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Geoffrey Burnstock – An accidental pharmacologist

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Abstract

Geoffrey Burnstock, the founder of the field of purinergic signaling research passed away in Melbourne, Australia on June 3rd, 2020, at the age of 91. With his death, the world of biomedical research lost one of its most passionate, creative and unconventional thought leaders. He was an inspiration to the many researchers he interacted with for more than 50 years and a frequent irritation to those in the administrative establishment. Geoff never considered himself a pharmacologist having being trained as a zoologist and becoming an autonomic neurophysiologist based on his evolving interests in systems and disease-related research. By the end of his life he had: published some 1550 papers; been cited more than 125,000 times; had an *h*-index of 156 and had supervised over 100 Ph.D. students. His indelible legacy, based on a holistic, data-based, multidisciplinary, unconventional “outside the box” approach to research was reflected in two of the seminal findings in late 20th century biomedical research: the purinergic neurotransmitter hypothesis and the concept of co-neurotransmission, both of which were initially received by his peers with considerable skepticism that at times verged on disdain. Nonetheless, while raising hackles and threatening the status quo, Geoff persevered and prevailed, becoming a mentor for several generations of biomedical researchers. In this review we provide a joint perspective on Geoff Burnstock’s legacy in research.

Keywords

Burnstock; Purine hypothesis; Pharmacology

1. Introduction

With the death of Geoffrey Burnstock in Melbourne, Australia on June 3rd, 2020 at the age of 91, biomedical research has lost one of its more passionate, creative and unconventional thought leaders. Trained as a zoologist at King’s College and University College London

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Francesco Di Virgilio: Conceptualization, Writing - original draft, Writing - review & editing. **Kenneth A. Jacobson:** Conceptualization, Writing - original draft, Writing - review & editing. **Michael Williams:** Conceptualization, Writing - original draft, Writing - review & editing.

(UCL), Geoff received his Ph.D. in 1957. After postdoctoral fellowships at the National Institute for Medical Research in Mill Hill, London, the Department of Pharmacology at Oxford University and the Department of Physiology at the University of Illinois, Champagne-Urbana, Geoff moved to the Department of Zoology at the University of Melbourne in Australia in 1959.

This was prompted by his view – based on interactions with his “great mates” from Australia - that “in England, if you want to do something new, the first response is, ‘it can’t be done’..... Whereas, in Australia, the first thing they say is, ‘give it a go, mate’ “[1]. His research activities in Melbourne resulted in two seminal research concepts, the first, the discovery of non-adrenergic, non-cholinergic (NANC) neurons in the gut which were identified as purinergic nerves and led to the now well-established concept of purinergic transmission [2-4] despite considerable skepticism from his peers that at times verged on disdain, e.g., “the purimagine hypothesis” [5]. The second was another controversial topic, namely Geoff’s re-examination in a seminal 1976 review [6] of the phenomenon known as Dale’s Principle, an appellation coined by Eccles who is considered to have misinterpreted Dale’s original concept. Dale’s Principle, wherein neurons were thought to synthesize, store, and release only a single neurotransmitter, was questioned by Geoff in terms of its universal applicability since a body of evidence was emerging that supported the concept of co-transmission - specifically where ATP was frequently found to be stored and released together with the classical neurotransmitters, acetylcholine and norepinephrine [6]. The technologies required to generate the data to establish the concept of co-transmission did not become available until after the era of Dale or Eccles. These were used by Geoff and others to establish and extend the concept of co-transmission to include GABA, glutamate and a variety of endogenous neuroactive peptides [7].

In 1975, Geoff moved back to London as the Chair of the Department of Anatomy and Physiology at UCL, a position he held until 1997 when he became Director of the Autonomic Neuroscience Institute, Royal Free & University College London and in 2004, President of the Autonomic Neuroscience Center at the Royal Free, a position he held until his formal retirement in 2017. He then returned to Melbourne where he became an Honorary Professorial Fellow of the Florey Institute of Neuroscience and Mental Health and, until his death, was closely affiliated with the Department of Pharmacology and Therapeutics, University of Melbourne.

2. Geoff as a researcher

For over a decade, Geoff occupied the top position in the world of scientific citations in pharmacology and toxicology [8]. On his passing he had published more than 1,550 papers, mentored over 100 Ph.D. students [1], generated 125,000 citations, had an h-index of 156 and was one of the most highly regarded neuroscientists of his generation [9]. In addition, Geoff had received a number of prestigious awards that recognized his achievements in the field of biomedical research, which have been documented in detail by Abbrachio et al. [10]. Despite these successes, Geoff’s approach to the conduct of research was a frequent cross for those in the administrative establishment to begrudgingly bear, while his research activities remained a source of irritation to, and were continuously challenged by, his peers.

In the former regard, one of Geoff's Deans at the UCL Faculty of Life Sciences, A.R. Lieberman noted in 1997 at Geoff's first non-retirement celebration (https://www.ucl.ac.uk/biosciences/sites/biosciences/files/cdb-about-short-history.pdf) that "Geoff has been the most awkward, the most argumentative, the most difficult and the most demanding Head of Department in the [UCL] Faculty and I would guess, the entire College. And quite right too! That's why he has been such a success and such a good Head of Department. Geoff, difficult though it has sometimes been, it has been overall a pleasure and unquestionably a privilege to work with you." In the latter regard, peer irritation was often the result of Geoff's ideas being simply too far 'out of the box' in challenging the existing status quo [1,5]. On one occasion, Geoff was even challenged by a member of the public. At a Purine meeting held at the main location of the University of Milan in an area close to the center of the city by the Duomo, Geoff was in the middle of his presentation when this uninvited individual walked into the main lecture theater. He abruptly launched into an animated speech in Italian that at times appeared quite hostile in tenor. Geoff graciously allowed this individual to continue talking until the authorities came and took him away. An English translation of what had been said has not become available although the meeting organizers indicated at the time that the individual may have been emotionally disturbed and that none of what he had said was specifically directed to either Geoff or the validity of the purinergic hypothesis, although as memory serves Geoff did comment that his presentation was relatively benign and had not been intended to upset anyone.

It took some 25 years for the concept of purinergic neurotransmission to be widely accepted even after receptor cloning became a routine research tool in the last decades of the 20th century and clear evidence for the existence of P1 (adenosine) and P2 (ATP receptors) was obtained [11,12]. Given the long standing interest in the physiological effects of adenosine that dated back to 1929 [13], a nomenclature for adenosine receptors, A_1, A_{2A}, A_{2B} and A_3, had evolved on a pharmacological basis ahead of that for P2 receptors [14] such that heated discussions arose when Geoff's suggestion of the P1/P2 receptor nomenclature was the subject of debate with some key figures arguing for an alternative A (adenosine) and N (nucleotide) receptor system. This latter notion obviously never gained traction, in part because of the potential confusion with the receptor nomenclature for the nicotinic cholinergic receptor family. With additional research on P2 receptors in multiple laboratories using a variety of techniques, data emerged for the existence of P2X and P2Y families [15], the P2X which are ligand-gated ion channel receptors that form homo- and hetero-trimers (P2X1-7) and the P2Y that are G protein-coupled receptors and are currently eight in number ($P2Y_{1/2/4/6/11/12/13/14}$) [16]. Thus, within roughly two decades of the formal proposal of the concept of purinergic neurotransmission [2], three families of purinergic receptors had been identified that number nineteen in total [16].

As a result of Geoff's tenacity, his openness to collaborate and his willingness "to help everybody else coming into the field" [1], in time he was proved to be prescient with regard to both the purinergic hypothesis and the concept of co-transmission. His insights were based on a thorough knowledge of the literature, historical and emerging, an extensive investigator network through which Geoff was in constant contact with those conducting cutting edge research, his own research activities and, above all, a willingness to refine and change his personal views and hypotheses based on data. The latter contrasts with the

reductionists dogma that characterizes much of 21st century biomedical research relevant to which Geoff - as highlighted recently by both Verkhratsky et al. [4] and Jacobson et al. [17] – described as the rationale for his questioning of Dale's Hypothesis. This bears further dissemination here, to quote Geoff: "1 would like to remind the reader of the extraordinary influence of fashionable concepts in science. Gifted and meticulous workers will perform remarkable contortions to fit their data into accepted dogma, especially if established by powerful and brilliant personalities at the forefront of the field. They will often dismiss or ignore data that fall outside interpretation by current theory, searching hard for technical or artefactual explanations. Once a new attitude becomes acceptable, then the same data can be miraculously redeployed to support it" [6].

Noteworthy in regard to the concept of 'accepted dogma', another major insight into human disease pathophysiology that also originated in the Antipodes was the Nobel Prize work of Warren and Marshall [18,19]. This had shown that peptic ulcers can be caused by *H. pylori* infection of the stomach, a discovery that flew in the face of established medical dogma and revolutionized the treatment of peptic ulcers. A still emerging body of work from another Australian, the late Robert Moir has indicated a potential role for infectious agents in the pathophysiology of the neurodegenerative disease, Alzheimer's disease (AD) [20]. Like the purinergic hypothesis and peptic ulcer causation, Moir's antimicrobial protection hypothesis of AD, which is still in the process of being validated, goes against mainstream dogma, in this particular instance that AD is caused by the presence of toxic amyloid deposits in the brain [21]. Like Geoff, Moir had noted that "strong data with no obvious flaws are rejected out of hand because they do not fit current dogma and are dismissed for perfunctory reasons" [22]. These examples, proven and emerging, suggest that the Australian scientific community remains a fertile environment in which to productively explore ideas that lie outside the scientific mainstream. In this context, one may wonder that if Geoff had not made his fateful decision to move to Melbourne - rather than return to London - after his postdoctoral research, whether the weight of opposing opinion reflected in the conservative status quo of the English biomedical research establishment of the 60s and 70s might not have overwhelmed his spirit and dissuaded him in achieving his goals, an outcome that would have been a tragedy for the world of biomedical research. However, given Geoff's effervescence, optimism and innate resilience, personal attributes that were often reflected in his dealing with the arcane post WW II British class system where "the wrong background, the wrong accent, the wrong clothes" [23] still defined the identity, acceptance and perceived value of an individual [24], Geoff's capitulation while possible, would have been highly unlikely.

3. Personal reminiscences

3.1. FDV

My association with Geoff started in a rather odd fashion. I became aware of extracellular ATP and with ATP receptors long before I heard of a scientist named Geoff Burnstock or was aware of a "purinergic hypothesis". In 1982 I was at University College London (UCL) as a Honorary Research Assistant (basically as a Post Doc) in the laboratory of Bastien Gomperts, who was the first researcher to thoroughly describe ATP-mediated

permeabilization of the plasma membrane and hypothesize that this was a receptor-mediated event, the putative receptor for which he termed “the ATP^{4-} receptor”. Bastien thus made a fundamental contribution to understanding the signaling mediated by extracellular ATP. I was not directly involved in this work, but remember spending quite some time with Bastien trying to understand the physiological significance of such an odd phenomenon. I was new to this field, had very little knowledge of the literature, the expression “purinergic signaling” was never used during all our long discussions, and the name “Geoff Burnstock” was never mentioned.

In 1986 I moved to Columbia University in New York to work in the laboratory of Sam Silverstein. There I met the late Tom Steinberg who was investigating some peculiar effects of extracellular ATP on mouse macrophages. Knowing that I had spent time in Gomperts’ laboratory, Tom involved me in the analysis and possible interpretation of the strange responses triggered in macrophages by extracellular ATP (the now well-known phenomenon of plasma membrane permeabilization), and he first mentioned to me that his observations might be consistent with the general “purinergic signaling” theory put forward by Geoff Burnstock. I was very surprised that I had never heard of Burnstock before, especially since the Anatomy Department, at UCL where Geoff was Chair and the Experimental Pathology Department, where Bastien was Lecturer, were within a few hundred meters of each other. However, I finally became aware of the “purinergic hypothesis” and was able to appreciate the revolutionary nature of this concept.

I met Geoff Burnstock for the first time at the *5th International Symposium on Adenosine and Adenine Nucleotides* organized by Luiz Belardinelli and Amir Pelleg in Philadelphia in May of 1994. Geoff presented an appraisal of his early studies on the identification of ATP as the principal active substance responsible for nonadrenergic, noncholinergic (NANC) transmission, and elaborated on the newly proposed subdivision of P2 receptors into P2Y (slow) and P2X (fast) ATP receptors [25]. Since the Philadelphia Symposium I regularly met with Geoff at Purine Meetings around the world, most notably the Ciba (then Novartis) Foundation Meetings held in the enchanting location of Portland Place in London in 1995 and 2005, the remarkable German-Italian Joint Meetings started in the warm and hospitable setting of Chieti (in the once remote Abruzzi) by Peter Illes and Francesco Caciagli, and continuing until 2017 (Rome, see Fig. 1), and finally the celebrated Ferrara Meetings (in 1998, chaired by Pier Andrea Borea and the late Pier Giovanni Baraldi, and in 2006 again chaired by Pier Andrea, Pier Giovanni and by myself). Pedata has published a brief appraisal of the history of the Purine Meetings and National Purine Clubs [26].

When the journal *Purinergic Signalling* was started in 2004, Geoff asked me to join the Editorial Board to cover the topics of Immunology and Inflammation, and later genetics with a special emphasis on the P2X7 receptor, which had increasingly become my “pet receptor”. He was very keen that Editorial Board Members were not just colleagues dealing with a common scientific interest, but also friends who shared the same enthusiasm. Geoff was very aware of the importance of gathering together around a bowl of soup (and a glass of good wine) to strengthen common interests and friendship. At every international Purine Meeting therefore, Geoff organized a dinner for the Editorial Board Members that became the place to discuss recent developments in purinergic science, future directions in the

editorial policy of the journal, and also the personal issues that always enrich our scientific life. One thing that I always admired in Geoff was his informal attitude and his openness to listen and to talk to anyone who needed his help and advice. Young people were always welcomed by Geoff. Talking to him was like taking a ride on a “time-machine” to be taught by one of the world renown Masters as to how world first class science was in the early days, and use the lessons to guide research in the present and future. A special occasion for me was the award to Geoff of the Copernicus Gold Medal of the University of Ferrara in 2009 (Fig. 2). Nicolaus Copernicus became a *Juris Canonici Doctor* (Doctor in Canon Law) at the University of Ferrara (circa 1503) and the University awards the prestigious gold medal named after him to celebrate outstanding scientists. I believe that none of the Copernicus awardees interpreted the essence of Copernicus’ work better than Geoff. Both Copernicus and Geoff opened entirely new perspectives for human knowledge by “simply” stating something that should have been obvious to anyone, and is now indeed obvious, i.e., for Copernicus that the earth rotates around the sun, and for Geoff that ATP is a ubiquitous extracellular messenger. Both individuals were the subject of hostility and reprobation. It is also curious that both Copernicus and Geoff (who was a convinced atheist) received a degree in a religion-related topic: Copernicus a *Juris Canonici Doctor* at the University of Ferrara, and Geoff an undergraduate degree in theology at King’s College London.

Geoff never took established knowledge for granted: he always believed that challenging existing paradigms was at the heart of being a good scientist. I do not know if Geoff foresaw the impact that the purinergic hypothesis would have in fields as remote from neurotransmission as oncology and immunology, but I imagine that he did. This was the topic of many a discussion with him as he was very intrigued by the role of ATP as a signal of distress, and indeed had no reservations to accept the idea that ATP might be the prototypical danger signal (DAMP [27,28]), and in fact his deep understanding of biological evolution made it obvious to him that ATP had all the properties of an alarm factor. I deeply regret that Geoff was able to witness only the beginning of the impact that these concepts are now having in the wider fields of inflammation and oncology.

3.2. KAJ

Geoff was a scientific inspiration to me from our very first meeting. In August 1987, Geoff and I were both attendees at the Tenth International Union of Pharmacology (IUPHAR) Congress in Sydney, Australia and the IUPHAR satellite meeting *Physiology and Pharmacology of Adenosine and Adenine Nucleotides* in Auckland, New Zealand. The latter was my first opportunity to lecture on my early work on adenosine receptors at an international conference, with the encouragement of my NIH mentor in adenosine, the late John W. Daly. Geoff gave an opening plenary lecture in the Concert Hall of the Sydney Opera House on late Sunday afternoon, which was sparsely attended. I was enthralled with his description of adenosine signaling as only one component of a larger purinergic signaling system that included ATP and potentially other nucleotides, as later reviewed [29]. This is when I decided that I would like to work on P2 receptors as well as adenosine (P1) receptors, even though at that time Geoff was the sole champion of P2 receptors. Unlike P2 receptors, P1 receptor medicinal chemistry was already established scientifically with a number of international pharmaceutical development efforts [17,30]. Following Geoff’s

lecture, it appeared to me that it was important to broaden my own work to explore the entire purinergic signalome. Geoff evidently inspired many early career researchers to follow the same path to study P2 receptors.

When John Daly and I organized our own meeting on *Purines in Cellular Signaling: Targets for New Drugs* in Bethesda two years later, Geoff was presented with an award for his discovery of purinergic transmission [30], although the vast majority of the presentations at that meeting still neglected P2 receptors in favor of adenosine receptors. Even among purine receptorologists, there was still some doubt that ATP signaling was important; perhaps it was only a minor phenomenon or an artifact altogether [31] with many researchers assuming that the observed effects of ATP were, given its intrinsic lability, most likely mediated by adenosine. The tide did not fully turn toward P2 receptors until about a decade later, when at least half of the presentations at each of the then biannual international purine meetings involved P2 receptor signaling.

Establishing my own P2 research program to complement the adenosine work lagged by at least two years from the time I was sure that was where I wanted to head. At the time there were no other chemistry labs in the world reporting on novel P2 receptor ligands. A talented high school-then-college student intern in my lab, Jeffrey Zimmet (now Professor of Medicine at the University of California San Francisco), wanted to travel abroad, so I suggested that he continue working on our burgeoning P2 project, but remotely from the lab of Edith Heilbronn of Stockholm University, where he synthesized some ATP-2-alkylthio ethers as our earliest P2Y agonists [32]. At about the same time, we implemented a highly productive collaboration with pharmacologist T. Kendall Harden and his colleague, José L. Boyer at the University of North Carolina, Chapel Hill. In 1992, I recruited Bilha Fischer from the Bar-Ilan University, Ramat Gan in Israel as a postdoc in our lab at the NIH to work on synthesis of ATP derivatives. Trained as a synthetic organic chemist, the unconventional methods used to prepare nucleoside triphosphates in highly polar solvents and without protecting groups came as something of a shock to her. However, she became an expert in the art and eventually established her own successful research group in the P2 field at Bar-Ilan, where she heads the Department of Chemistry. The many derivatives Bilha and others in our lab prepared [33,34] were tested at P2Y receptors using PLC and other second messenger assays, and Geoff was very interested to examine many of the same compounds at P2X and P2Y receptors in his smooth muscle assays [33,34]. In the absence of P2 receptor radioligands, it was not feasible for our own lab to characterize the compounds pharmacologically. Philip van Galen (a former student of Ad IJzerman at Leiden University, The Netherlands) then a postdoc in our lab tried several nucleotide radioligands and struggled to find a source for turkey blood to obtain erythrocytes (a preferred system at that time for studying the $P2Y_1$ receptor), because it was soon after Thanksgiving, 1991 and the farms neighboring Bethesda were fresh out of turkeys. After considerable effort we gave up on the development of an erythrocyte ghost or brain membrane $P2Y_1$ binding assay, as the pharmacology did not fit the expected behavior of the receptors [35]. We then focused on the medicinal chemistry and were entirely dependent on collaborators to perform pharmacology on our synthetic products in the early years. The progression to P2 receptor antagonists was a result of Boyer's discovery in 1996 that adenosine bis-phosphates acted as partial agonists or antagonists of the $P2Y_1$ receptor [36]. Thus, it was the first clue that P2 agonists could be

modified to become antagonists, an approach we employed in the design of widely used bis-phosphate $P2Y_1$ antagonists, 2′-deoxynucleotide MRS2179 and a rigidified methanocarba nucleotide, MRS2500 [37]. We later extensively studied the structure activity relationships of non-nucleotide P2Y and P2X antagonists [17].

With the cloning of the chick $P2Y_1$ receptor in 1993 [38], the first sequence in the P2Y receptor family to be determined, we thought that molecular modeling might provide significant insights into the three-dimensional structure of the receptor. Geoff was quite enthusiastic about modeling the structure and the binding site of P2Y receptors, so he provided a stipend for Michiel Van Rhee (another IJzerman student) to begin work toward that goal, as a postdoc in our lab. If successful, this could eventually provide a major boost to the drug discovery process, via the rational design of ligands, but there were technical limitations in computational modeling approaches at the time. At the time, homology modeling was not yet well accepted for GPCRs, although it is now a common approach in receptor structural studies. Furthermore, there was no sequence-related structural template onto which to map the $P2Y_1$ receptor sequence. The high-resolution structure of bovine rhodopsin was still unknown, so we only had low-resolution rhodopsin as a template. Nevertheless, we published the first molecular models of a P2 receptor using this relatively crude approach [39,40], which was refined later in stages, including the first $P2Y_1$ receptor site-directed mutagenesis [40,41], leading up to our eventual determination, in collaboration with Ray Stevens (University of Southern California) and Beili Wu (Shanghai Inst. Materia Medica), of the X-ray structure of the human $P2Y_1$ receptor in complex with the subnanomolar $P2Y_1$ antagonist, MRS2500 [42].

Geoff remained a valued collaborator through the many stages of our work on P2 receptors, and even when we stopped actively collaborating, I relied heavily on his advice and insights. I looked forward to seeing him at the Purine Club meetings, and also visiting his lab frequently. I was hosted by Geoff and Nomi at their home in London on a few occasions. Geoff was always eager to help in the effort, for example in critiquing new research ideas, as he was the unequalled champion of the P2 field and wanted to see the fruits of his key hypothesis and associated discoveries spread to new domains and techniques. He was particularly keen on the idea that medicinal chemists might actually discover new drugs for his beloved receptors, as is being accomplished for P2X3, P2X4, P2X7 and other receptor antagonists [17,43].

Now, we usually prefer a structure-based approach [44] over the empirical trial and error approach to purinergic receptor ligand design, as envisioned in our early modeling efforts. Having introduced many of the widely used P2Y and adenosine receptor pharmacological probes, we are seeing the fruits of this labor in the translation of some of our ligands toward clinical studies for various chronic diseases [45-48]. The furthest advanced are A_3 receptor agonists that are in Phase II/III clinical trials from CanFite Biopharma for autoimmune inflammatory diseases (IB-MECA, piclodenoson) and hepatocellular carcinoma and nonalcoholic steatohepatitis (Cl-IB-MECA, namodenoson). $P2Y_1$ and $P2Y_{14}$ antagonists from our lab have shown promise, respectively, for the treatment of thrombosis and neurodegeneration [44,46], and for inflammatory conditions, asthma and chronic pain [47,48]. A_3 receptor agonists are a particularly promising approach for chronic pain

treatment that is nonaddictive and actually, when used in combination, counteracts many of the serious adverse effects of chronic opioids [49].

3.3. MW

I met Geoff Burnstock in June 1978 at the *1st International Symposium on Adenosine and Adenine Nucleotides* in Banff, Alberta. During the time I was working on my Ph.D. at the Institute of Psychiatry, University of London. I had studied the effects of adenosine on cAMP and protein phosphorylation in guinea pig brain slices [50], work that was based on the seminal findings in the early 1970s and 1980s on the methylxanthine-sensitive stimulation by adenosine of adenylyl cyclase in brain tissue by Sattin and Rall [51] and the excitotoxic release of adenosine by Pull and McIlwain [52]. I had met John Daly when he visited London where he had provided valuable critique and suggestions to my ongoing thesis work. After moving to the US where I did a postdoc at the University of North Carolina, I subsequently joined the pharmaceutical industry at the Merck Institute for Therapeutic Research outside of Philadelphia, PA to develop a radioligand-based screening lab to facilitate the characterization of newly synthesized compounds.

I remained in regular contact with John at the NIH and he encouraged me to attend the Banff meeting where I was impressed by the scope of the science, especially that related to the role of ATP as a neurotransmitter. Since my background in purine research had been confined to the biochemical studies already mentioned [51,52], I was unfamiliar with Geoff's more physiologically-oriented work on non-adrenergic, non-cholinergic neurotransmission (NANC) processes. While the purinergic hypothesis was still lacking a solid pharmacological basis and many of the tools required to firmly establish the concept, I became convinced that the work that Geoff presented at the meeting was intriguing and that his particular genius was to enthusiastically develop the big picture and then seek to fill in the details by pulling together early concepts to allow these to drive multiple efforts using multiple disciplinary approaches within the purinergic community to test emerging hypotheses. Geoff's remarkable, in-depth knowledge of the literature was a major strength in moving forward. This may seem somewhat quaint in the present era of instant PubMed and Google Scholar search engines where whole research areas can be explored in a matter of days, if not hours – at least at the abstract level, a practice that rapidly generates an ephemeral understanding but not the necessary context and considered insights necessary for a true grounding in a research area. Geoff however, frequently knew the experimental details of the papers he read - often by heart - and was well aware of the nuances (and shortcomings) of published work and the more obscure literature - and its relevance.

A less pleasant memory of the Banff meeting was a session where two individuals, both from the UK, surprisingly announced during the discussion following a roundtable presentation by Geoff their intention of disproving the 'so-called' purinergic nerve hypothesis, even though they had no data to present or cite, to refute or add to what Geoff had been talking about. Although I did not know it, this was not the first time that Geoff had experienced such opprobrium and in his audio interview [1], he quotes similar instances, one of which occurred at the Royal Society after his return to London where a discussant to a presentation from a member of Geoff's lab on the purinergic hypothesis unexpectedly

"showed four incomprehensible slides which, he said, destroyed the purinergic hypothesis. People looked at me – there were 15s left – and I said, 'I have never seen this work, it has not been published and I need to study it carefully and take it into consideration, but it doesn't look to me like a major thing'. But, for years after, people would come up to me and say, 'wasn't the purinergic hypothesis destroyed at that Royal Society meeting 10 years ago?' It was so painful and so unfair".

On returning from Banff, I researched Geoff's background and invited him to become a consultant for the Neuroscience group at Merck in 1979. Meanwhile, having maintained an interest in adenosine neurotransmission, I spent what spare time I had at Merck (mostly weekends) in developing a radioligand binding assay for adenosine receptors. I was aware from John Daly that he, together with Sol Snyder, was supervising Fred Bruns to develop an adenosine radioligand binding assay, for which the NIH/Hopkins team identified the secret ingredient. This was the inclusion of adenosine deaminase (ADA) to remove the copious amounts of adenosine that were released on tissue homogenization. The Bruns assay using [^{3}H] N^6-cyclohexyladenosine and [^{3}H]1,3-diethyl-8-phenylxanthine as radioligands [53] and the Merck assay using [^{3}H]2-chloroadenosine [54] were published in *PNAS* within a month or so after one another towards the end of 1980.

From 1979 until 2000 when I left Abbott Laboratories, Geoff was a consultant for all the companies that I worked for and along with developing several important tool compounds (target selective entities that lacked drug-like properties), we were focused, like many other pharmaceutical companies at that time, on advancing purine-targeted compounds to clinical development status. This was a far from easy task as Geoff had repeatedly noted [55] due to the ubiquity and importance of P1 receptor signaling, a point reinforced a decade later by Mike Jarvis [56]. As Geoff continued to add to the evidence for the scope of purinergic signaling and its role in human disease pathophysiology, specifically that involving the growing families of P2 receptors, at the same time he provided valuable insights into the ongoing P1-based drug discovery efforts which were deemed more chemically tractable than those involving P2 receptor targets. At Merck, such efforts were focused on defining the potential role for adenosine as a novel approach to Parkinson's disease, depression and anxiety. At Nova Pharmaceuticals, the biotech company founded in 1983 on the receptor binding techniques emerging from Sol Snyder's lab at Johns Hopkins Medical School in Baltimore, MD, we expanded our work on adenosine receptors to understand species differences in P1 receptor pharmacology and to support medicinal chemistry activities on developing novel P1 receptor selective agonists and antagonists. The latter became part of Nova's first research collaboration with Marion Laboratories in 1984 that involved the "development and subsequent marketing of new pharmaceutical compounds to treat cardiovascular, respiratory and other diseases." [57]. Subsequently at CIBA-Geigy in New Jersey (now Novartis), our research focus was again on P1 receptor agonists and antagonists, building on work that had been initiated by John Francis and Geetha Ghai with the synthesis and characterization of the novel, non-xanthine adenosine antagonist, CGS 15943 [58]. This compound was targeted as an anti-asthmatic drug candidate but had formulation issues that precluded its first in human evaluation. Further research with a team that now included Mike Jarvis and Al Hutchison, led to the design and characterization of CGS 21680, a potent A_{2A} receptor agonist [59] that was an early stage clinical candidate as a novel antihypertensive.

For strategic reasons, development of this compound was discontinued and CGS 21680 became a useful research tool and the first selective radioligand for A_{2A} receptors [60]. Later at Abbott Laboratories in Abbott Park. IL, our initial efforts in the purinergic area were focused on developing site and event specific adenosine kinase (AK) inhibitors [61] that, due to their ability to prevent extracellular adenosine breakdown at sites involved in pain related events, increased local levels of the endogenous purine and acted as analgesics [56,62]. This effort culminated in ABT 702, a novel, non-nucleoside AK inhibitor [63,64], that was a potent, long lasting analgesic that was approved for clinical trials in early 1999. Almost immediately, the compound ran into side effect issues that were identified as target class related and led to the cancellation of the planned first in human studies. These events have been documented in detail by Jarvis [56].

Based on advances that the Abbott group had made in the practical use of ion channel technologies, e.g., the POET patch clamp automation system [65], in a parallel drug discovery program focused on nicotinic receptor agonists for the treatment of Alzheimer's disease, cognitive and attentional disorders, and pain [66], the purinergic research focus at Abbott shifted to the design, synthesis and exploration of P2X3, P2X2/3 and P2X7 receptor antagonists for pain [67]. These efforts yielded A-317491 (P2X3/P2X2/3 antagonist [68]) and A-740003 (P2X7 antagonist [69]). A-317491 was not orally bioavailable with the structure activity properties of the parent chemotype not being amenable to improvement in this key pharmacokinetic property. For A-740003, the predominately glial localization of P2X7 receptors and a mechanism of action that was indirectly related to the P2X7 target led to the discontinuation of this lead candidate.

On leaving Abbott at the end of 2000, my research interests and activities necessarily changed and while I was able to attend a few purine focused meetings, my ability to actively participate in purinergic-based drug discovery was severely curtailed. Nonetheless, the major drug discovery efforts in cancer at Cephalon which I had joined in 2003 were focused on protein kinase inhibitors that shared the common element of ATP with P2 receptors. Given that our medicinal chemistry efforts at Cephalon were focused on designing compounds that interfered with ATP binding to the kinase catalytic site, we wondered if we might inadvertently have also been making P2 receptor modulators given the ATP motif in both drug targets. Given that we had no ready access to P2 receptor screens we had our research partner, Ambit Biosciences, run a small panel of published P2 receptor ligands selected with input from Ken Jacobson through the 400 or so member panel of human kinome screens that were physically available at that time (circa 2005). To our surprise, the P2 ligands showed minimal ability to interact with the kinase targets. Regrettably, only limited data were generated that were insufficient to be able to publish these findings. Incidentally, I chaired a session on drug discovery at the human kinome at the last purine meeting I attended in Ferrara in 2006. This was the last time that I saw Geoff.

One final point is that, in addition to his consulting activities with major pharmaceutical companies, Geoff was never averse to listening to new ideas and issues on purinergics and providing his insights and help to biotech startup companies. In doing so, Geoff often indirectly facilitated their discussions with major pharmaceutical companies that in turn provided insights into the drug discovery process and collaborations that these companies

might never have had access to. Long after leaving the field, I obliquely ran into Geoff as I did due diligence assessments on purine-based biotechs that Geoff had consulted for. Revisiting my involvement with him has brought forth many fond memories not the least of which were the dinners hosted by Maria Pia Abbrachio and Flaminio Cattabeni at the restaurant, Hot Meeting Pizza, close to the main campus of the University of Milan where he introduced newcomers to the purinergic field to their 'fabulous' beef carpaccio with rocket and parmesan.

4. Purinergic drug discovery

Reading the purinergic literature over the past 35 years, the reader will be immediately impressed, if not overwhelmed, by the potential breadth of human disease states that have been identified as being amenable to treatment with compounds that can interact with P1 and P2 receptors. The reality, as in most areas of drug discovery where 90% of compounds advanced to clinical trials still fail despite best efforts at de-risking [70,71], was less impressive with very few drug candidates being clinically approved. In the P1 area, this has been due to the ubiquity of P1 receptors [55,56,72] that, coupled with their important role in tissue homeostasis, has been plagued with side effects [73].

Three P1 receptor ligands have received regulatory approval only two of which were out of the many thousands synthesized. Adenosine itself, as Adenocard™ was approved for use in the treatment of paroxysmal supraventricular tachycardia (PSVT) [74] and as Adenoscan™, an adjunct to diagnostic cardiac imaging for coronary artery disease [75]. Regadenoson (CVT-3146), a selective A_{2A} receptor agonist that was conceptualized by Luiz Belardinelli in collaboration with CV Therapeutics [76] was also approved for use in diagnostic cardiac imaging [76,77]. It is a more potent vasodilator than adenosine and is selective for the coronary versus the renal, peripheral and mesenteric circulations [78]. Its P1 receptor selectivity versus adenosine also has the potential to reduce the negative chronotropic, dromotropic and inotropic effects associated with A_1 receptor activation and the bronchospasm and mast cell degranulation associated with A_3 receptor activation. Of note is the fact that the A_3-induced mast cell degranulation that occurs in rodents is absent in higher species including human [17]. However, in real world clinical use adenosine had a lower occurrence of adverse effects and a lower rate of a rescue agent use than regadenoson, a finding that has been attributed to differences in the pharmacokinetic properties of the two P1 agonists, adenosine having a short half-life of 30–40 secs while regadenoson has a triphasic half-life that can extend to 15–30 min [79].

The A_{2A} receptor antagonist, istradefylline (KW 6002), was another successful P1 compound that had been championed by Fumio Suzuki at Kyowa Hakko. It was able to reverse motor disability in MPTP-treated marmosets without producing dyskinesia [80] and was approved in Japan in 2013 and the US in 2019 to treat L-dopa-associated off periods in Parkinsonian patients [81].

For P2 receptors, two anti-platelet drugs, that are both irreversible $P2Y_{12}$ receptor antagonist prodrugs, clopidogrel (approved in 1998) and a competitor compound, prasugrel (approved in 2009) that is more potent than clopidogrel with a faster onset of action and lower

interindividual variability in platelet response [82], are used, together with aspirin, in the treatment of acute coronary syndromes to prevent thrombus formation. An earlier $P2Y_{12}$ receptor antagonist prodrug, ticlopidine, that was introduced as an antiplatelet drug in 1978, is rarely used due serious side effects including agranulocytosis, thrombotic thrombocytopenic purpura and aplastic anemia. Of relevant interest to the topic of purine-based drug discovery is the fact that this class of thienyl $P2Y_{12}$ antagonist prodrug was initially approved for human use [83] long before their target, the $P2Y_{12}$ receptor, was identified in 2001 [84]. Their therapeutic potential thus preceded by several decades the serendipitous discovery of its target such that its contribution to the roster of successes in purinergic drug discovery is more honorary than real. In the first half of 2012 clopidogrel was reportedly the top selling drug in terms of sales volume in the US, a success that was extremely short lived given the arrival of generic versions of the drug later that year. Nonetheless, given the size of the anti-platelet drug market which was substantial, the knowledge of the therapeutic target for clopidogrel facilitated other drug discovery efforts in the area. This led to the development and approval of ticagrelor a reversible, direct acting, allosteric $P2Y_{12}$ receptor antagonist that is reportedly more efficacious than clopidogrel with a faster, more consistent $P2Y_{12}$ inhibitory effect than clopidogrel [85].

One of the more exciting fields of purinergic drug development is cancer [86,87]. It is now clear that a major avenue for tumor escape from immune-mediated killing is the generation of an immunosuppressive micro-environment. Multiple factors that impair the immune response accumulate in this protected extracellular space, among which is adenosine. Adenosine potently inhibits T- and NK-lymphocyte responses acting via A_{2A} receptors producing marked immunosuppression in the tumor microenvironment (TME) [88,89]. These observations paved the way to the development of A_{2A} antagonists for the treatment of cancer, either as mono- or combination-therapy. Five A_{2A} antagonists are currently in clinical trials, with an acceptable toxicity profile and encouraging preliminary results [87]. The realization that ATP is a fundamental biochemical constituent of the extracellular milieu has further implications in cancer therapy. ATP concentrations in the tumor microenvironment can be 3–4-fold higher than in the healthy tissue interstitium [86,90] forming the basis for the development of novel anticancer drugs designed to be active only in the presence of near-millimolar ATP concentrations, and therefore highly tumor selective [91]. Further details on the potential for purinergics in cancer treatment can be found elsewhere in this special issue *of Biochemical Pharmacology*.

While many other P1 and P2 ligands and modulators of purine metabolism have shown evidence of therapeutic potential in animal models of a variety of human disease states, these have had a poor record of translation with the majority having failed their clinical evaluation for a variety of reasons. Nonetheless, this has not diminished the enthusiasm for their development [92,93]. Many of the more advanced of these lead compounds or candidate drugs have been discussed above or are the subject of other articles in this special issue *of Biochemical Pharmacology*. These include purinergic based therapeutics for diabetes, various CNS conditions including hearing loss and pain, disorders involving inflammation and immunity, joint function, bone homeostasis, COPD (chronic obstructive pulmonary disease) and host defense. The purinergic compound closest to regulatory approval is the P2X3 antagonist, gefapixant which is in Phase III for chronic cough [43]. This compound

and other P2X3 antagonists are also in clinical evaluation for their potential use in endometriosis-associated pain [94].

5. The accidental pharmacologist

The accolades and honors attesting to Geoff Burnstock's contributions to biomedical science are myriad [10] and were reflected in his occupying for the decade 1994-2004 the top position in the scientific world for citations in pharmacology and toxicology [8]. While zoologist, physiologist, anatomist, neurobiologist and electrophysiologist are immediate descriptors of Geoff's scientific skill sets, some have been puzzled how this would lead to him being the top pharmacologist/toxicologist *in the world*, especially since ASPET (American Society for Pharmacology and Experimental Therapeutics) defines the discipline thus - "Pharmacology is the science of how drugs act on biological systems and how the body responds to the drug. The study of pharmacology encompasses the sources, chemical properties, biological effects, and therapeutic uses of drugs" [95].

From the outset, Geoff's primary research interests were in the discovery and definition of biological systems and their properties - how they interacted and their role in development and disease etiology with drugs – the primary output of pharmacology – being an accidental, albeit inevitable and welcome, product of this focus. This has led to Geoff being described as an 'accidental pharmacologist' [23], an apt reflection of his 'big picture' approach to research which parallels the similar 'big picture' aspects of pharmacology - an integrative, hierarchically structured research framework that uses cells, tissues, whole animal, human models and a multipicity of enabling technologies to provide context and relevance to understanding drug actions and support the drug discovery process [96].

Geoff's approach to research rarely concerned itself with the details of the core concepts of pharmacology - the Law of Mass Action, receptor occupancy, receptor reserve and receptor theory, etc. [97-99], but nonetheless he routinely tapped into the integrative nature of the discipline – the use of any and all discipline-based technologies to answer key questions related to a biological phenomenon – itself a contrast to positing and answering reductionistic questions based on the capabilities of a single discipline-based technology. As a result, Geoff enthusiastically engaged the skills and interest of medicinal chemists, molecular biologists, *in vitro* and *in vivo* pharmacologists – if not their passion - in his quest to unequivocally delineate the purinergic hypothesis. His willingness to talk to anyone – already alluded to – irrespective of their position in the scientific hierarchy of a laboratory made many graduate students, technicians and junior staff look forward to Geoff's visits further involving them in the research process and its outcomes. Geoff's infectious enthusiasm made research fun.

In closing this tribute, the authors would like to acknowledge Geoff's autobiography "Against The Odds" (Fig. 3) that was self-published with support from the Physiological Society, in 2018. In it, Geoff provided a lively, informative, and often amusing (if not always PC) account of his life in science and as innovator, facilitator, motivator, raconteur and bon vivant. While this monograph is unfortunately unavailable for purchase with only a few fortunate friends and colleagues receiving a personal copy from Geoff, the reader is

encouraged to borrow a copy. They will be rewarded by copious and often whimsical insights on an era in biomedical research that is fast disappearing and to a life that was lived to its fullest. To have known and worked with Geoff was a privilege.

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Fig. 1.

Geoff together with Felicita Pedata (back left), Christa Muller (next to him on the left), Francesco Di Virgilio (far right) and (from the left) Alba Clara Sarti, Anna Lisa Giuliani, Elena Adinolfi, Elena De Marchi, Anna Pegoraro, Valentina Vultaggio-Poma.

Fig. 2.

Award of the Copernicus Gold Medal and Diploma d' Onore of the University of Ferrara. Ceremony held in Ferrara on June 4th 2009. Geoff is receiving the "Diploma d'Onore" from the Rector of the University of Ferrara Prof. Patrizio Bianchi. Francesco Di Virgilio on the right.

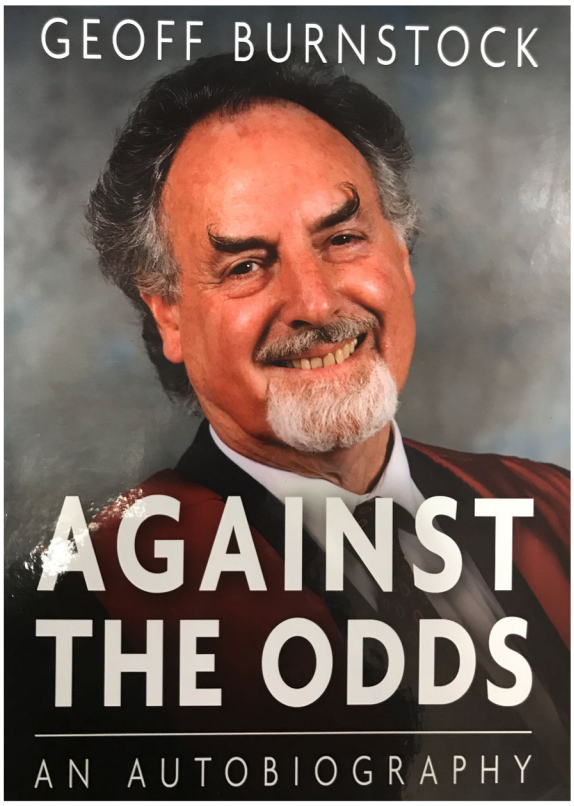

Fig. 3.
Cover of Geoff's self-published autobiography.