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Review

Purinergic mechanisms and pain—An update

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ABSTRACT

There is a brief summary of the background literature about purinergic signalling. The review then considers purinergic mechanosensory transduction involved in visceral, cutaneous and musculoskeletal nociception and on the roles played by P2X3, P2X2/3, P2X4, P2X7 and $P2Y_{12}$ receptors in neuropathic and inflammatory pain. Current developments of compounds for the therapeutic treatment of both visceral and neuropathic pain are discussed.

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1. Introduction

The concept of purinergic signalling was proposed in 1972 (Burnstock, 1972) following hints in the early literature, notably by Drury and Szent-Györgyi (1929), about the potent extracellular actions of purines on the heart and blood vessels and from Pamela Holton (1959) about the release of adenosine 5'-triphosphate (ATP)

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during antidromic stimulation of sensory nerve collaterals, together with the evidence that ATP is the transmitter in non-adrenergic, non-cholinergic nerves supplying the gut (Burnstock et al., 1970) and bladder (Burnstock et al., 1972). It is now recognised that ATP is a cotransmitter in nerves in both peripheral and central nervous systems (see Burnstock, 2007a) and that receptors for purines and pyrimidines are widely expressed on non-neuronal as well as nerve cells (see Burnstock and Knight, 2004). A P1 receptor family activated by adenosine and P2 receptor family activated by ATP and ADP were recognised early (Burnstock, 1978) and two subtypes of P2 receptors proposed in 1985, P2X and P2Y (Burnstock and Kennedy, 1985). In the early 1990s P1, P2X and P2Y receptors were cloned and characterised. Four subtypes of P1 receptors were identified, A_1, A_{2A}, A_{2B} and A_3, then in 1993 the first two P2Y G protein-coupled receptors were reported and a year later the first two P2X ion channel receptors identified (see Ralevic and Burnstock, 1998). Currently 7 subtypes of P2X receptors and 8 subtypes of P2Y receptors are established (see Burnstock 2007b).

Apart from vesicular release from nerves, it was usually assumed that the only source of extracellular ATP acting on purinoceptors was damaged or dying cells, but it is now recognised that ATP release from healthy cells is a physiological mechanism (see Bodin and Burnstock, 2001; Lazarowski, 2012). ATP is released from many non-neuronal cell types during mechanical deformation in response to shear stress, stretch or osmotic swelling, as well as hypoxia and stimulation by various agents. The transport mechanism(s) involved in ATP release include vesicular exocytosis, ATP-binding cassette transporters, connexin or pannexin hemichannels, plasmalemmal voltage-dependent anion channels and P2X7 receptors. After release, ATP and other nucleotides undergo rapid enzymatic degradation (Yegutkin, 2008). Ectonucleotidase families include the E-NTPDases (ecto-nucleoside triphosphate diphosphohydrolases), E-NPP (ecto-nucleotide pyrophosphatase/phosphodiesterases), alkaline phosphatases and ecto-5'-nucleotidase. It is possible that, while adenosine is largely produced by ectoenzymatic breakdown of ATP, there may be subpopulations of neurons and/or astrocytes that release adenosine directly (Wall and Dale, 2007).

Both P2X and P2Y receptors are expressed by sensory neurons, including those from dorsal root (DRG), trigeminal, nodose, and petrosal ganglia (see Burnstock, 2009b). All P2X subtypes are found on sensory neurons, although the P2X3 receptor has the highest level of expression (both in terms of mRNA and protein). P2X2/3 heteromultimers are particularly prominent in the nodose ganglion. RT-PCR showed that $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptor mRNA is also expressed on neurons of DRG, nodose and trigeminal ganglia and receptor protein for the $P2Y_1$ receptor is localised on over 80% of mostly small neurons (Ruan and Burnstock, 2003). Double immunolabelling showed that 73–84% of P2X3 receptor positive neurons also stained for the $P2Y_1$ receptor, while 25–35% also stained for the $P2Y_4$ receptor.

There were early reports that ATP might be involved in pain, including the demonstration of pain produced by injection of ATP into human skin blisters (Collier et al., 1966; Bleehen and Keele, 1977), ATP involvement in migraine (Burnstock, 1981) and ATP participation in pain pathways in the spinal cord (Jahr and Jessell, 1983; Salter and Henry, 1985). A significant advance was made when the P2X3 ionotropic ion channel purinergic receptor was cloned in 1995 and shown to be localised predominantly on small nociceptive sensory neurons in DRG together with P2X2/3 receptors (Chen et al., 1995; Lewis et al., 1995). Burnstock (1996) put forward a unifying purinergic hypothesis for the initiation of pain, suggesting that ATP released as a cotransmitter with noradrenaline and neuropeptide Y from sympathetic nerve terminal varicosities might be involved in sympathetic pain (causalgia and reflex sympathetic dystrophy); that ATP released from vascular endothelial cells of microvessels during reactive hyperaemia is associated with pain in migraine, angina and ischaemia; and that ATP released from tumour cells (which contain very high levels), damaged during abrasive activity, reaches P2X3 receptors on nociceptive sensory nerves. This has been followed by an increasing number of papers expanding on this concept (see Burnstock and Wood, 1996; Burnstock, 2009a; Jarvis, 2010; Tsuda et al., 2010) and also on the involvement of adenosine (Burnstock and Sawynok, 2010). Immunohistochemical studies showed that the nociceptive fibres expressing P2X3 receptors arose largely from the population of small neurons that labelled with the lectin IB_4 (Bradbury et al., 1998). The decreased sensitivity to noxious stimuli associated with the loss of IB_4-binding neurons expressing P2X3 receptors indicates that these sensory neurons are essential for the signalling of acute pain. The central projections of these primary afferent neurons were shown to be in inner lamina II of the dorsal horn and peripheral projections demonstrated to skin, tooth pulp, tongue and subepithelial regions of visceral organs. A schematic illustrating the initiation of nociception on primary afferent fibres in the periphery and purinergic relay pathways in the spinal cord was presented by Burnstock and Wood (1996) (Fig. 1). While P2X3 and P2X2/3 receptors, expressed on sensory neurons, were the predominant P2 receptor subtypes first recognised to be involved

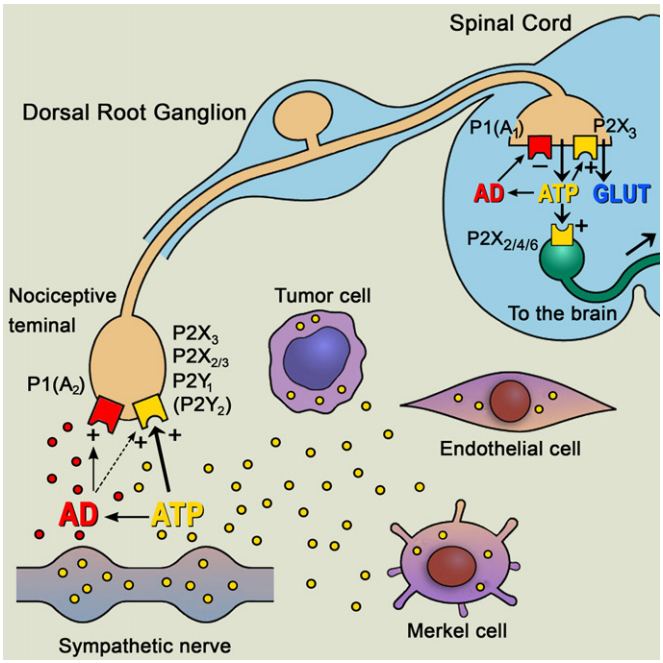

Fig. 1. Hypothetical schematic of the roles of purine nucleotides and nucleosides in pain pathways. At sensory nerve terminals in the periphery, P2X3 and P2X2/3 receptors have been identified as the principal P2X purinoceptors present, although recent studies have also shown expression of $P2Y_1$ and possibly $P2Y_2$ receptors on a subpopulation of P2X3 receptor-immunopositive fibres. Other known P2X purinoceptor subtypes (1–7) are also expressed at low levels in dorsal root ganglia. Although less potent than ATP, adenosine (AD) also appears to act on sensory terminals, probably directly via $P1(A_2)$ purinoceptors; however, it also acts synergistically (broken line) to potentiate P2X2/3 receptor activation, which also may be true for 5-hydroxytryptamine, capsaicin and protons. At synapses in sensory pathways in the CNS, ATP appears to act postsynaptically via P2X2, P2X4 and/or P2X6 purinoceptor subtypes, perhaps as heteromultimers, and after breakdown to adenosine, it acts as a prejunctional inhibitor of transmission via $P1(A_2)$ purinoceptors. P2X3 receptors on the central projections of primary afferent neurons in lamina II of the dorsal horn mediate facilitation of glutamate and probably also ATP release. Sources of ATP acting on P2X3 and P2X2/3 receptors on sensory terminals include sympathetic nerves, endothelial, Merkel and tumour cells. Yellow dots, molecules of ATP; red dots, molecules of adenosine. (Modified from Burnstock and Wood, 1996 and reproduced with permission of Elsevier).