Pain, Purines and Geoff

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Summary

The story of purinergic neurotransmission and regulation is intimately linked to studies of the somatosensory system. Burnstock's contributions to the discovery of ATP as a primary afferent neurotransmitter, as well as a signal of peripheral tissue damage that depolarised sensory neurons initiated a new period of pain research. The neuro-immune interactions that occur after tissue damage and are important for pain have now also been found to involve purinergic signalling, and adenosine has been demonstrated to have significant analgesic effects. In the pain field as in so many other areas of neuroscience and physiology, Burnstock's contributions have been critical to the expansion of our knowledge about the significance of purines. His mechanistic insights have profound significance for understanding the pain system and further underscore his stature as a pioneer and force for progress in biomedicine.

A Personal Introduction

Geoffrey Burnstock was a larger-than-life swashbuckling bon viveur, a family man and an incisive intellectual. His piercing intellect and nose for the right answer with limited information combined with energy and perceptive insights contributed so much to physiology and neuroscience. On a personal note, Geoff generously saved my career when he sponsored me for a Wellcome University award. I often saw him striding across the front quad, magnificently be-suited, as he surveyed with appreciation the summer sun-bathing students. Geoff wisely took me on as an each-way bet. He arranged for my transfer from his Department (Anatomy) to the Biology Department at the point where UCL would have to pick up some of my salary! Fortunately, Chih-Cheng Chen, a graduate student, and Armen Akopian, a postdoc with me, repaid him by cloning P2rX3. Both now lead excellent reach teams in Taiwan and San Antonio respectively. Outside science, Geoff was a wise and entertaining friend whose loyalty to his staff was admirable. Many excellent obituaries of Geoff's contribution to the pain field have been written (e.g. Tam and Salter 2021).

ATP as a signal of tissue damage

The universal presence of millimolar levels of intracellular ATP mean that this molecule is a clear signal of tissue damage and cell lysis (Burnstock and Wood 1996, Inoue and Tsuda 2020). Interestingly, other ubiquitous cellular components, for example chromatin proteins released from lysed cells also have a role in signalling to the immune and sensory systems that a response is required. Thus HMGB1 is both a chromatin protein and an alarmin playing a key role in septic shock (Jiang et al. 2020). Early studies on human blisters showed that AMP, ADP and ATP could all evoke a sensation of pain and in 1966 Collier had summarised evidence that ATP could cause pain both on blister bases as well as in mice (Collier et al. 1966, Bleehan and Keele 1997). The mechanism that underpinned these important observations had to wait for the development of the molecular genetic tools that identified purinergic receptors. In 1981 Burnstock showed that injections of ATP into human volunteers evoked a sensation of pain (Coutts et al. 1981) , an observation explored mechanistically by Jahr and Jessell on cultured dorsal root ganglion and spinal cord neurons (Jahr and Jessell 1983). These experiments showed that both sets of neurons could be depolarised by ATP. These studies extended the original observations of Holton that had energised Geoff to focus on ATP as a signalling molecule within the nervous system (Holton and Holton 1954) . Geoff ascribed many pain mechanisms to ATP in a Lancet article (Burnstock 1996) but this turned out to be slightly speculative!

After the initial discovery and cloning of the first two members of the family of P2X receptors (Brake et al., 1994, Valera et al., 1994), five more genes encoding mammalian P2X receptors, most of which may exist as various splice variants, were identified. Eight P2Y G-protein coupled receptors with affinity for ATP have also now been identified. Interestingly, the P2Y1 receptor is expressed at high levels by low threshold mechanoreceptive sensory neurons, and a case for a role as a mechanosensor has been made (Nakamura and Strittmatter, 1996). More recently Stucky has provided evidence that ATP released from keratinocytes may play a role in mechanosensation and even temperature sensation via P2X4 receptors on sensory neurons (Moehring et al. 2018, Sadler et al. 2018). However, more attention has focussed on the role of P2X receptors in pain pathways. In particular, attention focussed on the $P2X_3$ receptor because it is expressed selectively in a subset of predominantly nociceptive sensory neurons, (Chen et al. 1995, Burnstock and Wood, 1996). Here it may exist as a homomeric trimer, or a heteromultimer with P2Xr2.

The role of transgenic knock-out mice has become central to understanding the roles of particular purinergic receptors. First studies using P2Xr3 knock-outs showed that the rapidly desensitising current that is characteristic of dorsal root ganglion sensory neurons is completely ablated (Souslova et al. 2000). Roche collaborated with Burnstock to confirm these findings and explore P2X3 in bladder function . In the majority of small diameter sensory neurons isolated from wild-type mice, ATP evoked a large fast activating, rapidly inactivating inward current. In neurons from P2Xr3 knock-out mice, ATP evoked a detectable inward current in a smaller proportion of cells and the profile of such current was significantly different from wild-type neurons: the current was smaller, slower to activate, and less inactivating (Cesare et al., 2000) Null mutants have normal sensorimotor function. Behavioural responses to noxious mechanical and thermal stimuli are also normal, although formalin-induced pain behaviour is reduced. In contrast, deletion of the P2X3 receptor causes enhanced thermal hyperalgesia in chronic inflammation. Notably, although dorsal-horn neuronal responses to mechanical and noxious heat application are normal, P2X3-null mice are unable to code the intensity of non-noxious 'warming' stimuli and this may relate to the recent findings of Stucky (Souslova et al. 2000).

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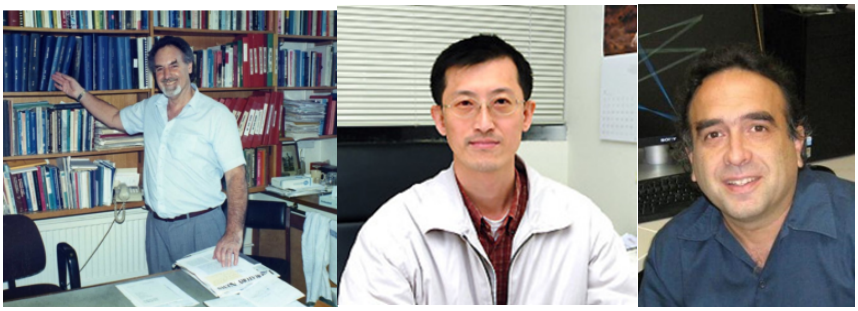

Geoff in his Anatomy Department office next to numerous PhD theses. Chih-Cheng Chen and Armen Akopian cloned P2X3 from sensory neurons in a difference cloning exercise, and only then discovered Geoff's purinergic hypothesis!

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ATP as a nociceptor neurotransmitter

Geoff's interest in pain was certainly enhanced by the identification of P2X3 in his department following on from his seminal work distinguishing ionotropic and GPCR receptors for ATP (Chen et al. 1995). ATP receptors had been initially classified by Burnstock in terms of the rank order of potency of various ATP congeners into two major subsets, the P2X and P2Y receptors (Abbrachio and Burnstock, 1994). Strikingly, these receptor subtypes correspond to ligand-gated ion channels and G-protein coupled receptors, respectively. P2X receptors consist of two transmembrane subunits with a large extracellular domain, which shows conserved cysteine residues in all members of the family. These channels are broadly distributed throughout somatic and nervous tissues.

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Studies of spinal cord slices, as well as co-cultures of dorsal root ganglion and dorsal horn neurons, have provided insights into the role of ATP in primary afferent transmission (Salter and Henry 1985). ATP application at central nociceptive nerve terminals elicits glutamate release and generated EPSCs in the neurons from the spinal dorsal horn (Gu and MacDermott, 1997). Hence ATP was postulated to be capable of activating pain pathways at central terminals or altering the response of the spinal dorsal horn to peripheral input. In the same study, αβmeATP (a stable agonist at P2X1, P2X3 and

P2X2/3 receptors) reproduced the glutamate release response evoked by ATP, suggesting the possible involvement of these receptor isoform(s) at presynaptic terminals. In the spinal cord, ATP release has been reported in response to primary afferent excitation, but may well be a neuromodulator rather than an excitatory neurotransmitter (Li and Perl, 1995). suppression of dorsal root-evoked EPSCs occurred with glutamate receptor antagonists but not suramin or PPADs and ATP the potentiates glutamate responses (Li and Perl, 1995). Studies by Gu and MacDermott (1997) are consistent with this view. However, despite the predominant role of glutamate as the principal primary afferent neurotransmitter, P2X receptor mediated currents do occur in dissociated dorsal horn neurons. The P2X receptors on acutely dissociated dorsal horn neurons are non-desensitizing, insensitive to αβmeATP, and show sensitivity to the antagonists suramin and PPADS. These characteristics are consistent with a heterogeneous population of P2X receptors, the composition of which includes $P2X_2$, $P2X_4$, and $P2X_6$ receptor subtypes. ATP-activated P2X receptors in lamina II of the rat spinal cord could thus play a role in modulating nociceptive information. (Bardoni et al. 1997, Jo and Schlicter, 1999). The ATP that activates these currents has been suggested to be released from GABAergic interneurons (Jo and Schlicter (1999). ATP was not co-detected with glutamate, but was co-released with the inhibitory neurotransmitter GABA. Adenosine, which may derive from the extracellular breakdown of ATP, also has regulatory actions on GABAergic inhibitory postsynaptic currents. Differential modulation of excitatory versus inhibitory components of this mixed co-transmission could potentially underlie changes in central processing in pathological pain states (Jo and Schlicter, 1999), although no subsequent evidence has been obtained for such events.

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Neuro-immune interactions and pain

The role of ATP receptors on immune cells particularly microglia has been shown to be intimately linked with a number of pain conditions. P2X4 receptors and P2X7 receptors are involved in these interactions. A P2Xr7 knockout has been described as essentially pain-free, but this exciting observation has not been followed up with the development of useful analgesic antagonists (Chessell et al. 2005) . The channel changes its properties to become a permeant pore with high concentrations of ATP, and its physiological role has remained opaque. Considerable interest in P2x7 antagonists is still apparent and further null mutants are being analysed to try to extend the earlier findings on the global null mutant (Young et al. 2018).

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P2X4 is found on microglia and plays a key role in the development of neuropathic pain through the upregulation of BDNF in the dorsal horn (Tsuda et al . 2003, Trang et al. 2009) There, effects on NMDA receptors and ion cotransporters sensitise central pain pathways (Ulman et al. 1997). An intriguing element in the P2Xr4 story is that this pathway seems to be only significant in male rather than female mice. The situation in humans with respect to this signalling pathway is still uncertain. (Inoue and Tsuda 2020)

Future prospects

As our grasp of the redundancy and plasticity of the nervous system increases and the computer mimetic analogy of a hard-wired digital brain fades away, it is hardly surprising that manipulating purinergic signalling for therapeutic ends has proved problematic. Gene deletion experiments often highlight unsuspected multiple roles for the encoded proteins (e.g. Jiang et al. 2020). Many signalling systems are involved in multiple organ functions – for example cardiovascular elements of purinergic signalling mitigate against the development of antagonists for P2X4 despite its importance in pain. A P2X3 antagonist, gefapixant named in honour of Geoff has proved potentially useful for cough rather than pain treatment and a decision on approval by the FDA should be made in 2021. Adenosine signals through four distinct receptors found in neurons, glia and other tissues. A_1R is interesting in a pain context as it is a $G_{i/o}$-coupled receptor that is found in nociceptors (Zylka 2011, Bai et al. 2017, Sawynock et al. 2018). However, adenosine has been shown to cause pain in humans when injected systemically, whilst some evidence for A1R agonists as analgesics exists. Other studies have shown useful perioperative pain relief with adenosine compounds (Vincenzi et al. 2020). The field is complicated by cardiovascular problems associated with adenosine. Thus the future challenge in this, as in other pathologies is to target drugs to the specific cells of interest – in our case immune cells, nociceptors or the neurons in the dorsal horn that are involved in pain pathways. This is a challenging task that is feasible with gene therapy using tissue specific promoters. However, such an approach is at present unattractive in cost terms. Nonetheless the insights provided by Geoff Burnstock and his collaborators have shown a whole new world of pain signalling mechanisms that will eventually help us to treat pain. Geoff Burnstock has provided the fundamental insights that will eventually promote success.

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