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The roles of purinergic signaling during gastrointestinal inflammation

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Abstract

Extracellular purines play important roles as neurotransmitters and paracrine mediators in the gastrointestinal (GI) tract. Inflammation of the GI tract causes marked changes in the release and extracellular catabolism of purines, and can modulate purinoceptor expression and/or signaling. The functional consequences of this include suppression of the purinergic component of inhibitory neuromuscular and neurovascular transmission, increased release of purines from immune and epithelial cells, loss of enteric neurons to damage through P2X₇ purinoceptors, and enhanced activation of pain fibres. The purinergic system represents an important target for drug therapies that may improve GI inflammation and its consequences.

Keywords

colitis; synaptic transmission; ectonucleotidase; immunomodulation; visceral pain

Introduction

The proposal that purine nucleotides act as neurotransmitters to the smooth muscle of the guinea-pig taenia caecum was received with strong scepticism when first advanced by Burnstock and colleagues [1–4]. The molecular characterization of purinoceptors throughout the body removed any doubt about their importance; purines are now recognized as important regulators of neural, muscular and glandular functions in the gastrointestinal (GI) tract and elsewhere. The accepted roles of extracellular purines have expanded beyond those of neurotransmitters and neuromodulators with the realisation that non-excitable cells release purines as paracrine and autocrine mediators [5–7]. P2 purinoceptors comprise a large family of ionotropic (P2X_{1–7}) and metabotropic (P2Y_{1–14}) receptors that bind ATP or

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related purines [8–12]. Access of ligands to these receptors is tightly controlled by diverse families of ectonucleotidases, which regulate the concentration of extracellular purines and limit receptor desensitization [13–16]. In addition, some metabolites of ATP activate adenosine receptors (A_{1-3}), which also have important physiological effects in the GI tract [17,18]. In this review we consider how plasticity in purinergic signaling and P2 receptor activation during GI inflammation may contribute to symptom generation and disease pathogenesis.

One of the challenges faced in analysis of the roles of purinoceptors in GI function is the dearth of receptor-selective agonists and antagonists. Moreover, there is considerable controversy regarding the identity of the purinergic neurotransmitter within the gut, with evidence in favour of roles for ATP, β -nicotinamide adenine dinucleotide and ADP ribose [12,19–22]. Resolving the identity of the purinergic neurotransmitter(s) may have important implications when considering the actions of mimicking drugs or competitive antagonists of purinoceptors, the effects of ectonucleotidases and the roles of transmitter metabolites.

Purinergic ganglionic transmission

In the intestines, intrinsic reflex circuits of the enteric nervous system (ENS), involving the myenteric and submucosal plexuses, are responsible for initiating and regulating important digestive functions such as motor activity and mucosal secretion. Purinergic fast and slow synaptic transmission between enteric neurons has been shown to contribute to these events in mouse and guinea pig [11,23,24]. Furthermore, Hons and colleagues have recently demonstrated that purines may also act as a retrograde transmitter to facilitate presynaptic transmitter release in the mouse [25].

Acetylcholine acting on nicotinic receptors is the predominant mediator of fast synaptic transmission in the ENS; however, inhibition of nicotinic transmission reveals purinergic and serotonergic components of the fast excitatory postsynaptic potential (EPSP) under physiological conditions. Purinergic components of the fast EPSP, mediated by $P2X_2$ receptors, are observed in myenteric neurons in the ileum [26–28] and colon [28–30], with the higher proportion of purinergic transmission found in the ileum [28], at least in guinea pig. In the ileum, purinergic fast synaptic transmission is present primarily in descending pathways, including transmission to interneurons and inhibitory motor neurons [31], whereas in the distal colon, purinergic fast EPSPs are detected in ascending and descending neurons [30]. In the submucosal plexus, purinergic fast EPSPs are rarely observed in the small or large intestines [32,33]. Tachykinins, acetylcholine, and serotonin are the primary mediators of slow EPSPs in the ENS, but purinergic slow EPSPs mediated by $P2Y_1$ receptors have been reported in myenteric [34,35] and submucosal [32,36] ganglia. Neurotransmission from enteric and sympathetic neurons to enteric glial cells involves purinergic activation of $P2Y_1$ and $P2Y_4$ receptors [37–39], and is regulated by ectonucleotidases [16,37]. The (patho)physiological significance of this observation remains to be fully determined.

Facilitation of both fast and slow excitatory synaptic transmission has been reported in the submucosal and myenteric plexuses during trinitrobenzene sulphonic acid (TNBS)-induced colitis in guinea pigs. This model of colitis induces a transmural inflammation of the distal colon, associated with ulceration, granulocyte infiltration and thickening of the smooth muscle. In TNBS-colitis, the proportion of fast EPSPs that have a non-nicotinic component rises from 29% to 78% in the submucosal plexus [40]. Pharmacological analysis revealed that the enhanced component of the fast EPSP is due to purinergic and serotonergic contributions mediated by $P2X$ and $5-HT_3$ receptors, respectively. The increased participation of purines and 5-HT likely involves enhanced presynaptic release rather than

postsynaptic sensitivity because responses to exogenous agonists are comparable in the normal and inflamed preparations. There is no change in the pattern of axonal 5-HT immunoreactivity, which suggests the change is not due to axonal sprouting. Also, there is no change in the paired-pulse ratio, indicating that synaptic efficacy is not enhanced in inflamed preparations. Regardless of the mechanism, it is interesting to note that the inflammation-induced increase in fast synaptic transmission in submucosal ganglia persists beyond the recovery from inflammation [41].

In the myenteric plexus, inflammation-induced fast synaptic facilitation is not associated with a change in the pharmacological properties of the EPSPs, but it does appear to involve a presynaptic mechanism [29]. In myenteric nerve terminals, the increase in synaptic strength likely involves an increase in protein kinase A activity and an increase in the readily releasable pool. The mechanism(s) responsible for enhanced slow excitatory synaptic transmission have not yet been resolved, but it may involve enhanced purinergic transmission.

Purinergic neuromuscular transmission

Neurally released purines influence motor activity in the intestines in at least two ways: through tonic inhibition of the smooth muscle at rest, and by contributing to the descending, inhibitory limb of the peristaltic reflex. It is notable that inhibitory neuromuscular transmission involves co-transmitters, primarily nitric oxide (NO) and purines [42,43]. There is also evidence for a contribution from carbon monoxide (CO) [44]. Purinergic neuromuscular signaling in the intestines is mediated by P2Y₁ receptors in the mouse, rat, guinea pig and human [20,45–50], and activation of these receptors has an inhibitory effect on smooth muscle via the opening of small conductance, Ca²⁺-activated K⁺ (SK3) channels [51–53]. Accordingly, enteric purinergic neuromuscular transmission can be inhibited by P2Y₁ receptor antagonists as well as SK3 channel blockers, such as apamin. In the resting bowel, spontaneous inhibitory junction potentials (IJP) that are sensitive to P2Y₁ receptor antagonists or the SK3 channel blocker apamin are common, and these events likely contribute to the tonic relaxant effect of the ENS on the intestinal smooth muscle.

Purinergic neuromuscular transmission contributes to the relaxation of the receiving segment of bowel and to sphincter relaxation that allows passage between gut regions. Stimulation of the bowel results in aboral IJP that consist of a fast purinergic component and a slower nitrgic component [54]. The purinergic relaxation likely contributes to propulsive motility because P2Y₁ receptor inhibition reduces pellet velocity in a guinea pig *in vitro* assay[55], and *in vivo* transit of fecal pellets is delayed in mice lacking the P2Y₁ receptor [49].

There has been considerable debate as to whether purinergic neuromuscular transmission involves a direct action of purines on smooth muscle cells, or if some other cell type transduces this response. There is evidence that inhibitory transmission may be mediated at least in part by interstitial cells of Cajal [56], and recent findings indicate responses are also mediated by a newly classified interstitial cell, the fibroblast-like cell (FLC). These cells have long been described by morphologists, but have been difficult to study functionally until recently. FLCs contain platelet derived growth factor- α (PDGFR- α) receptors, and a new reporter mouse line has tagged this receptor with green fluorescent protein, allowing for functional studies [57]. PDGFR- α cells respond to purines, express SK3 channels, are directly coupled to smooth muscle cells by gap junctions, and are often found near enteric motor nerve varicosities. Based on these lines of evidence from mice, it is likely that these cells are playing a role in mediating purinergic neuromuscular transmission.

Purinergic neuromuscular transmission is attenuated in the inflamed colon, and this may contribute to dysmotility during colitis. In a study of neuromuscular transmission in the guinea pig TNBS model of colitis, Strong and colleagues found that purinergic IJPs were significantly attenuated, while the slow nitrergic component of the IJP was normal and muscarinic excitatory junction potentials were also unaltered [55]. As described above, inhibition of P2Y₁ receptors slows propulsive motility, and propulsive motility is particularly disrupted in ulcerated regions where purinergic IJPs are deficient. This deficit may contribute to post-inflammatory dysmotility as well as to disruption during active inflammation.

Decreased innervation is unlikely to contribute to the deficit in purinergic neuromuscular transmission, because there was lack of disruption in cholinergic and nitrergic neuromuscular transmission, and because axon density is not altered in inflamed regions [55]. Furthermore, decreased responsiveness of effector cells is unlikely, because relaxation in response to purine receptor agonists is not altered in TNBS-colitis [55]. Another possibility is that purinergic signaling is selectively attenuated due to oxidative stress in the inflamed area, as the mitochondria, the principal organelles responsible for intracellular purine metabolism, are particularly susceptible to free radical damage. Consistent with this model, myenteric nitrergic neurons produce reactive nitrogen species as well as reactive oxygen species, making these inhibitory motor neurons particularly susceptible to oxidative stress [58]. Oxidative stress in the muscularis externa is a feature of two mechanistically distinct rat models of inflammatory bowel disease (IBD): TNBS- and dextran sulphate sodium (DSS)-induced colitis in the rat [59], which involve transmural and mucosally limited inflammatory responses, respectively. Furthermore, Galligan and colleagues have recently demonstrated that purine transmission is disrupted in mesenteric arteries of deoxycorticosterone acetate-salt hypertensive rats in association with oxidative stress, and that purinergic transmission is restored when these rats receive antioxidant treatment [60]. Future studies are necessary to better define the links between oxidative stress and reduced purinergic transmission.

Purinergic signaling in the vasculature

The activation of purinoceptors can have multiple effects on vascular compliance, depending on the source of the purine, the type of receptor activated and the localisation of the receptor. The most prominent effect of purines on GI vasculature is arteriolar constriction following neurotransmission from sympathetic varicosities [61–63], and activation of P2X₁ receptors on vascular smooth muscle [64]. However, purinoceptors are also present on vascular endothelium, where they are thought to participate in flow-induced vasodilation of mesenteric vessels [65]. ATP released from endothelial cells in response to shear forces can activate endothelial P2Y₁ [66] and P2X₁ [67] receptors, which in turn promotes endothelial NO generation and vasodilation.

The vasoconstrictor response to ATP is substantially reduced in patients with IBD and in mouse models of IBD [62,63]. In the DSS model of colitis in mice, loss of ATP responsiveness within submucosal arterioles has been attributed to an upregulation of the purine catabolising ectonucleotidase, nucleoside triphosphate diphosphohydrolase-1 (NTPDase1, also known as CD39) [62,68]. Decreased vascular responsiveness to ATP during GI inflammation may be mediated by the resultant hypoxia activating hypoxia-inducible factor (HIF). Studies of the vascular response to hypoxia in mice have demonstrated that HIF activation leads to a coordinated upregulation of CD39 and ecto-5'-nucleotidase (CD73), which act together to break down ATP and ADP to adenosine [69,70]. The expression of protective adenosine 2B receptors is also enhanced following HIF activation. Although the hypoxia-induced alterations in purinergic and adenosine signaling

promote a protective decrease in vascular endothelial permeability, they may also reduce vasomotor regulation in the splanchnic and other vascular beds that are innervated by purinergic sympathetic neurons. It is noteworthy that endothelial generation of NO is also impaired in submucosal arterioles of IBD patients and in mouse models of IBD [71,72]. Thus, it appears that the dynamic regulation of vasoconstriction and vasodilation is compromised during GI inflammation.

The increase in purine catabolism observed during colitis may be compounded by a decrease in purine release from sympathetic varicosities. In mouse and rat models of colitis due to TNBS or DSS administration, inhibition of the N-type voltage-gated Ca^{2+} current in postganglionic sympathetic neurons led to a decrease in release of noradrenaline, a co-transmitter of purines, from sympathetic varicosities innervating the GI tract [73–75]. Inhibition of purinergic neurotransmission to submucosal arterioles during DSS-induced colitis is consistent with the decrease in neuromuscular transmission [55], but contrasts with the increase in the purinergic contribution to fast EPSPs in the submucosal plexus [41]. Also, it has been reported that TNBS colitis in rats leads to an enhanced mucosal release of ATP [76]. The findings of opposite effects of inflammation on purinergic signalling in adjacent tissues suggest that purinergic signalling within the gut is impressively compartmentalized.

Purinergic signaling in the immune system

Purinergic signaling is utilised extensively in the immune system [6,77]. Release of purines from immune cells via connexin hemichannels or pannexin channels and their catabolism by ectonucleotidases contribute importantly to antigen presentation, cytokine release and T-cell activation [7,78–80]. Furthermore, autocrine purinergic signaling has been implicated in the modulation of neutrophil chemotaxis [81]. Under normal circumstances, the extracellular concentration of ATP is low (10–100 nM), due to limited release of ATP and the actions of ectonucleotidases. Low concentrations of ATP are associated with activation of P2Y_{11} receptors on immune cells and a Th_2 pattern of cytokine release that is considered to be immunosuppressive [82]. However, during infection or inflammation, the concentration of ATP and related purines can rise rapidly [6,82], a consequence of regulated release of purines from immune and epithelial cells, and the leakage of purines from damaged cells. At higher concentrations of extracellular ATP (>100 μM), P2X_7 receptors are activated and combine with components of the inflammasome to drive caspase-dependent processing and release of interleukin (IL)-1 β and IL-18 [77]. Importantly, P2X_7 receptor and pannexin-1 have recently been localised to mouse enteric neurons and play a critical role in the loss of enteric neurons that occurs during experimental colitis [37].

The large fluxes in extracellular ATP concentration can be mitigated to some extent by a compensatory increase in ATP catabolism during GI inflammation [68,83]. Interestingly, CD39 mRNA is upregulated in colonic biopsies from IBD patients compared to controls, polymorphisms in the gene encoding CD39 are linked to increased susceptibility to Crohn's disease, and mice that lack CD39 have more severe inflammation in DSS-colitis [83]. However, it should be noted that the effect of blocking CD39 on inflammation is not consistent in other mouse models of IBD, as mice that lack CD39 exhibit decreased disease severity in TNBS-colitis, whereas loss of CD39 has no effect on disease outcomes during oxazolone-induced colitis [83,84]. Together, these data indicate that CD39 may play an important role in IBD pathogenesis but that extrapolation of findings on the impact of CD39 on GI inflammation from rodent models to human IBD is not straightforward.

Purinergic signaling may modulate the severity of GI inflammation indirectly, via effects on epithelial cells. Intestinal epithelial cells express P2X_7 , P2Y_2 and P2Y_6 receptors, which can

promote epithelial release of cytokines and chemokines [85–88], and even drive T-lymphocyte differentiation [89]. Interestingly, the expression of these epithelial purinoceptors is augmented by inflammation [85,86,88].

Purinergic signaling and nociception

Visceral pain is a hallmark symptom of GI inflammation, and the role of purinergic signaling in visceral nociception is an active area of research. One means through which purinergic signaling contributes to visceral pain during GI inflammation is by a direct action of locally released purines on the excitability of the peripheral endings, in the gut, of dorsal root ganglia (DRG) neurons. The most well-studied purinoceptors expressed by DRG neurons are the P2X₃ and heteromultimeric P2X_{2/3} receptors, which can be activated by the distension-induced release of ATP from mucosal epithelial or enteroendocrine cells [76,90].

During TNBS-induced colitis in rats, distension-induced release of ATP from the mucosa is enhanced and the expression of P2X₃ subunit-containing channels is increased in the DRG [76]. Cation influx through activated P2X_{2/3} and P2X₃ receptors on DRG neurons can evoke action potential discharge. Consequently, an increase in ligand and receptor during colitis may contribute to increased sensory and nociceptive traffic from the inflamed region. Rong and colleagues found that the mechanosensitivity of intestinal afferents is not affected by P2X receptor agonists in uninflamed mice, whereas the *Trichinella Spiralis* model of post-infectious hypersensitivity unmasks an ability of P2X_{2/3} receptors to increase afferent neuron mechanosensitivity [90], indicating that purines may modulate the mechanosensitivity of sensory terminals of extrinsic afferent neurons in the gut. Together, these findings suggest that GI inflammation increases afferent traffic to the spinal cord by enhancing the expression of pro-nociceptive P2X receptors and by increasing ATP release, which directly activates nociceptive neurons and/or increases their mechanosensitivity.

The involvement of purinoceptors in visceral inflammatory pain is not restricted to direct effects of purines on nociceptor excitability or mechanosensitivity. Recent studies have clearly identified roles for P2X₄ and P2X₇ receptors on immune cells in regulating the release of inflammatory mediators, including IL-1 β and PGE₂, which act directly on DRG neurons to elicit pain [80,91,92]. Purinoceptors may also influence visceral pain via effects on gut wall compliance. The decrease in purinergic relaxation of the circular muscle of the colon during colitis [55] may reduce the distension threshold for activation of visceral afferent neurons, thereby contributing to visceral pain during GI inflammation.

Concluding remarks

Extracellular purines utilise an intricate array of extracellular enzymes, receptors and intracellular signaling pathways to modulate gut function during health and disease. Purinergic signaling is remarkably plastic during GI inflammation, and is impressively compartmentalised in that it can increase or decrease in adjacent cell types within the same tissue. The plasticity of purinergic signaling during inflammation suggests that the development of drugs which selectively target individual purinoceptors or nucleotidases may provide new therapies for gut inflammation.

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Highlights

- Purinergic signaling in the gut is remarkably plastic and compartmentalized.
- Inflammation decreases purinergic neurovascular and neuromuscular transmission.
- Purine release from immune and epithelial cells is increased in colitis.
- P2X₇ receptor and pannexin channel activation in colitis leads to neuronal loss.
- Inflammation-induced nociception involves spinal afferent P2X receptor activation.

Figure 1.

Inflammation-induced changes in intestinal purinergic signaling. Inter cellular communication that utilises purines is enhanced during inflammation due to ¹increased purine release via synaptic transmission or pannexin channels, ²leakage of purines from damaged cells, and ³changes in purinoceptor expression, signalling and/or catabolism. However, colitis has also been reported to decrease purinergic signalling by ⁴impaired neuromuscular purine release and ⁵augmented catabolism of extracellular purines. Purines can be released from neurons, enteroendocrine cells, enterocytes and immune cells, and from damaged cells ↑ = increase; ↓ = decrease.