The P2Y/P2X divide: how it began

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Abstract

Extracellular purine and pyrimidine nucleotides produce their pharmacological effects through P2 receptors. These were first named by Geoff Burnstock in an extensive review in 1978. They were then subdivided into P_{2X} and P_{2Y} purinoceptors by Burnstock and Kennedy in 1985, based on applying pharmacological criteria to data generated by functional studies in smooth muscle tissues. Several other P2 subtypes, P_{2T}, P_{2Z}, P_{2U} and P_{2D} were subsequently identified in the following years, again using pharmacological criteria. The number and identity of subtypes were clarified and simplified by the cloning of seven ATP-sensitive ligand-gated ion channel subunits and eight adenine and/or uracil nucleotide-sensitive G protein-coupled receptors from 1993 onwards. The former were all classified as members of the P2X receptor family and the latter as members of the P2Y receptor family. More recently, high resolution imaging of the tertiary and quaternary structures of several P2X and P2Y receptor subtypes has provided a much greater understanding of how and where agonists and antagonists bind to the receptors and how this leads to changes in receptor conformation and activity. In addition, medicinal chemistry has produced a variety of subtype-selective agonists and antagonists, some of which are now in clinical use. This progress and success is a testimony to the foresight, intelligence, enthusiasm and drive of Geoff Burnstock, who led the field forward throughout his scientific life.

1. Introduction

I spent four years at University College London (UCL) working with Geoff Burnstock (Figure 1) and learned so much from him during that time that helped and guided me through my career. I was there because I had been introduced to non-adrenergic, non-cholinergic neurotransmission in the gastrointestinal tract during my undergraduate pharmacology studies at Aberdeen University and quickly realised that purinergic receptors and neurotransmission were the most interesting and exciting areas of research that I had come across. I, therefore, applied for a PhD position with him and started my studies in the autumn of 1981. Because of Geoff's guidance I read widely and from him learned about what we have to do to produce high quality data and how best to advertise and disseminate these data to the scientific community. Geoff always had a large group of PhD students, postdocs, research technicians and visiting scientists and through them I learned about the benefits of collaboration. The UCL labs was a great environment in which to undertake a PhD and I am forever grateful to Geoff for this opportunity and for the experience that he gave me.

2. P_1 and P_2 purinoceptors

2.1 Initial characterisation: Adenosine 5'-triphosphate (ATP) and related nucleotides were discovered in 1929, the year of Geoff Burnstock's birth [1,2] and the first demonstration of pharmacological activity was published shortly afterwards [3]. It was another 49 years, however, before pharmacological criteria were applied to classify the receptors through which these purines act. In 1978, Burnstock proposed a subdivision of receptors in which ATP, adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP) and adenosine act at P_1 and P_2 purinoceptors, as follows [4]. P_1 purinoceptors are stimulated selectively by adenosine and AMP, which leads to changes in cytoplasmic cAMP levels. They are also selectively antagonised by

methylxanthines, such as theophylline. Conversely, P_2 purinoceptors are stimulated selectively by ATP and ADP, are unaffected by methylxanthines and have no effect on intracellular cAMP levels. This proposal of separate adenosine receptors and ATP receptors received much support and was broadly accepted.

Soon afterwards, it was proposed that the P_1 purinoceptor could be subdivided into A_1 and A_2 adenosine receptors, based on pharmacological, biochemical and receptor-binding data [5,6], which also received much support. A big advantage for researchers in the P_1 purinoceptor field was the early and continuing development of drugs, particularly antagonists, which displayed selectivity between the different receptor subtypes. This development led to a faster and greater increase in our knowledge and understanding of the number of P_1 purinoceptor subtypes and their pharmacological properties compared to our knowledge and understanding of P_2 purinoceptors.

2.2 P_2 purinoceptor agonists and antagonists: In the early 1980s the number of pharmacological tools available to study P_2 purinoceptors was very limited. The agonists, ATP, ADP and the enzymatically-stable analogues, α,β-methyleneATP and β,γ-methyleneATP were commercially-available, but although 2-methylthioATP had been synthesised and its effects reported [7,8], it was only available through collaboration with a synthetic chemist. Furthermore, no P_2 purinoceptor antagonists were commercially-available. The photolysable analogue, $ANAPP_3$, had been synthesised and studied by David Westfall's group, who found that it irreversibly inhibited some of the effects of ATP, including the fast, transient contractile component of neurotransmission in the vas deferens [9,10,11]. $ANAPP_3$ was not, however, available commercially and its active component was generated by irradiation by a tungsten halogen projector lamp for 20 minutes, which generated very high local temperatures. A peptide neurotoxin found in bee venom, apamin, had recently been shown to block purinergic actions in gastrointestinal tract smooth muscle, but subsequently this was shown to be due to it blocking

small conductance Ca^{2+}-dependent K^+ channels that are activated by ATP in these tissues [12].

It was around this time that Lubo Kasakov, who was undertaking a sabbatical from the Bulgarian Academy of Sciences with Geoff, made a major breakthrough. Lubo was characterising P_2 purinoceptors present in the guinea-pig urinary bladder and their role in the atropine-resistant, neurogenic contractions. When using α,β-methyleneATP, which was much more potent than ATP at evoking contractions, he saw that these contractions faded very quickly in the continued presence of the agonist. Also, long intervals and repeated washes between additions were needed in order to generate reproducible responses. Lubo quickly realised that this desensitising action of α,β-methyleneATP could be a useful pharmacological tool and proceeded to use it to study the contribution of P_2 purinoceptors to parasympathetic neurotransmission in the urinary bladder. He found that repeated administration of α,β-methyleneATP depressed the atropine-resistant component of neurogenic contractions and also contractions evoked by exogenous ATP, but not acetylcholine or histamine [13]. These results showed that α,β-methyleneATP was selective for P_2 purinoceptors and clearly demonstrated for the first time that ATP and acetylcholine act as cotransmitters from parasympathetic nerves in the urinary bladder.

The high potency, ease of use and commercial availability of α,β-methyleneATP meant that others soon used it to study purinergic neurotransmission in other tissues. Lorna Meldrum, who was also a PhD student with Geoff, found that α,β-methyleneATP-induced desensitisation greatly suppressed the initial phasic contraction of sympathetic nerve-mediated contractions of the guinea-pig vas deferens [14], confirming the results obtained using $ANAPP_3$. Desensitisation of P_2 purinoceptors by α,β-methyleneATP also revealed a cotransmitter action of ATP when released from vascular sympathetic nerves [15,16]. In the following years, this technique was employed many times to study the actions of ATP, particularly as a cotransmitter [see17,18]. Thus α,β-methyleneATP proved to be a very useful, selective inhibitor of P_2 purinoceptors.

3. P2 receptor subtypes

As was often the case with Geoff, my PhD project reflected his typically challenging approach, which was to first read the published literature and identify any gaps in our knowledge and understanding, then to find and develop *something interesting* about P_1 and P_2 purinoceptors. Initially I carried out some experiments on the guinea-pig taenia coli, reflecting my initial interest in purinergic neurotransmission in the gastrointestinal tract, but they came to nothing. I then moved on to the portal vein, which, at that time, in the rabbit was the best example of purinergic inhibitory neurotransmission outside of the gastrointestinal tract [19,20]. These experiments did produce two papers on the modulatory actions of purines on sympathetic neurotransmission [21,22], but they did not meet the challenge of being *interesting*.

As I entered the final year of my PhD I looked for a fresh approach (and enough data to complete a PhD thesis). Because I had taken Geoff's advice and was very well acquainted with the literature, I realised that there was a gap in our knowledge and understanding of the pharmacological properties of P_2 purinoceptors present in vascular smooth muscle. Whilst there were numerous reports relating to P_2 purinoceptors present in vas deferens and urinary bladder smooth muscle, this was not the case for arteries and veins. In addition, Bob Furchgott had recently discovered endothelium-dependent vasodilation [23] and at that time in 1983, only one report on the endothelium-dependent effects of ATP had been published [24]. Thus I had identified a gap in our knowledge and understanding and I proceeded to characterise the pharmacological properties of the P_2 purinoceptors that mediate ATP-induced contraction and relaxation of vascular smooth muscle.

In the rat isolated femoral artery at basal tone, α,β-methyleneATP evoked concentration-dependent contractions, with an EC_{50} of ~4 μM (Figure 2) [25]. ATP also evoked concentration-dependent contractions, but with substantially lower potency and its concentration-response curve did not reached a clear maximum. A similar profile was seen in the rabbit isolated ear

artery with respect to the relative potency of α,β-methyleneATP and ATP at inducing vasoconstriction [26]. Vasodilation was studied by first raising the muscle tone with noradrenaline and under these conditions ATP produced relaxation at low concentrations and transient contractions followed by a maintained relaxation at higher concentrations (Figure 3). Physical removal of the endothelial cells abolished the relaxations and now ATP only evoked contraction. α,β-methyleneATP, on the other hand, only ever elicited vasoconstriction of the femoral artery (Figure 4) and ear artery [26], regardless of whether the endothelial layer was intact or had been removed. Thus the P_2 purinoceptor present on the smooth muscle of the rat femoral artery and mediating vasoconstriction appeared to have different pharmacological properties from the P_2 purinoceptor present on the endothelium and mediating vasodilation.

Further experiments were then performed on the rabbit isolated portal vein longitudinal muscle, when tone had been raised by ergotamine. Under these conditions, ATP elicited relaxation (Figure 5), whereas α,β-methyleneATP evoked contraction [27]. As discussed above, 2-methylthioATP was at that time not commercially available, but I had a great stroke of fortune when I found a single vial of 2-methylthioATP solution at the back of a packed freezer. It had been synthesised by Noel Cusack, a chemist at King's College London, for an earlier collaboration [28]. 2-methylthioATP also relaxed rabbit isolated portal vein longitudinal muscle and was more potent than ATP (Figure 5). When I discussed this piece of luck with Noel a few years later we realised that this may have been the only sample of 2-methylthioATP available anywhere in the world at that time.

The data from these two studies clearly indicated two very different P_2 purinoceptor pharmacological profiles. One P_2 purinoceptor mediated contraction and α,β-methyleneATP was much more potent than ATP, whereas the other mediated relaxation in response to 2-methylthioATP, which was more potent than ATP, and α,β-methyleneATP was inactive. Consequently I felt that I had discovered *something interesting* and when Geoff saw the data it was clear from his excitement that I had.

4. P_{2X} and P_{2Y} purinoceptors

When Geoff and I then re-examined the published literature with these two P_2 purinoceptor profiles in mind, we saw that numerous reports contained individual pieces of data that agreed with our hypothesis that P_2 purinoceptors were not a single homogenous group. The strongest evidence was that 1) α,β-methyleneATP and β,γ-methyleneATP had previously been reported to be more potent than ATP, which was equipotent with 2-methylthioATP, when contraction of the guinea-pig isolated vas deferens and urinary bladder was measured. This is consistent with the vascular contractile data discussed above. 2) 2-methylthioATP was more potent than ATP, which in turn was more potent than α,β-methyleneATP when relaxation of rabbit isolated portal vein, pig isolated aorta and guinea-pig isolated taenia-coli was measured. 3) Repeatedly administering α,β-methyleneATP or pretreating tissues with $ANAPP_3$ inhibited contractions of the vas deferens and urinary bladder, but had no effect on relaxations of the portal vein or taenia-coli. On the basis of these data we posed the question "*Is there a basis for distinguishing two types of P_2-purinoceptor*" [29]

We named the two subtypes P_{2X} and P_{2Y} purinoceptors for several reasons. Greek letters have been used to label subtypes of other receptors, for example μ, δ, κ in the opioid field and α and β in the adrenoceptor field and were considered, but we chose not to follow suit. Of the remaining Greek symbols that are not in common use, how many of us can remember how to pronounce ξ, ζ or ν? We also considered calling them P_{2A} and P_{2B} purinoceptors, but evidence had recently been presented for a subdivision of the adenosine A_2 receptor into A_{2A} and A_{2B} subtypes and we felt that P_{2A} and P_{2B} would potentially be very confusing. Consequently, we settled on P_{2X} and P_{2Y} purinoceptors because these terms were not in use elsewhere and we thought that they would be easy to remember. In hindsight, we probably should have gone with P_{2A} and P_{2B} purinoceptor, as it would have avoided the subsequent "random walk through the

alphabet" that occurred when further subtypes were proposed (see below). Nonetheless, the P_{2X} and P_{2Y} nomenclature has survived to this day.

5. More P_2 purinoceptor subtypes

The following year John Gordon identified and named two further subtypes, adopting and extending our nomenclature. He called ADP-sensitive receptors that are present in platelets and mediate aggregation, P_{2T} purinoceptors, and ATP-sensitive receptors that are expressed in mast cells and mediate degranulation, P_{2Z} purinoceptors [30]. Over the next few years these proposed subdivisions slowly gained support as their pharmacological properties were characterised further. Evidence also accumulated that β,γ-methylene-L-ATP and ADP-β-F activated P_{2X} and P_{2Y} purinoceptors respectively, with a degree of selectivity. Subtype-selective antagonists were still some way off in the future, but the demonstration that the trypanocidal agent, suramin, was also a non-selective P_2 purinoceptor antagonist was a major development for purinergic research in general, as it was inexpensive and easy to use. Also, electrophysiological and biochemical studies showed that P_{2X} purinoceptors are ligand-gated cation channels (LGIC) and P_{2Y} purinoceptors are G protein-coupled receptors (GPCR). This is not surprising, as it had been known for some time that P_{2X} purinoceptors mediated the fast neurotransmitter actions of ATP, which implied that they were ion channels, and that responses mediated by P_{2Y} purinoceptors tended to be slower, indicating activation of second messenger signalling pathways.

The final advances in this stage of the evolution of P_2 purinoceptors was the identification of receptors that were activated by uracil nucleotides, such as uridine 5'-triphosphate (UTP). These were also known to be pharmacologically active and a separate pyrimidoceptor was proposed [31]. In 1991, the P_{2U} purinoceptor was proposed to be activated by both ATP and UTP [32]. Finally, adenine dinucleotides were proposed to produce some of their pharmacological effects by stimulating the P_{2D} purinoceptor [33,34].

6. The cloning of P2 receptors

The identification and naming of P_2 purinoceptor subtypes was revolutionised by the cloning of seven ATP-sensitive LGIC subunits and eight adenine and/or uracil nucleotide-sensitive GPCR from 1993 onwards [35,36]. In recognition that not only purines, but also pyrimidines, were pharmacologically active and that in some cases they acted at a common site, the term *P_2 purinoceptor* was dropped and all nucleotide-sensitive subtypes were thereafter referred to as *P2 receptors*. In addition, all LGIC, including the P_{2Z} purinoceptor, were now called P2X receptors and all GPCR, including the P_{2U} purinoceptor, were considered to be P2Y receptors. Thus cloning greatly simplified P2R nomenclature.

All of the subtypes have now been cloned in multiple species and the *Ensembl Gene* database currently lists 151-350 species orthologues of each P2X subunit and 191-295 of each P2Y receptor. They are all present in placental mammals, birds, reptiles, fish and primitive organisms, such as amoeba and algae, but interestingly, not *Caenorhabditis elegans*, *Drosophila melanogaster* or *Saccharomyces cerevisiae*. Interestingly, the $P2Y_{11}$ subtype is not present in rats and mice [37].

7. P2X and P2Y receptors today

The properties of the cloned P2X [38,39] (Table 1) and P2Y [40,41] (Table 2) receptors have been discussed in detail in several recent comprehensive reviews. I will, therefore, briefly summarise here our current knowledge and understanding of their structure, pharmacological properties and potential for therapeutic application.

7.1 Structure: P2X receptor subunits comprise a single polypeptide chain that forms two helical transmembrane spanning regions, with intracellular N- and C-termini and a large extracellular loop and form homomeric and heteromeric, non-selective cationic channels, with a relatively high permeability to Ca^{2+} [35]. High resolution tertiary structures generated by X-ray

crystallography have now been obtained from multiple species, in ligand-free, ligand-bound, activated and desensitised states [39]. They confirm previous indirect structural studies that three subunits interact to form a functional receptor. They further reveal that the subunits wrap round each other to produce a chalice-like structure, that the large extracellular domain protrudes above the plane of the plasma membrane by ~70 Å and that the transmembrane spanning regions are α-helices that cross the plasma membrane at an angle of nearly 45º relative to its plane. The grooves formed by the sites of interaction between the three subunits contain binding pockets for both orthosteric ligands, at ~40 Å above the plane of the plasma membrane [42] and above them, for P2X7 negative allosteric modulators [43].

P2Y receptors are a member of the δ group of class A, rhodopsin-like GPCR and when activated they couple to heterotrimeric G proteins, as follows: the $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$ subtypes couple to $G\alpha_{q/11}$, the $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ receptors couple to $G\alpha_{i/o}$ and the $P2Y_{11}$ receptor also couples to $G\alpha_s$ [36] (Table 2). High resolution tertiary structures of the human $P2Y_1$ [44] and $P2Y_{12}$ [45,46] receptors in ligand-bound states confirm that they have the canonical GPCR structure of a single polypeptide chain that crosses the plasma membrane seven times and has extracellular N- and intracellular C-termini. At present, the agonist-bound state has only been solved for the $P2Y_{12}$ receptor and the orthosteric binding site is within the transmembrane spanning region bundle, just beneath the plane of the plasma membrane [46]. Binding of antagonists within the orthosteric binding site has been shown for both subtypes and a unique binding site for the negative allosteric modulator, BPTU, was identified on the outer surface of the $P2Y_1$ receptor, at its interface with lipids of the plasma membrane [44]. It is likely that BPTU gains access to this site through the lipid bilayer, which is consistent with its high lipophilicity.

7.2 Selective P2X ligands and therapeutic applications: Currently, there are no subtype-selective P2X agonists, which, given the highly conserved nature of the ATP-binding pocket, is not

surprising. Positive allosteric modulators may be the best approach for selectively stimulating P2X subtypes (see Table 1) and the best known example is ivermectin, which potentiates ATP-induced ionic currents through P2X4 [47] and human, but not rat P2X7 [48] receptors.

Development of selective antagonists has been much more successful, particularly against P2X3 and P2X7 receptors [38,39,49] (Table 1). Currently, the most promising clinical application for P2X3 antagonists is in treatment of chronic cough by Gefapixant (AF-219, MK-7264), named after Geoff Burnstock [50] and BLU-5937 [51]. P2X7 receptors have an important role in inflammation and numerous patents have been filed and clinical trials registered for P2X7 antagonists in the treatment of chronic systemic inflammatory diseases, such as rheumatoid arthritis, allergies, asthma, COPD and autoimmune diseases [52]. A very potent anti-P2X7 receptor, bivalent nanobody-Fc has also been developed and had beneficial effects in mouse models of allergic contact dermatitis and experimental glomerulonephritis [53]. P2X7 receptors are also expressed in numerous types of cancerous cell [52,54] and a recent phase 1 trial with an ointment containing an antibody, BIL010t, directed against the P2X7 receptor reduced the lesion area in 65% of patients with basal cell carcinoma [55]. An anti-P2X7R vaccine (BIL06v), is under assessment in patients with advanced solid tumours [52]. Finally, neuropathic pain is another therapeutic area of interest, due to the expression of P2X4 receptors in microglial cells of the spinal cord dorsal horn and NC-2600, a new P2X4 antagonist, had no serious side effects in a phase 1 trial [56]. Interestingly, there is evidence that P2X4 receptors do not contribute to pain hypersensitivity in females, suggesting that their involvement in chronic pain is sexually dimorphic [57].

7.3 Selective P2Y ligands and therapeutic applications: Compared to P2X receptors, more selective P2Y receptor agonists and antagonists have been developed [40,41], which is not a surprise given that the agonist-binding pocket of P2Y receptors is less well conserved between subtypes. Diquafasol (UP4U, INS365), a selective $P2Y_2$ receptor agonist, is licensed in South

Korea and Japan for the treatment of dry eye syndrome , as it stimulates secretion of water and mucin by conjunctival epithelial and goblet cells in the eye. Subtype-selective antagonists include RS2179, MRS2279, MRS2500 and BPTU at $P2Y_1$ receptors, AR-C118925XX at $P2Y_2$ receptors [58,59], MRS2578 at $P2Y_6$ receptors [60], NF340 at $P2Y_{11}$ receptors [61], MRS2211 at $P2Y_{13}$ receptors [62] and PPTN at $P2Y_{14}$ receptors [63]. By far the most clinically useful class of P2 receptor ligands, however, are P2Y12 antagonists, such as clopidogrel, prasugrel, ticlodipine ticagrelor, cangrelor and AZD1283, which inhibit platelet aggregation and thrombus formation and are used widely as anti-thrombotic agents [40,41].

8. Conclusion

In the decades since Geoff Burnstock first proposed the existence of P2 receptors that mediate the pharmacological actions of purine nucleotides, they have developed from being a single, poorly defined receptor, with few selective ligands and no antagonists to two large families of multiple subtypes, each of which has clear and definable properties. In addition, recent high resolution imaging of the tertiary and quaternary structures of several P2X and P2Y receptor subtypes has provided a detailed understanding of how and where agonists and antagonists bind to the receptors and how this leads to changes in receptor conformation and activity. Furthermore, highly selective agonists and antagonists have been developed that can be used to determine the physiological and pathophysiological roles of P2X and P2Y receptors, some of which are now in clinical use. We also have a greater understanding of associated processes, such as the cellular release of ATP, which can now be followed in real time [64] and the metabolism of extracellular purines and pyrimidine nucleotides by a family of ecto-enzymes [65]. Behind it all lies Geoff Burnstock's intelligence, enthusiasm and drive.

References

1. Lohmann K (1929) Über die pyrophosphate fraktion im muskel. Naturwiss 17:624-625. doi: 10.1007/BF01506215.
2. Fiske CH, SubbaRow Y (1929) Phosphorus compounds of muscle and liver. Science 70:381-382. doi: 10.1126/science.70.1816.381-a.
3. Drury AN, Szent-Györgi A (1929) The physiological activity of adenine compounds with special reference to their action upon the mammalian heart. J Physiol 68:213-237. doi: 10.1113/jphysiol.1929.sp002608
4. Burnstock G (1978) A basis for distinguishing two types of purinergic receptor. In Straub, R.W. (Ed.), Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach, Raven Press, New York, pp107-118.
5. Van Calker D, Muller M, Hamprecht B (1979) Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. J Neurosci 33:999-1005. doi: 10.1111/j.1471-4159.1979.tb05236.x.
6. Londos C, Cooper, DMF, Woolf J (1980) Subclasses of external adenosine receptors. Proc Natl Acad Sci USA 77:2551-2554. doi: 10.1073/pnas.77.5.2551
7. Gough GR, Maguire MH, Satchell DG (1973) Three new adenosine-triphosphate analogs. Synthesis and effects on isolated gut. J Med Chem 16:1188-1190. doi: 10.1021/jm00268a028
8. Satchell DG, Maguire MH (1975) Inhibitory effects of adenine nucleotide analogs on the isolated guinea-pig taenia-coli. J Pharmacol Exp Ther 195:540-548.
9. Hogaboom GK, O'Donnell JP, Fedan JS (1980) Purinergic receptors: photoaffinity analog of adenosine triphosphate is a specific adenosine triphosphate antagonist. Science 208:1273-1274. doi: 10.1126/science.6103581.
10. Fedan, JS, Hogaboom, GK, O'Donnell JP, Colby J, Westfall DP (1981) Contributions by purines to the neurogenic response of the vas deferens of the guinea-pig. European J Pharmacol 69:41-53. doi: 10.1016/0014-2999(81)90600-2.
11. Sneddon P, Westfall DP, Fedan JS (1982) Cotransmitters in the motor nerves of the guinea pig vas deferens: electrophysiological evidence. Science 218:693-695. doi: 10.1126/science.6291151.
12. Shuba MF, Vladimirova IA (1980) Effects of apamin on the electrical responses of smooth muscle to adenosine 5-triphosphate and to non-adrenergic, non-cholinergic nerve stimulation. Neurosci 5:853-859. doi: 10.1016/0306-4522(80)90154-2.
13. Kasakov L, Burnstock G (1982) The use of the slowly degradable analogue α,β-

methyleneATP, to produce desensitisation of the P_2-purinoceptor: effect on non-adrenergic, non-cholinergic responses of the guinea-pig urinary bladder. European J Pharmacol 86:291-294. doi: 10.1016/0014-2999(82)90330-2.

14. Meldrum LA, Burnstock G (1984) Evidence that ATP acts as a cotransmitter with noradrenaline in the sympathetic nerves supplying the guinea-pig vas deferens. European J Pharmacol 92:161-163. doi: 10.1016/0014-2999(83)90126-7.
15. Von Kügelegen I, Starke K (1985) Noradrenaline and adenosine triphosphate as co-transmitters of neurogenic vasoconstriction in rabbit mesenteric artery. J Physiol 367:435-455. doi: 10.1113/jphysiol.1985.sp015834.
16. Kennedy C, Saville V, Burnstock G (1986) The contributions of noradrenaline and ATP to the responses of the rabbit central ear artery to sympathetic nerve stimulation depend on the parameters of stimulation. European J Pharmacol 122:291-300. doi: 10.1016/0014-2999(86)90409-7.
17. Kennedy C, Tasker P, Gallagher G, Westfall TD (2007) Identification of atropine- and $P2X_1$ receptor antagonist-resistant, neurogenic contractions of the urinary bladder. J Neurosci 27:845-851. doi: 10.1523/JNEUROSCI.3115-06.2007
18. Kennedy C (2015) ATP as a cotransmitter in the autonomic nervous system. Auton Neurosci: Basic and Clinical, 191:2-15. doi: 10.1016/j.autneu.2015.04.004
19. Hughes J, Vane JR (1967) An analysis of the responses of the isolated portal vein of the rabbit to electrical stimulation and to drugs, British J Pharmacol 30:46-66. doi: 10.1111/j.1476-5381.1967.tb02112.x.
20. Hughes J, Vane, JR (1970) Relaxations of the isolated portal vein of the rabbit induced by nicotine and electrical stimulation. British J Pharmacol 39:476-489. doi: 10.1111/j.1476-5381.1970.tb10356.x.
21. Burnstock G, Crowe R, Kennedy C, Torok J (1984) Indirect evidence that purinergic modulation of perivascular adrenergic neurotransmission in the portal vein is a physiological process. British J Pharmacol 82:359-368. doi: 10.1111/j.1476-5381.1984.tb10770.x
22. Kennedy C, Burnstock G (1984) Evidence for an inhibitory prejunctional P_1-purinoceptor in the rat portal vein with characteristics of the A_2- rather than the A_1-subtype. European J Pharmacol 100:363-368. doi: 10.1016/0014-2999(84)90014-1
23. Furchgott RF, Zawadski JV (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288:373-376. doi: 10.1038/288373a0.
24. De Mey JG, Vanhoutte PM (1981) Role of intima in cholinergic and purinergic relaxation

of isolated canine femoral arteries. J Physiol 316:347-355.

25. Kennedy C, Delbro D, Burnstock G (1985) P_2-purinoceptors mediate both vasodilation (via the endothelium) and vasoconstriction of the isolated rat femoral artery. European J Pharmacol 107:161-168. doi: 10.1016/0014-2999(85)90055-x.
26. Kennedy C, Burnstock G (1985). ATP produces vasodilation via P_1-purinoceptors and vasoconstriction via P_2-purinoceptors in the isolated rabbit central ear artery. Blood Vessels 22:145-155. doi: 10.1159/000158592.
27. Kennedy C, Burnstock G (1985) Evidence for two types of P_2-purinoceptor in the longitudinal muscle of the rabbit portal vein. European J Pharmacol 111:49-56. doi: 10.1016/0014-2999(85)90112-8.
28. Burnstock G, Cusack NJ, Hills, JM, MacKenzie I, Meghji P (1983) Studies on the stereoselectivity of the P_2-purinoceptor. British J Pharmacol 79:907-913. doi: 10.1111/j.1476-5381.1983.tb10535.x.
29. Burnstock G, Kennedy C (1985) Is there a basis for distinguishing two types of P_2-purinoceptor? Gen Pharmacol 5:433-440. doi: 10.1016/0306-3623(85)90001-1.
30. Gordon JL (1986) Extracellular ATP: effects, sources and fate. Biochem J 233:309-319. doi: 10.1042/bj2330309.
31. Seifert R, Schultz G (1989) Involvement of pyrimidinoceptors in the regulation of cell functions by uridine and by uracil nucleotides. Trends Pharmacol Sci 10:365-369. doi: 10.1016/0165-6147(89)90009-6.
32. O'Connor SE, Dainty IA, Leff P (1991) Further subclassification of ATP receptors based on agonist studies. Trends Pharmacol Sci 12:137-141. doi: 10.1016/0165-6147(91)90530-6
33. Hilderman RH, Martin M, Zimmerman JK, Pivorun EB (1991) Identification of a unique membrane receptor for adenosine 5',5'''-P1,P4-tetraphosphate. J Biol Chem 266:6915-6918.
34. Castro E, Pintor J, Miras-Portugal MT (1992) Ca-stores mobilisation by diadenosine tetraphosphate, Ap4A, through a putative P2Y purinoceptor in adrenal chromaffin cells. British J Pharmacol 106:833-837. doi: 10.1111/j.1476-5381.1992.tb14421.x
35. Khakh BS, Burnstock G, Kennedy C, King BF, North RA, Seguela P, Voigt M, Humphrey PPA (2001) International Union of Pharmacology. XXIV. Current status of the nomenclature and properties of P2X receptors and their subunits. Pharmacol Rev 53:107-118.
36. Abbracchio MP, Burnstock G, Boeynaems JM, Barnard EA, Boyer JL, Kennedy C, Fumagalli M, Gachet C, Jacobson KA, Weisman GA (2006) International Union of Pharmacology. Update of the P2Y G protein-coupled nucleotide receptors: from molecular

mechanisms and pathophysiology to therapy. Pharmacol Rev 58:281-341. doi: 10.1124/pr.58.3.3.

37. Kennedy C (2017) $P2Y_{11}$ receptors: properties, distribution and functions. Adv Exp Med Biol: Prot Rev 1051:107-122. doi: 10.1007/5584_2017_89.
38. Alexander SPH, Mathie A, Peters JA, Veale EL, Striessnig J, Kelly E, Armstrong JF, Faccenda E, Harding SD, Pawson AJ, Sharman JL, Southan C, Davies JA; CGTP Collaborators (2019) THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: Ion channels. British J Pharmacol 176 Suppl 1,:S142-S228. doi: 10.1111/bph.14749.
39. Illes P, Müller CE, Jacobson KA, Grutter T, Nicke A, Fountain SJ, Kennedy C, Schmalzing G, Jarvis M, Stojilkovic S, King BF, Di Virgilio F (2021) Update of P2X receptor properties and their pharmacology; IUPHAR review. British J Pharmacol (in press).
40. Alexander SPH, Christopoulos A, Davenport AP, Kelly E, Mathie A, Peters JA, Veale EL, Armstrong JF, Faccenda E, Harding SD, Pawson AJ, Sharman JL, Southan C, Davies JA; CGTP Collaborators (2019) THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: G protein-coupled receptors. British J Pharmacol 176 Suppl 1, S21-S141. doi: 10.1111/bph.14748.
41. Jacobson KA, Delicado EG, Gachet C, Kennedy C, von Kügelgen I, Li B, Miras-Portugal MT, Novak I, Schöneberg T, Perez-Sen R, Thori D, Wu B, Yang Z, Müller CE (2020) Update of P2Y receptor pharmacology: IUPHAR Review 27. British J Pharmacol 177:2413-2433. doi: 10.1111/bph.15005.
42. Hattori M, Gouaux, E (2012) Molecular mechanism of ATP binding and ion channel activation in P2X receptors. Nature 485:207–212. doi: 10.1038/nature11010.
43. Karasawa A, Kawate T (2016) Structural basis for subtype-specific inhibition of the P2X7 receptor. eLife 5: e22153. doi: 10.7554/eLife.22153.
44. Zhang D, Gao ZG, Zhang K, Kiselev E, Crane S, Wang J, Paoletta S, Yi C, Ma L, Zhang W, Han GW, Liu H, Cherezov V, Katritch V, Jiang H, Stevens RC, Jacobson KA, Zhao Q, Wu B (2015) Two disparate ligand-binding sites in the human $P2Y_1$ receptor. Nature 520:317-321. doi: 10.1038/nature14287.
45. Zhang K, Zhang J, Gao ZG, Zhang D, Zhu L, Han GW, Moss SM, Paoletta S, Kiselev E, Lu W, Fenalti G, Zhang W, Müller CE, Yang H, Jiang H, Cherezov V, Katritch V, Jacobson KA, Stevens RC, Wu B, Zhao Q (2014) Structure of the human $P2Y_{12}$ receptor in complex with an antithrombotic drug. Nature 509:115-118. doi: 10.1038/nature13083

46. Zhang J, Zhang K, Gao ZG, Paoletta S, Zhang D, Han GW, Li T, Ma L, Zhang W, Müller CE, Yang H, Jiang H, Cherezov V, Katritch V, Jacobson KA, Stevens RC, Wu B, Zhao Q (2014) Agonist-bound structure of the human $P2Y_{12}$ receptor. Nature 509:119-122. doi: 10.1038/nature13288.
47. Khakh BS, Proctor WR, Dunwiddie TV, Labarca C, Lester HA (1999) Allosteric control of gating and kinetics at P2X4 receptor channels. J Neurosci 19:7289-7299. doi: 10.1523/JNEUROSCI.19-17-07289.1999.
48. Nörenberg W, Sobottka H, Hempel C, Plötz T, Fischer W, Schmalzing G, Schaefer M (2012) Positive allosteric modulation by ivermectin of human but not murine P2X7 receptors. British J Pharmacol 167:48-66. doi: 10.1111/j.1476-5381.2012.01987.x.
49. Jarvis MF (2021). Geoffrey Burnstock's influence on the evolution of P2X3 receptor pharmacology. Purinergic Signal (In press). doi: 10.1007/s11302-020-09744-9
50. Smith JA, Kitt MM, Butera P, Smith SA, Li Y, Xu ZJ, Holt K, Sen S, Sher MR, Ford AP (2020) Gefapixant in two randomised dose-escalation studies in chronic cough. Eur Respir J 55:1901615. doi: 10.1183/13993003.01615-2019.
51. Marucci G, Dal Ben D, Buccioni M, Martí Navia A, Spinaci A, Volpini R, Lambertucci C (2019) Update on novel purinergic P2X3 and P2X2/3 receptor antagonists and their potential therapeutic applications. Expert Opin Ther Pat 29:943-963. doi: 10.1080/13543776.2019.1693542
52. Lara R, Adinolfi E, Harwood CA, Philpott M, Barden JA, Di Virgilio F, McNulty S (2020) P2X7 in cancer: from molecular mechanisms to therapeutics. Front Pharmacol 11:793. doi: 10.3389/fphar.2020.00793.
53. Danquah W, Meyer-Schwesinger C, Rissiek B, Pinto C, Serracant-Prat A, Amadi M, Iacenda D, Knop JH, Hammel A, Bergmann P, Schwarz N, Assunção J, Rotthier W, Haag F, Tolosa E, Bannas P, Boué-Grabot E, Magnus T, Laeremans T, Stortelers C, Koch-Nolte F (2016) Nanobodies that block gating of the P2X7 ion channel ameliorate inflammation. Sci Transl Med 8:366ra162. doi: 10.1126/scitranslmed.aaf8463.
54. De Virgilio F (2021) P2X7 is a cytotoxic receptor….maybe not. Implications for cancer. Purinergic Signal (In press). doi: 10.1007/s11302-020-09735-w
55. Gilbert SM, Gidley Baird A, Glazer S, Barden JA, Glazer A, Teh LC, King J. (2017). A phase I clinical trial demonstrates that nfP2X7 -targeted antibodies provide a novel, safe and tolerable topical therapy for basal cell carcinoma. British J Dermatol 177:117-124. doi: 10.1111/bjd.15364.
56. Inoue K (2021) P2X receptors and pain. Purinergic Signal (In press). doi: 10.1007/s11302-

020-09743-w.

57. Tam TH, Salter MW (2021). Purinergic signalling in spinal pain processing. Purinergic Signal (In press). doi: 10.1007/s11302-020-09748-5
58. Rafehi M, Müller CE (2018) Tools and drugs for uracil nucleotide-activated P2Y receptors. Pharmacol Ther 190:24-80. doi: 10.1016/j.pharmthera.2018.04.002.
59. Muoboghare MO, Drummond R, Kennedy C (2019) Characterisation of $P2Y_2$ receptors in human vascular endothelial cells using AR-C118925XX, a potent and selective $P2Y_2$ antagonist. British J Pharmacol 176:2894-2904. doi: 10.1111/bph.14715.
60. Mamedova LK, Joshi BV, Gao ZG, Von Kügelgen I, Jacobson KA (2004) Diisothiocyanate derivatives as potent, insurmountable antagonists of $P2Y_6$ nucleotide receptors. Biochem Pharmacol 67:1763-1770. doi: 10.1124/mol.113.085654.
61. Meis S, Hamacher A, Hongwiset D, Marzian C, Wiese M, Eckstein N, Royer HD, Communi D, Boeynaems JM, Hausmann R, Schmalzing G, Kassack MU (2010) NF546 [4,4′-(carbonylbis(imino-3,1-phenylene-carbonylimino-3,1-(4-methyl-phenylene)-carbonylimino))-bis(1,3-xylene-alpha, alpha'-diphosphonic acid) tetrasodium salt] is a non-nucleotide P2Y11 agonist and stimulates release of interleukin-8 from human monocytes. J Pharmacol Exp Ther 332:238-247. doi: 10.1124/jpet.109.157750.
62. Kim YC, Lee JS, Sak K, Marteau F, Mamedova L, Boeynaems JM, Jacobson KA (2005). Synthesis of pyridoxal phosphate derivatives with antagonist activity at the $P2Y_{13}$ receptor. Biochem Pharmacol 70:266-274. doi: 10.1016/j.bcp.2005.04.021.
63. Barrett MO, Sesma JI, Ball CB, Jayasekara PS, Jacobson KA, Lazarowski ER, Harden TK (2013) A selective high-affinity antagonist of the $P2Y_{14}$ receptor inhibits UDP-glucose-stimulated chemotaxis of human neutrophils. Mol Pharmacol 84:41-49. doi: 10.1124/mol.113.085654.
64. Dale N (2021) Real-time measurement of adenosine and ATP release in the central nervous system. Purinergic Signal (In press). doi: 10.1007/s11302-020-09733-y
65. Zimmermann H. (2021) Ectonucleoside triphosphate diphosphohydrolases and ecto-5'-nucleotidase in purinergic signaling: How the field developed and where we are now. Purinergic Signal (In press). doi.org/10.1007/s11302-012-9309-4

Figure legends

Figure 1. Geoff Burnstock

Geoff Burnstock is seen at his desk in his office at UCL in 1997.

Figure 2. Contractions of rat isolated femoral artery

Contractions evoked by a) α,β-methyleneATP (10^{-7}-10^{-4} M) and b) ATP (10^{-5}- $3x10^{-4}$ M) at resting tone when endothelium was intact are shown. c) Log concentration-response curves for contractions evoked by α,β-methyleneATP (10^{-7}-10^{-4} M) (◊,♦) and ATP (10^{-5}-10^{-3} M) (○,●) at resting tone when endothelium was intact (open symbols) or removed (closed symbols) (n=6) are shown. Vertical bars show sem. Reproduced from [25], with permission from Elsevier.

Figure 3. Two types of response to ATP in rat isolated femoral artery

a) The effects of ATP (10^{-6}- $3x10^{-4}$ M) in tissues precontracted by 10^{-6} M noradrenaline (NA) when endothelium was intact (o) or removed (●) (n=4) are shown. Vertical bars represent sem. b) Endothelium removed, contraction to 10^{-5} M ATP. c) Endothelium intact, relaxations to ATP (10^{-3}-$3x10^{-4}$ M). Reproduced from [25], with permission from Elsevier.

Figure 4. One type of response to α,β-methyleneATP in rat isolated femoral artery

a) The effect of α,β-methyleneATP ($3x10^{-7}$-10^{-4} M) in tissues precontracted by 10^{-6} M noradrenaline (NA) when endothelium was intact (o) or removed (●) (n = 6) are shown. Vertical bars show sem. b) Contractions evoked by α,β-methyleneATP when the endothelium was intact are shown. Reproduced from [25], with permission from Elsevier.

Figure 5. Relaxations of rat isolated portal vein longitudinal muscle

a) Relaxations of precontracted tissues induced by 2-methyithioATP (10^{-4} M) and ATP (10^{-4} M) in the same preparation are shown. b) The mean peak amplitude of relaxations evoked by 2-methylthioATP (10^{-6}-10^{-4} M) (Δ) and ATP (10^{-5}-$3x10^{-4}$ M) (O) (n =6) are shown. Vertical bars show sem. Adapted from [27], with permission from Elsevier.