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COMMENTARY

A holistic view of adenosine in the control of intestinal neuromuscular functions: the enteric 'purinome' concept

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Adenosine is involved in the modulation of enteric neuromuscular functions, operating a fine tuning of smooth muscle contractility, peristaltic reflex and transit. In this issue of the *BJP*, Zizzo *et al.* report novel findings on the expression of adenosine receptors in mouse duodenum, extending our knowledge of their involvement in the control of spontaneous and neurogenic intestinal motility. In this study, particular attention was paid to the differential activation of adenosine receptors, as a result of their interplay with regulatory systems, modulating the availability of endogenous adenosine in a compartmentalised manner. This evidence will contribute to the holistic evaluation of the role played by adenosine in the regulation of intestinal motility, in accordance with the novel concept of the enteric 'purinome'. This commentary discusses the role of the 'purinome' in the modulation of enteric neuromuscular activity, pointing out its involvement in the intestinal neuroplasticity associated with bowel dysmotility.

LINKED ARTICLE

This article is a commentary on Zizzo *et al.*, pp. 1580–1589 of this issue. To view this paper visit http://dx.doi.org/10.1111/j.1476-5381.2011.01498.x

Abbreviation

ENS, enteric nervous system

Intestinal functions are the resultant of integrated and complex interplays between the enteric nervous system (ENS), smooth muscle and the mucosal immune system, aimed at maintaining body homeostasis through finely regulated and coordinated motor, secretory and absorptive activities. In particular, the ENS is endowed with a wide array of restorative, maintenance and adaptive functions, which can be activated or modulated in response to different physiological or noxious stimuli. In this regard, the tight interactions between the neuronal elements of ENS and the enteric immune cells, carrying out an active patrolling of gut lumen, ensure effective motor and secretory adaptive changes, with the purpose of counteracting pathogenic assaults, as well as normalizing abnormal intestinal activities (Antonioli *et al.*, 2008).

This complex enteric framework is carefully controlled by several mediators and increasing evidence points to a prominent role played by adenosine in the modulation of gastrointestinal neuromuscular functions, with an array of fine tuning processes operated by this nucleoside at the level of contractility, peristaltic reflex and transit. Consistent with this view, several investigations have demonstrated wide and heterogeneous distributions of receptors for adenosine throughout the gut as well as the presence of adenosine synthetic and catabolic enzymes and transporters in the neuromuscular compartment and in the mucosa and submucosal layer of the digestive tract in both humans and rodents, supporting the involvement of adenosine in the modulation of gastrointestinal functions (Antonioli *et al.*, 2008).

In this issue of the *British Journal of Pharmacology*, the article by Zizzo *et al.* (2011) reports their findings on the expression of adenosine receptor subtypes in mouse duodenum and their involvement in the modulation of spontaneous and neurogenic contractile activity in this section of the

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gut. First, the authors showed that transcripts coding for all four adenosine receptors were expressed in the mouse duodenum, and that A_{2B} receptors were confined to the intestinal mucosa, in line with the role that has been postulated for this receptor subtype in the autocrine and paracrine regulation of enteric secretion (Antonioli *et al*., 2008). Then, by means of functional experiments, they went on to demonstrate that adenosine exerted an inhibitory control on duodenal motility, either by activation of A_1 inhibitory receptors, located on smooth muscle, or by modulation of neurotransmitter release, via A_1 or A_{2A} receptors located on enteric nerves. In particular, A_1 receptors were found to act via direct inhibition of enteric cholinergic nerves, while A_{2A} receptors were shown to exert indirect modulating actions on cholinergic excitatory pathways through the recruitment of inhibitory nitrergic nerves. Notably, these authors obtained solid evidence that, although both A_1 and A_{2A} receptors were fully available for pharmacological activation by specific ligands, exogenously applied adenosine relaxed the duodenal preparations through a preferential activation of A_1 receptors, while the actions of endogenous adenosine, as elicited by electrical stimuli, were restricted to the preferential activation of neuronal A_{2A} receptors. Of interest, Zizzo *et al*. highlighted a pivotal role played by nucleoside transporters in this selective activation of A_{2A} receptors by endogenous adenosine, through the segregation of purine bioavailability in discrete microenvironments within the duodenal neuromuscular layer.

The evidence presented by Zizzo *et al*. is in full agreement with the novel concept of the 'purinome', consisting of a dynamic molecular network, involving adenosine synthetic and catabolic enzymes, transporters and receptors, aimed at triggering, maintaining and terminating the purinergic signalling in specific locations under different pathophysiological conditions. Indeed, it is being increasingly appreciated that adenosine receptor activation by the endogenous mediator is the resultant of a fine regulation operated by synthetic or catabolic enzymes and transporters, aimed at channelling the production of endogenous adenosine into discrete compartments, in order to selectively target specific receptor subtypes (Volonté and D'Ambrosi, 2009).

In the last few years, the intriguing hypothesis of the 'purinome' has broadened our understanding of the variety of roles played by adenosine in the intestinal neuromuscular compartment. In line with this concept, several authors are advocating a holistic evaluation of the 'enteric purinome' in an attempt to gain detailed information on the involvement of the purinergic system in the regulation of intestinal motility. In this regard, Duarte-Araújo *et al*. (2004) provided the initial evidence supporting the coexistence of both inhibitory A_1 and facilitatory A_{2A} adenosine receptors in the cholinergic nerves of rat ileum, thus raising the question of how endogenous adenosine could activate each receptor subtype to differentially regulate intestinal neurotransmission. These authors found the answer by a contextual evaluation of the role played by A_1 and A_{2A} adenosine receptors in relationship with two major determinants of extracellular adenosine levels, adenosine deaminase and the nucleoside transporters. Owing to the efficiency of these inactivating systems, Duarte-Araújo *et al*. (2004), in line with the recent findings reported by Zizzo *et al*., proposed that the selective activation of prejunctional facilitatory A_{2A} receptors could be a consequence of endogenous adenosine release or production restricted to a discrete region of the myenteric nerve synapse.

In line with the concept of the enteric 'purinome', it is noteworthy that the specific activation of purinergic receptors in the modulation of the myenteric plexus can be influenced also by synthetic enzymes, which, because of their differential tissue distribution and expression, together with differential substrate availability, may contribute differently to the modulation of enteric purinergic signalling. In this respect, an interesting study by Duarte-Araújo *et al*. (2009) focused its attention on the enzymes involved in the extracellular conversion of nucleotides into nucleosides, such as the ecto-nucleotidases. The main concept arising from this study was that ATP, released from activated smooth muscle cells, as well as from stimulated myenteric neurons, can be converted preferentially into AMP, and then dephosphorylated to adenosine by ecto-5′-nucleotidase. Although, under physiological conditions, this extracellular sequential catabolism of ATP, operated by ecto-nucleotidases, seems to contribute marginally to the overall interstitial adenosine concentration within the myenteric plexus, the authors claimed that this enzymatic pathway could become more important under pathological conditions, such as intestinal ischaemia and inflammation, characterized by increased extracellular ATP levels.

The evaluation of how and to what extent the enteric 'purinome' is involved in the pathophysiology of intestinal motor disorders represents an interesting point of investigation and discussion. De Man *et al*. (2003) provided the first evidence about a significant rearrangement of intestinal purinergic signalling in the presence of inflammatory disorders. These authors demonstrated that, in a model of chronic intestinal inflammation induced by *Schistosoma mansoni* in mice, the physiological inhibitory control exerted by A_1 receptors on contractions of the small intestine was no longer evident. The mechanism proposed to explain this loss of modulating action was the desensitization of A_1 receptors caused by their prolonged exposure to high concentrations of endogenous adenosine, released as a consequence of the infectious disorder. Recently, our research group reported a functional rearrangement of high affinity A_1 and A_{2A} receptors in the presence of experimental colitis (Antonioli *et al*., 2011). In particular, we observed a preferential activation of A_{2A} receptors over A_1 receptors in the presence of bowel inflammation. Such a differential receptor activation was ascribed to a channelled production of endogenous adenosine towards A_{2A} receptors operated by the synthetic enzyme, ecto-5′-nucleotidase, to which this receptor pathway seems to be functionally linked.

The presence of functional interplays between adenosine receptors and enzymatic pathways, critically involved in controlling the magnitude of purinergic responses to pathological events, is consistent with previous findings from our laboratory (Antonioli *et al*., 2010). In that study, we observed an impairment of A_3 receptor-mediated tonic inhibitory control by endogenous adenosine in rat colonic motility during bowel inflammation, despite obtaining evidence of an increased density of functioning and pharmacologically recruitable A_3 receptors. In an attempt to explain this intriguing discrepancy, we investigated the mechanisms underlying

the regulation of adenosine concentration, focusing our attention on adenosine deaminase, the main enzyme responsible for adenosine catabolism. As, in the presence of colitis, a marked increase of adenosine deaminase was observed in the neuromuscular layer, in accordance with the 'purinome' concept we proposed that such a mechanism could represent a regulating system, aimed at preventing A_3 receptors from being activated by endogenous adenosine. In this framework, the enhanced A_3 receptor expression was interpreted as the consequence of a compensatory response to a reduced availability of endogenous adenosine in the compartment of the A_3 receptor biophase.

In conclusion, the interesting findings reported by Zizzo *et al*. represent a step forward in the better understanding of how adenosine can influence the ENS functions under both normal and pathological conditions. Although these authors have previously highlighted the involvement of adenosine receptor subtypes in the regulation of mouse intestinal motility (Zizzo *et al*., 2006; 2007), with the present study they have made a first attempt of providing a comprehensive evaluation of the roles played by adenosine in the regulation of duodenal motility. In particular, consistent with the emerging concept of the enteric 'purinome', they propose the evaluation of the mechanisms involved in nucleoside metabolism, together with the activation of adenosine receptor subtypes, as an integrated unit aimed at modulating the activity of myenteric neurons, through a fine tuning of purinergic signalling. Accordingly, future investigations, addressing the possible role of the enteric 'purinome' in the processes of intestinal neuroplasticity, could be of pivotal importance in elucidating the pathophysiology of various pathological conditions associated with functional bowel disorders, including intestinal infection, inflammatory bowel diseases, irritable bowel syndrome, intestinal ischaemia and post-operative ileus, in order to channel investigative efforts towards novel and targeted therapeutic interventions in the adenosine system.

Conflict of interest

The authors declare no conflicts of interest.

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