosal disorders (Synnestvedt et al., 2002; Karhausen et al., 2004; Colgan et al., 2006; Crane et al., 2007; Louis et al., 2008) (Fig. 4).

A marked enhancement of $A_{2B}$ receptor expression has been reported in the presence of intestinal adverse conditions, and there is evidence to support an active role of this receptor in the pathogenesis of diarrhoea (Crane et al., 2002; Kolachala et al., 2005; Kong et al., 2006). It is currently acknowledged that the increase in adenosine levels, due to CD73 overactivity, leads to massive stimulation of $A_{2B}$ receptors, which then trigger a marked increase in $Cl^-$ secretion, resulting in an abnormal movement of isotonic fluids into the intestinal lumen (Tally et al., 1996; Wang et al., 2004; Kolachala et al., 2006). Sitaraman et al. (2001) investigated the role of adenosine in the epithelial response to immune activation during active intestinal inflammation. Using the intestinal epithelial cell line T84, these authors evaluated the effects of adenosine on the release of IL-6, a pro-inflammatory cytokine involved in the control of neutrophil degranulation and lymphocyte differentiation. Stimulation of T84 monolayers with adenosine induced an increase in IL-6 secretion, which was found to be mediated by $A_{2B}$ receptors via a time-dependent activation of the nuclear transcription factor CREB (cAMP response element-binding) (Fig. 4). Interestingly, Wang et al. (2004) investigated the cellular domain and the mechanism by which $A_{2B}$ receptors were recruited to the membrane of T84 cells upon adenosine stimulation. Under resting conditions, $A_{2B}$ receptors were localized mainly at the intracellular level, whereas low levels of this receptor could be detected on the apical membrane. The stimulation of surface $A_{2B}$ receptors with adenosine induced the translocation of intracellular receptors to the apical membrane by means of soluble *N*-ethylmaleimide-sensitive factor attachment receptor (SNARE) proteins, vesicle-associated membrane protein (VAMP)-2 and synaptosomal-associated protein (SNAP)-23. It was thus concluded that the intracellular localization of $A_{2B}$ receptors may be functionally relevant to prevent their inappropriate stimulation, which would be followed by adenosine-induced chloride secretion (i.e. secretory diarrhoea) and IL-6 release. Recently, Kolachala et al. (2006) have characterized the mechanism of $A_{2B}$ receptor activation in T84 cell line, showing that $A_{2B}$ receptor signalling is mediated by adenylate cyclase-6 isoform. As anticipated in previous sections, data from cultured cells should be translated to native cell populations with great caution, owing to possible differences in their pattern of receptor expression. Therefore, additional information, gained from complex and integrated experimental models, could provide more solid bases to interpret the regulatory actions of adenosine under pathological conditions.

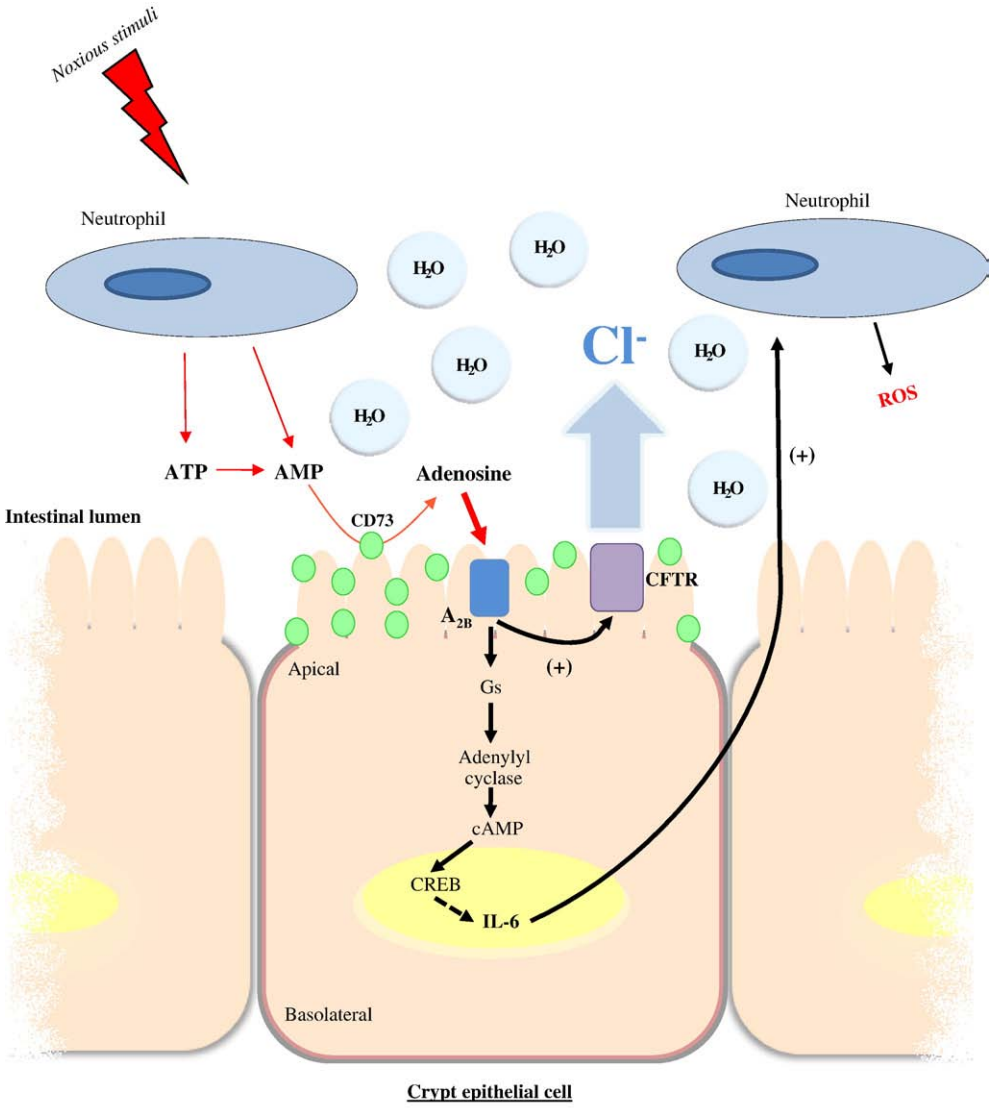


**Fig. 4.** Schematic representation showing the involvement of adenosine in the regulation of cross-talk between neutrophils and crypt epithelial cells in the presence of diarrhoeogenic stimuli. Neutrophils migrating across intestinal epithelia in response to noxious stimuli release large amounts of AMP or ATP, which is then broken down to AMP. AMP is rapidly converted into adenosine by CD73 located on the apical membrane of intestinal epithelial cells. This enhanced adenosine production, due to CD73 overactivity, results in massive stimulation of $A_{2B}$ receptors, which trigger a marked increase in $Cl^-$ secretion, giving rise to an abnormal movement of isotonic fluids into the intestinal lumen. Moreover, the stimulation of epithelial $A_{2B}$ receptors elicits an increase in intracellular cAMP followed by a time-dependent activation of the nuclear transcription factor CREB (cAMP response element-binding), which induces the production and secretion of interleukin-6 (IL-6). Once released into the intestinal lumen, IL-6 stimulates neutrophil activation, with a subsequent increment in reactive oxygen species (ROS) production. CFTR: cystic fibrosis transmembrane conductance regulator; Gs: stimulatory G-protein.

Overall, CD73 and $A_{2B}$ receptors appear to act as pivotal players in the regulation of the complex cross-talk between enteric immune system and epithelial cells, establishing a mutually enhancing interaction between neutrophil activation and the secretory response of surface epithelial cells. Moving from this concept, several authors have suggested the pharmacological modulation of adenosine pathways as a novel strategy for the management of diarrhoea. In support of this rationale, treatments with the CD73 inhibitor AOPCP, to reduce adenosine concentration in gut lumen, or selective $A_{2B}$ antagonists have been shown to exert promising beneficial effects in experimental models of diarrhoea (Strohmeier et al., 1995; Tally et al., 1996; Strohmeier et al., 1997; Colgan et al., 2006; Crane et al. 2007).

### 3.5. Abdominal pain

Abdominal pain represents the most common complaint responsible for patients seeking the care of gastroenterologist (Mulak, 2003). This symptom may occur as a consequence of gut inflammatory disorders (IBDs, diverticulitis) (Katz, 2007), abnormal distention of intestinal wall (intestinal obstruction), reduced blood supply (ischemia) (Kozuch & Brandt, 2005) or without detectable organic diseases (functional bowel disorders) (Andresen & Camilleri, 2006). Pain perception starts in the peripheral terminals of afferent nerves with the activation of nociceptive receptors. When the stimulus achieves a sufficient magnitude, an action potential is generated, resulting in neurotransmitter release in the dorsal horn of spinal cord. From the spinal level, sensory information is transferred via spinoreticular and spinothalamic pathways to the brainstem and thalamus for integration, and then to cortical and subcortical areas involved in processing sensory experience (Woolf & Salter, 2000; Grundy et al., 2006). In recent years, the observation that patients with intestinal disorders may display an abnormally low threshold for visceral perception (designated as "visceral hypersensitivity"), has fostered increasing interest for investigations into the sensory component of gut innervation (Boeckxstaens, 2002). Particular attention is being focused on the variety of chemical mediators released during adverse intestinal conditions and actively involved in sensory neuron

plasticity and pain perception. In this regard, Wynn et al. (2004) investigated the possible involvement of a purinergic component during mechanosensory transduction in a rat model of colitis, and they found an enhanced involvement of ATP, which could be ascribed to an increased distension-evoked ATP release in conjunction with up-regulation of $P2X_3$ receptor expression at the level of dorsal root ganglionic neurons innervating colorectum. Consistently with these findings, Yiangou et al. (2001) had previously demonstrated an increment of $P2X_3$ receptor expression in the inflamed intestine, and suggested a potential role of this receptor pathway in bowel dysmotility and pain. More recently, Xu et al. (2008) showed that visceral hyperalgesia is associated with an increase in ATP activity and enhanced expression of $P2X_3$ receptors in colonic sensory neurons, suggesting that these receptors may represent suitable targets for treatment of visceral hypersensitivity and pain.

The involvement of adenosine in pain transmission at peripheral and spinal sites has been largely demonstrated, and the possible antinociceptive effects resulting from the pharmacological modulation of this pathway have been discussed exhaustively in previous reviews (Sawynok, 1998; Sawynok & Liu, 2003; Skrabanja et al., 2005; Gan & Habib, 2007). It is currently acknowledged that endogenous adenosine exerts complex regulatory actions on pain transmission depending on the receptor subtypes called into play, their localization, and the availability of extracellular adenosine. Most attention has been dedicated to the role of $A_1$ receptors on algogenic nerve pathways. There is wide evidence that spinal analgesia induced by adenosine is mediated through $A_1$ receptors located both at presynaptic and postsynaptic level (Sawynok & Liu 2003). In particular, the stimulation of $A_1$ receptors induces postsynaptic inhibition of excitatory transmission through activation of $K^+$ channels and membrane cell hyperpolarization (Patel et al., 2001). There is also evidence of a presynaptic inhibitory action of $A_1$ receptors on the release of substance P, calcitonin gene-related peptides and glutamate via a decrease in $Ca^{2+}$ influx into afferent nerve terminals (Sawynok & Liu, 2003). A report by Johansson et al. (2001) showed that mice with disruption of $A_1$ receptor displayed hyperalgesia and a loss of the analgesic effect exerted by intrathecal adenosine. At present, there is little or conflicting information on the involvement of $A_{2A}$, $A_{2B}$ and $A_3$ receptors in pain transmission at spinal level. Ledent et al. (1997) examined the nociceptive threshold in mice lacking $A_{2A}$ receptors under different experimental conditions, and they observed that these animals displayed a higher nociceptive threshold when compared with wild type animals, thus suggesting a facilitatory role of $A_{2A}$ receptors in pain transmission. Hussey et al. (2007) have also shown that mice with $A_{2A}$ gene deletion are less sensitive to nociception elicited by formalin injection into the paw. Moreover, treatment of wild type animals with the $A_{2A}$ antagonist SCH58261 increased the nociceptive threshold, further supporting a role of these receptors in peripheral nociceptive pathways. Abo-Salem et al. (2004) investigated the effects of adenosine receptor antagonists in an acute model of pain in mice. They showed that $A_{2B}$ receptor blockade was associated with antinociceptive effects. By contrast, $A_1$ or $A_{2A}$ antagonists did not alter the pain threshold, and $A_3$ receptor inhibition induced hyperalgesia.

A dual control of adenosine on pain transmission has been described in an animal model of visceral pain induced by intraperitoneal injection of acetic acid, where the stimulation of $A_1$ or $A_{2A}$ receptors respectively induced an inhibitory or facilitatory effect on pain perception (Bastia et al., 2002; Sawynok & Liu, 2003). Since both receptors have high affinity for endogenous adenosine and they are often co-localized on the same nerve terminals, it is conceivable that the preferential recruitment of these receptors depends on variations of their expression pattern in relation to different pathophysiological conditions (Cunha et al., 1996).

Several studies support the involvement of adenosine pathways in opioid-induced antinociception. In particular, Cahill et al. (1993, 1995) showed that the activation of spinal μ opioid receptors was followed by an increase in adenosine levels at this site, and provided evidence for a contribution of this nucleoside to morphine-induced spinal antinociception. Keil and Delander (1995) strengthened further the existence of a purinergic-opioid axis, demonstrating that treatment with dilazep (an inhibitor of nucleoside transporter) significantly enhanced the antinociceptive effect of opioid receptor agonists in mice. Bailey et al. (2002) examined the pain sensitivity of $A_{2A}$ knock-out mice and the antinociceptive effects of μ, δ and κ opioid receptor agonists in this model. The results showed that mice with $A_{2A}$ gene deletion were less sensitive to pain. These animals displayed also a significant increase in the antinociceptive effects mediated by κ receptors and a reduction in δ-mediated antinociception, whereas the μ-mediated analgesic effect was unaffected, suggesting a functional interaction between spinal δ and κ opioid receptors and peripheral adenosine pathways in pain control. Overall, these findings support a possible clinical use of drugs acting on adenosine, alone or in combination with opioid drugs, in the management of pain.

Increasing evidence shows that mast cells, strategically located at the host–environment interface in close proximity to sensory nerves, play a significant role in abdominal pain/discomfort. In particular, mast cell mediators (histamine, tryptase, proteoglycans, leukotriene $C_4$, platelet activating factor and prostaglandin $D_2$) can activate sensory nerves, including those innervating the gastrointestinal tract, leading to visceral hyperalgesia/allodynia (Barbara et al., 2006, 2007). Barbara et al. (2004) demonstrated high density of degranulated mast cells in colonic mucosa from patients with irritable bowel syndrome (IBS), and reported significant correlation between the proximity of activated mast cells to mucosal nerve fibres and the severity of abdominal pain. In this regard, it is noteworthy that adenosine can enhance visceral pain sensation through $A_{2B}$ and $A_3$ receptors expressed on mast cells, with subsequent release of histamine and sustained sensitization of peripheral nociceptors (Sawynok et al., 1997). Since these receptors display lower affinity for adenosine than $A_1$ and $A_{2A}$ receptors, they are more likely to be activated under pathological conditions associated with local adenosine accumulation (Sawynok & Liu, 2003). The nerve–mast cell interaction represents an important mechanism for abdominal pain generation, and a deeper knowledge of the regulatory role exerted by adenosine on mast cells activity and sensory nerve endings could be an emerging field for investigating novel therapeutic options for treatment of visceral pain.

Besides the involvement of adenosine in the transmission of abdominal pain, there is evidence supporting a pivotal role of ATP in the control of visceral hypersensitivity during pathological conditions. In particular, P2X receptor blockade has been reported to attenuate abdominal pain in IBS patients and, since this pharmacological intervention would be expected to decrease also bowel propulsion and secretion, it might be useful for treatment of diarrhoea-predominant IBS. On the other hand, P2Y receptor stimulation has been suggested to promote beneficial effects in patients with constipation-predominant IBS (Galligan, 2004).

## 4. Conclusions and perspectives

The existence of the purinergic system has been demonstrated for over forty years, but the roles played by ATP and adenosine under physiological and pathological conditions have only been highlighted in recent years. The current body of knowledge indicates complex regulatory actions of these mediators on immune, functional and sensory systems of the gastrointestinal tract, as well as a significant participation of purinergic pathways in the pathophysiology of various digestive diseases. Nucleotide receptors are widely expressed in the intestinal tract, and there is increasing evidence supporting a significant involvement of ATP and its receptors (P2X and P2Y families) in the physiological modulation of secretory and motor activity, as well as in the pathophysiology of several gut disorders including IBS, IBD and abdominal pain, thus representing potential therapeutic targets for

treatment of such pathological conditions. In experimental settings, the pharmacological modulation of adenosine pathways has been shown to produce beneficial effects in bowel inflammation, ischemic conditions and functional disorders. Despite these encouraging results, several aspects pertaining to the regulation of digestive functions by adenosine remain unclear and deserve extensive investigation. In recent years, a number of drugs acting on adenosine pathways have been patented for treatment of extra-digestive diseases (rheumatoid arthritis, asthma) and some are currently under clinical investigation, but none of these drugs is being developed for treatment of human gastrointestinal disorders. Nevertheless, the recent interest on adenosine and the emerging awareness of its significance in the control of enteric functions are expected to foster the development of drugs targeted on adenosine as novel therapeutic approaches to the management of intestinal diseases characterized by high incidence and major socioeconomic impact.

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