Institution: University College London / Birkbeck College

Unit of Assessment: 5 - Biological Sciences

Title of case study: Identification and cloning of the P2Y receptor class leads to new therapies targeting purinergic signalling

1. Summary of the impact

Professor Geoffrey Burnstock and colleagues' establishment of the molecular structure of the P2Y class of receptor led to the cloning of several receptors within this class, which are increasingly seen as therapeutic targets for a variety of disorders. Indeed, drugs acting at these receptors are already improving patient health worldwide by reducing the risk of thrombotic events in people suffering from myocardial infarction or ischaemic stroke (via $P2Y_{12}$ receptor antagonists) and by relieving the symptoms of dry eye disorder (via $P2Y_2$ receptor agonists). Burnstock and colleagues also cloned the P2X3 receptor which mediates pain information, and P2X3 antagonists are being developed as novel analgesics. As well as clear clinical benefits, these drug developments are associated with substantial economic and commercial benefits.

2. Underpinning research

Adenosine 5'-triphosphate (ATP) was originally considered to be exclusively an intracellular energy source, with no role in extracellular signalling. Against consensus opinion at that time, Burnstock (in UCL's Department of Anatomy, now Cell & Developmental Biology) alone pioneered the notion that ATP and its derivatives can also act as extracellular messengers. Burnstock championed this idea through years of persistence, until, several decades of research later, we now know that such "purinergic" signalling occurs in many cell types throughout the body, including neurons, smooth muscle cells, epithelial cells, erythrocytes, immune cells and sperm. Purinergic signalling is now a vast and expanding field of biomedical research – over 5,000 articles are returned when PubMed is searched for "purinergic signaling".

Burnstock was the first to define three distinct classes of purinergic receptor (for review, see **[1]**): P1 receptors (G protein-coupled receptors activated by adenosine), P2Y receptors (G protein-coupled receptors activated by several nucleotides including ATP and ADP), and P2X receptors (ATP-gated ion channels). Important roles for these receptor types have already been identified in development (from cell cycling to migration), normal physiology (from pain sensation to bladder contraction), and pathology (from arthritis to cancer).

In 1993, Burnstock collaborated with Eric Barnard at the Royal Free Hospital and Trevor Smart at the School of Pharmacy (both now part of UCL) to clone the first P2Y G protein-coupled nucleotide receptor **[2]**. This work established for the first time the molecular structure of this class of receptor and has led to the cloning of several receptors within this class. There are now eight recognised subtypes of P2Y receptors. Further work in the Burnstock lab identified roles for P2Y receptors in, among other things, human platelet shape change ($P2Y_{12}$, **[3]**) and mucus secretion ($P2Y_2$, **[4]**). These discoveries were influential in the successful development of new drugs to prevent thrombotic events and to treat the symptoms of dry eye, respectively.

There are seven recognised subtypes of the P2X receptor. In 1995, collaboration with John Wood at UCL led to the first cloning of the P2X3 ion channel receptor located on nociceptive sensory nerves **[5]**. In 1999, Burnstock proposed a purinergic hypothesis for pain in visceral organs, whereby ATP released from lining epithelial cells during distension acts on P2X3 receptors on subepithelial sensory nerve endings to send nociceptive messages to the brain **[6]**. Supporting evidence, including epithelial release of ATP, immuno-localization of P2X3 receptors on subepithelial nerves, and activity recorded in sensory nerves during distension that is mimicked by ATP and reduced by P2X3 receptor antagonists, was later reported by Burnstock's lab in the bladder, ureter, and gut **[7a-c]**. In 2000, the lab demonstrated that pain-related behaviour is reduced in P2X3-deficient mice **[8]**. These observations have been influential in the development

of novel selective analgesics.

3. References to the research

[1] Burnstock G. Discovery of purinergic signalling, the initial resistance and current explosion of interest. Br J Pharmacol. 2012 Sep;167(2):238-55. http://doi.org/pnq

[2] Webb TE, Simon J, Krishek BJ, Bateson AN, Smart TG, King BF, Burnstock G, Barnard EA. Cloning and functional expression of a brain G-protein-coupled ATP receptor. FEBS Lett. 1993 Jun 14;324(2):219-25. http://dx.doi.org/10.1016/0014-5793(93)81397-I
In context: this paper reported the cloning of the first member of the P2Y family of purinergic receptors, thus allowing other related receptors to be cloned by searching for homologous genes.

[3] Jagroop IA, Burnstock G, Mikhailidis DP. Both the ADP receptors P2Y1 and P2Y12, play a role in controlling shape change in human platelets. Platelets. 2003 Feb;14(1):15-20. http://dx.doi.org/10.1080/0953710021000062914

[4] Burnstock G, Brouns I, Adriaensen D, Timmermans JP. Purinergic signaling in the airways. Pharmacol Rev. 2012 Oct;64(4):834-68. http://dx.doi.org/10.1124/pr.111.005389

[5] Chen CC, Akopian AN, Sivilotti L, Colquhoun D, Burnstock G, Wood JN. A P2X purinoceptor expressed by a subset of sensory neurons. Nature. 1995 Oct 5;377(6548):428-31. http://dx.doi.org/10.1038/377428a0
In context: this paper reported the cloning of P2X3 receptors, which were later shown to mediate nociception.

[6] Burnstock G. Release of vasoactive substances from endothelial cells by shear stress and purinergic mechanosensory transduction. J Anat. 1999 Apr;194 (Pt 3):335-42. http://dx.doi.org/10.1046/j.1469-7580.1999.19430335.x *and see also* Burnstock G. Purinergic mechanosensory transduction and visceral pain. Mol Pain. 2009 Nov 30;5:69. http://dx.doi.org/10.1186/1744-8069-5-69

[7] a. Vlaskovska M, Kasakov L, Rong W, Bodin P, Bardini M, Cockayne DA, Ford AP, Burnstock G. P2X3 knock-out mice reveal a major sensory role for urothelially released ATP. J Neurosci. 2001 Aug 1;21(15):5670-7. http://www.jneurosci.org/content/21/15/5670.long

b. Rong W, Burnstock G. Activation of ureter nociceptors by exogenous and endogenous ATP in guinea pig. Neuropharmacology. 2004 Dec;47(7):1093-101. http://doi.org/bcvp8f

c. Wynn G, Burnstock G. Adenosine 5'-triphosphate and its relationship with other mediators that activate pelvic nerve afferent neurons in the rat colorectum. Purinergic Signal. 2006 Sep;2(3):517-26. http://dx.doi.org/10.1007/s11302-005-5305-2

[8] Cockayne DA, Hamilton SG, Zhu QM, Dunn PM, Zhong Y, Novakovic S, Malmberg AB, Cain G, Berson A, Kassotakis L, Hedley L, Lachnit WG, Burnstock G, McMahon SB, Ford AP. Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. Nature. 2000 Oct 26;407(6807):1011-5. http://dx.doi.org/10.1038/35039519
In context: this paper showed that knock-out of P2X3 receptors reduces pain, prompting a search for therapeutic agents to block the receptor.

4. Details of the impact

1) Impact derived from the identification and cloning of the P2Y receptor class

i. $P2Y_{12}$ receptor: New antagonists to treat thrombosis and stroke

Every year, arterial blood clots lead to approximately 90,000 heart attacks and 150,000 strokes in the UK. Historically, anticoagulants such as aspirin and warfarin are used to reduce the likelihood of blood clotting in high risk cases, but these drugs can have widespread unwanted side effects. Burnstock's characterisation of the P2Y receptor class made it possible to identify specific antagonists for the $P2Y_{12}$ receptor expressed on platelets. Two new drugs acting on this receptor,

clopidogrel and its derivative ticagrelor, have been developed to prevent platelet aggregation. They are being used widely and successfully for the treatment and prevention of thrombosis and stroke.

Clopidogrel (trade name Plavix, marketed by Bristol-Myers Squibb and Sanofi) **[a]** is indicated for the prevention of thrombotic events in patients suffering from symptomatic atherosclerosis, acute coronary syndromes, (including myocardial infarction with and without ST elevation and unstable angina), ischaemic stroke, or after placement of intracoronary stent or as an alternative antiplatelet drug for patients who are intolerant to aspirin. Plavix is marketed worldwide to nearly 110 countries, with global sales of $7.09bn in 2011 **[b]**.

Ticagrelor (trade names Brilique, Possia and Brilinta, produced by AstraZeneca) **[c]**, which acts faster than clopidogrel, is indicated for the prevention of thrombotic events (such as stroke or heart attack) in patients with acute coronary syndrome, or patients who have recently had an ischaemic stroke. Treatment of acute coronary syndrome with ticagrelor significantly reduces the rate of death from vascular causes, myocardial infarction or stroke. Brilinta is now included in seven sets of cardiovascular treatment guidelines by leading medical organisations across the world: two in Europe, four in the USA and one in Canada. It has been approved in 65 countries and its use is currently being reimbursed in 21 of them. Brilinta is now on formulary at more than 80% of the top 400 hospitals throughout the USA and its 2012 sales were $89m **[d]**.

ii. $P2Y_2$ receptor: New agonists to treat dry eye

Keratoconjunctivitis sicca, or dry eye syndrome, is a relatively common eye disease caused by either decreased tear production or increased tear film evaporation. Dry eye syndrome can be intensely uncomfortable and painful, with some patients going to the lengths of surgery to reduce the area available for tear evaporation. Burnstock's characterisation of the P2Y receptor class made it possible to identify specific agonists of the $P2Y_2$ receptors, which evoke mucus secretion. A new such agonist, diquafasol (Diquas), has been developed by Inspire Pharmaceuticals (to whom Burnstock has been a consultant) **[e]**. Diquas was launched by Santen in Japan in 2010, with Inspire Pharmaceuticals set to receive a milestone payment of $1.25m in the fourth quarter of 2010 **[f]**.

2) Impact derived from the identification and cloning of the P2X3 receptor

More than 270m people worldwide suffer from chronic pain, but there has been little recent success in advancing truly novel therapies for pain relief. Existing therapies, including opioids, antiepileptic drugs and non-steroidal anti-inflammatory drugs all have documented drawbacks. Through the cloning of the P2X3 ion channel receptor located on nociceptive sensory nerves and later the discovery of the involvement of these channels in signalling pain, Burnstock's work has uncovered a new target for pain relief, which is receiving attention from the pharmaceutical industry. Roche (with whom Burnstock was a Consultant and Research Collaborator with over £2m support for over six years for this project) and, more recently, Afferent Pharmaceuticals (for whom he acts as a member of the Scientific Advisory Board), have developed AF-219, a specific P2X3 receptor antagonist, which is a promising new analgesic.

AF-219 is currently in Phase 2 clinical trials for the treatment of three painful disorders: (a) osteoarthritis, which is the most common form of arthritis; (b) interstitial cystitis / bladder pain syndrome, which is experienced by 3-7% of women; and (c) idiopathic chronic cough, which accounts for up to 15% of coughs. Four Phase 1 studies of AF-219 have demonstrated that the compound is safe and well tolerated and completion of Phase 2 clinical trials is expected this year **[g]**.

Antagonism of P2X3 purinoceptors on primary afferent neurons is a novel analgesic approach that has been pursued by leading pharmaceutical companies for the last 15 years. Over 600 patents are currently held which relate to the P2X3 receptor and pain **[h]**.

3) Collaboration with clinicians and the pharmaceutical industry

As a result of his research on ATP, Burnstock has had links with 35 pharmaceutical companies, for whom he acted as a consultant or provided research support. He has collaborated with clinicians around the world on over 70 projects, including work on skin inflammation, motor neuron disease, diabetes, traumatic shock, liver and colorectal tumours, bladder incontinence, vitamin E deficiency, hypertension, and pancreatic, bladder and prostate cancer. Burnstock is currently the Senior Advisor for the Neuroallianz established by the German Government to link basic science, clinical science and the drug industry.

5. Sources to corroborate the impact

[a] Clinical impact of clopidogrel

- NHS patient information for clopidogrel: http://www.nhs.uk/conditions/Anti-platelets-clopidogrel
- NHS drug overview for Plavix: http://www.nhs.uk/medicine-guides/pages/MedicineOverview.aspx?condition=blood%20clotting&medicine=plavix&preparation=plavix%20300mg%20tablets
- NICE technology appraisal guidance for the NHS: http://www.nice.org.uk/nicemedia/live/13285/52030/52030.pdf

[b] Commercial / economic impact of clopidogrel

- Drug in Focus: Clopidogrel (Generics Web): http://www.genericsweb.com/index.php?object_id=1086
- Generic Clopidogrel – An Update for Health and Social Care Board (2011): bit.ly/15WVpVk

[c] Clinical impact of Ticagrelor

- NHS drug overview for Brilique: http://bit.ly/GHPfg6
- NICE technology appraisal guidance for the NHS: http://www.nice.org.uk/nicemedia/live/13588/56819/56819.pdf
- "Despite therapeutic advances, CV disease remains the number one cause of death worldwide. The benefit of ticagrelor on CV thrombotic events, including CV mortality, observed in patients who have had an ACS event supports continued study in other areas of cardiovascular disease." – Judith Hsia, Executive Director Clinical Research, AstraZeneca http://www.pharmatimes.com/Article/12-07-18/AZ_s_Brilinta_goes_head-to-head_with_clopidogrel_in_trial.aspx
- Wallentin et al. (2009) Ticagrelor versus clopidogrel in patients with acute coronary syndromes. The New England Journal of Medicine. 361, 1045-1057. http://doi.org/cw5mfq

[d] Commercial / economic impact of ticagrelor

- PharmaTimes (Feb 2012): http://www.pharmatimes.com/article/12-02-23/AstraZeneca_gets_Brilinta_boost_in_the_USA.aspx
- Financial Times (March 2013): http://on.ft.com/1ealzTx

[e] Clinical impact of diquafosol

- Santen Vision and Innovation Annual Report 2011: http://www.santen.com/en/ir/document/pdf/ar2011.pdf
- Matsumoto et al. (2012) Efficacy and safety of diquafosol ophthalmic solution in patients with dry eye syndrome: a Japanese Phase 2 clinical trial. Ophthalmology. 119, 1954-60. http://doi.org/f2hdxn

[f] Commercial / economic impact of diquafosol

- "Inspire Announces Launch of Diquas by Santen in Japan" (Dec 2010): http://bit.ly/dX6UyC

[g] Impact of AF-219. Afferent Pharmaceuticals:

- Clinical Development page: http://www.afferentpharma.com/therapeutic-ClinicalDevelopment.php
- P2X3 Antagonism page: http://www.afferentpharma.com/therapeutic-approach.php

[h] Patents held on the P2X3 receptor: Wipo patent database search for "P2X3 pain" in Any Field returns 632 hits.