TODAY'S NEUROSCIENCE, TOMORROW'S HISTORY

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Interviewed by Richard Thomas

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Interview transcript

School and university years

I think I was born in Portobello Road in London, but we then moved to Ealing, Greenford, and went to Greenford County Grammar School. Iit was a new school, during the war. It was okay. I was a good and a bad boy there, for several reasons. I was very keen on table tennis, and before long I had the whole school playing table tennis. The teachers against the students, the students against each other. The whole school was playing table tennis, and the headmaster had to call me in and say, 'Look, you've developed table tennis here but this school is about scholarship and I'm going to stop it.' I think I have some quality that gets people involved and excited in things, and maybe that is one of the things that has carried on through my scientific career.

I had to look after my old mother. I didn't have a grant. I worked in a graveyard every weekend digging graves, weeding and so forth, and I had to get in somewhere. I couldn't do what youngsters do these days and say, 'I'll take a year off and travel or something.' I had to do it, and the only place that would take me was King's (College, London), who allowed me to do this degree in Theology - although I was an atheist - and a degree in Pure Maths and Physics, I think, simultaneously. It was a strange business. People did want to take me on for a PhD. I think I showed imagination at these things - experimental examinations - that was my strength. Not my memory but the invention of inventing experiments to do. I ended up, by serendipity, working on gut motility, and I did some curious things on this with no serious supervision. I looked at the pharmacology of gut and then take segments out, and I thought, 'I ought to look at it *in vivo*.' So I chose fish because pike eat once every two months and goldfish eat all the time, and I put a window in them. My first paper, in 1957, was in *Nature* with a condom on a fish so that you could see the movements of the gut *in vivo*. Imaginative

but a bit ridiculous. In fact, when I finished my PhD thesis, it was absurd. I was the world expert on defecation in the brown trout. Not exactly a highly competitive area.

Developing the sucrose gap technique for smooth muscle

Since I was working on fish gut, which was an interest of J Z Young, I went to visit him at University College, and he might have spotted some imagination in me although the experiments were very diverse and some of them were crazy. But he took me on and finished being my supervisor for a PhD. And at that stage I decided that I really had to learn electrophysiology, and I visited Feldberg, who's another imaginative person that didn't mind oddballs. He even set up fish tanks for me at Mill Hill, in the Physiology Department, but there I got together with Ralph Straub and we - who had worked with Stämpfli, and developed the sucrose gap technique, and we developed it – amazingly – for smooth muscle. It only works because there are gap junctions, low resistance pathways, between smooth muscle cells, and if you don't have that it wouldn't work - but it did work, brilliantly. And so when Edith Bulbring in Oxford, who was running the major smooth muscle lab at that time, saw the results with sucrose gap, she asked if I would join her in Oxford because microelectrodes had very limited success with spontaneous smooth muscle. They kept breaking the tips and so forth. And so I went to Oxford then – Pharmacology - and built the sucrose gap apparatus for smooth muscle in my first six months there.

The fact was that the people I liked most in Oxford were Australians - Molly Holman, Mike Rand, were great - and I decided … I'd married by then a New Zealander, so I decided to try for a job in Melbourne, and got a senior lectureship in Zoology. And within four or five years I became head of department. I think I was thirty-three or something.

Discovering the NANC transmitter, 1962

So perhaps that set the stage of my life's work on the autonomic nervous system, mostly. The autonomic nervous (system), of course, is that part of the nervous system which controls all the visceral organs – the gut, the blood vessels, the heart and so on, the uterus - automatically. And the autonomic nervous system has three components to it. It has sympathetic nervous system, it has parasympathetic nerves, and it has enteric nerves – nerves in the gut. Most people are surprised to hear that there are hundreds of millions of neurones in the gut. More, in fact, than are found in the spinal cord - as well as glial cells, enteric glial cells, which are very similar to the astrocytes found in the brain.

So, when I was in Oxford, the preparation that was developed by Edith Bulbring - the taenia coli - is a strip of smooth muscle on the caecum actually, but it's enervated - has nerves

supplying it. And these are nerves which are a mixture of sympathetic nerves, probably some parasympathetic, and certainly enteric neurones, extensions of those neurones. That's developed from different neural crest tissue, from sympathetic and parasympathetic nerves. Completely separate system. So, all these nerves are in the preparation, so when you stimulate it transmurally or electrically, you're stimulating all the nerves. And the sucrose gap worked very well for the taenia coli - the one I'd developed – and so, when I got to Australia and got grants, and the sucrose gap set up, I had two young men working with me - Max Bennett, who was a part-time technician finishing an engineering degree, and Graham Campbell, who was doing a Masters degree with me.

Then, I think it was just before Christmas in 1962, my young colleagues and I decided, well, we'd knock out the classical transmitters, acetylcholine and noradrenaline, with drugs - atropine and gaunethidine - and probably stimulate the muscle directly. And we expected the preparation to depolarise the smooth muscle and to be followed by contraction. What we saw, to our amazement - and we all knew that something important had happened - but to our astonishment what we saw, in response to single stimuli, were hyperpolarising responses. And when you stop stimulating - this is at 1Hertz – then you get a contraction - a spike followed by a contraction. This was very puzzling. What on earth could this mean? How could this be due to direct stimulation of the muscle? This was debated in Oxford and other places, and nobody quite understood it. But I was lucky that I had a post doc from Japan who had a friend in Japan who'd just published a paper on the discovery of tetrodotoxin from the puffer fish. And tetrodotoxin is a marvellous drug because it blocks nerve conduction but doesn't affect smooth muscle activity, and tetrodotoxin completely blocked these hyper polarisations, so we knew they were inhibitory junction potentials in response to a non-adrenergic, non-cholinergic - later called NANC - transmitter. But it was serendipity in a way. I mean, it wasn't a hypothesis at the time at all, but we were quick to know that there was something important there. I think that's a key. It's the anomalies in science which are the exciting things. Its not the … what you predict.

Discovering that ATP (or related nucleotide) is the potential NANC transmitter, 1970

Having established NANC nerves in a number of preparations including the gut, which was our main test organ, the next obvious question was, 'What is the transmitter in the NANC nerves?' And we read Eccles, and other people - how to prove that something is a transmitter - and it needed five major criteria about synthesis and storage, showing that there was release - that you could collect the transmitter when it came out, showing that there was some inactivation system, and most importantly, showing that the exogenously applied substance exactly mimicked the neurogenic response. And finally, if you could find drugs that

produce parallel block of both the exogenously applied substance and the neurogenic response. And we started looking at everything we could think of. We looked at amino acids of various kinds - glutamate and GABA. We looked at various monoamines. We looked at all kinds of things. Many peptides by then were getting popular. We looked at those. None of them satisfied those criteria at all, and in my reading, I came across several papers that made me look at ATP. The first one was a paper, a classic paper by Drury and Szent-Gyorgy in 1929, who showed the purines were effective extracellularly when applied to heart and to blood vessels. And then there were papers by Feldberg, who showed that the ganglia were sensitive to extracellular ATP. And then there was this very important … two papers by Pamela Holden in the fifties, who showed that during antidromic stimulation of sensory nerves supplying the rabbit ear artery, there was release of ATP in sufficient amounts to change the tone of the blood vessel in the ear. All these things together, I thought, 'Well, why don't I try ATP? Seems unlikely.' But ATP really satisfied the criteria quite remarkably well.

So here's the NANC response in the taenia coli - rapid relaxation followed by rebound contraction. ATP mimicked it beautifully, and by then we knew that there was a NANC excitatory response to the bladder, and again, ATP mimicked it better than anything else because while you stimulate, it's still coming back. And ATP had the same time course. It turned out that there is a dye, a fluorescent dye, called quinacrine which selectively labels high levels of ATP bound to peptides in large granular vesicles, and subpopulations of neurones in the myenteric plexus of the gut. And in the bladder - intrinsic neurones in the bladder shine up showing high levels of ATP. So we published the paper in 1970 in *British Journal of Pharmacology*, describing ATP or related nucleotide, as the potential transmitter in NANC nerves. I'm very glad that we left it open that there might be other nucleotides, because in recent years, when the P2Y receptors were cloned - these are G protein coupled receptors - some of them, which we'll come onto later, I'm sure - P2Y2 and 4 - are sensitive to pyrimidines. UTP and ATP are equipotent on P2Y2 and 4, and P2Y6 is selectively hit by UDP. So there are other nucleotides involved.

The ATP transmission hypothesis

So in 1972 they let me publish a big review in *Pharmacological Reviews* called 'Purinergic Nerves' in which I really collected together every scrap of evidence in every system I could find for NANC nerves and for the possibility that ATP was the transmitter in these nerves. But unfortunately, hardly anybody believed it. There was a huge resistance for more than twenty years, following this paper, and I think that I can understand it, in a way, because really, ATP belonged to the biochemists. It was an intracellular energy molecule involved in the Krebs Cycle and many other metabolic processes. Why should such a ubiquitous molecule be an

extracellular signalling molecule? I think that about eighty per cent of the opponents - maybe sixty per cent - it was just an emotional rejection. It didn't seem possible. They never bothered to read the papers even. It just seemed so unlikely. Another component were very objective. They accepted … they said, 'We don't know whether it's right or not yet. We don't think there's enough evidence to support such a major idea, and we're going to sit on the fence until we're convinced.'

Von Euler had a Nobel Prize for discovering noradrenaline and gave me marvellous advice. He said, 'Geoff, I don't know whether the hypothesis is right or wrong, but negative people don't stay around for long, so don't worry about that. You must be objective. When there's new data, look at it carefully. See if it fits your hypothesis. See if it can fit another hypothesis. And if your hypothesis is wrong, *you* should be the first to say." So what this hypothesis said, that ATP is stored in vesicles which migrate to the surface during an action potential, and release the ATP by exocytosis, which then passes the synaptic cleft to act post-junctionally on receptors that we know a lot more about now. And then the ATP is broken down by exoenzymes, by ATP-ases, first to ADP, then to AMP, and then to adenosine. And adenosine is taken up by a high affinity uptake pump and reconstituted into ATP cells for further use.

Discovering co-transmission of ATP, 1976

When I went to California on sabbatical leave to work with Che Su and John Bevan in Pharmacology at UCLA, what we did was to set up the taenia coli and look at ATP release using tritiated ATP. But what we did is we had a dual control preparation. We had sympathetic nerves supplying it from the outside - peri-arterial nerves - as well as transmural stimulation of the NANC nerves, and to our astonishment, we got ATP released from sympathetic nerve stimulation as well. And it wasn't supposed to be there! *The Purinergic Nerves* was the name of the review in '72. We thought there were adrenergic, cholinergic and purinergic nerves, but suddenly ATP is coming out from sympathetic nerves. What's this about? I thought this was a distraction of my hypothesis. If it's coming out from sympathetic nerves, I mean, what the hell's going on? But as I say, I wrote all night discrediting my hypothesis, but when the sun rose in the morning, I suddenly thought, 'Could it be that ATP is a co-transmitter with noradrenaline in sympathetic nerves?' And then I started work on parasympathetic nerves and other nerves - enteric nerves - and later on, people worked on the brain. And it turns out that ATP is a co-transmitter with every known nerve in both the periphery and the central nervous system.

It is a co-transmitter, but it's not an accompanying 'something'. You know, they treat it not as a transmitter. 'Oh, maybe ATP comes out with it,' like it's not a transmitter. Sometimes people used to call it a neuromodulator, which was nonsense. In other words, a neuromodulator in their definition was something that wasn't a proven transmitter, whereas a neuromodulator is quite precise - it's something that acts prejunctionally to change the release of transmitter, or postjunctionally to alter the action of the transmitter. That's a neuromodulator, not a wishy washy 'maybe' transmitter. ATP is a transmitter now. When it's combined with other transmitters, that's fine. And that together with a lot of hints in the literature, and some new experiments, made me come up with this commentary in *Neuroscience* in '76 which spelled out formally the concept of co-transmission. I think the title was something like, 'Do some nerve cells release more than one transmitter?' Well fortunately for me, it turns out that there's no known nerve in either the peripheral or central nervous system that doesn't use co-transmitters. In fact, it's the rule rather than the exception, and ATP in particular is present in *every* one of those nerves as a co-transmitter. Sometimes a major co-transmitter, sometimes a very minor one, *except* in pathological conditions where sometimes it becomes much more dominant.

Co-transmission in sympathetic and parasympathetic nerves

If we start, for instance, with sympathetic co-transmission, here we see both ATP and noradrenaline coming up, but ATP acts on - as we'll see later - ion channel receptors, to give excitatory junction potentials and a fast contraction, whereas the noradrenaline works through alpha-1 receptors - G protein coupled receptors - which lead to a slow contraction. NPY, which is part of the chemical coding, as it's called, in co-transmission in sympathetic nerves contained in large granular vesicles, doesn't act mostly as a genuine co-transmitter, but rather either as a prejunctional or postjunctional neuromodulator.

For parasympathetic nerves, we know that acetylcholine and ATP in the urinary bladder are co-transmitters. In fact, in animal models - in all the experimental models used in the labs - about fifty to sixty per cent of the parasympathetic response - contractile response - of the bladder is purinergic. The rest is cholinergic mediated by muscarinic receptors, and then there are sensory motor nerves, which have turned out to be extremely important. These are the nerves involved in the axon reflex which was identified in the thirties. Here, what happens is that the sensory collaterals release calcitonin gene-related peptides - Substance P - and in many of them ATP as well, to cause vasodilatation of skin vessels but also of all the vessels in the body, and often in the skin, the flare reaction that follows.

Co-transmission in the NANC nerves

And then if we come back to the gut, we now know that the NANC inhibitory nerves in the gut utilise three transmitters in very different proportions and different regions of the gut in different species. ATP gives the fastest response, nitric oxide the second fastest - and in some a tonic, slow response due to vasoactive intestinal polypeptide. And some sphincters for instance, it's largely nitric oxide; others it's largely VIP; and in very big regions of the colon and the small intestine, ATP is the principle transmitter. But the exciting thing, of course, is in the last few years the central nervous system people have caught up with this. It took them a while to accept co-transmission but now it is quite clear, and there are papers showing acetylcholine, glutamate, GABA, dopamine, 5HT, which are co-transmitters with ATP in different neurones in the brain.

Transmitter synergism

When I go to neuroscience meetings, the *huge* emphasis is on the central nervous system. There really is limited interest in the peripheral nervous system although it sometimes surprises CNS neuroscientists to hear that there are more neurones in the gut - in the intrinsic plexuses in the gut - than there are in the spinal cord. I mean, it's a massive nervous system and it shouldn't be neglected. The way co-transmission was proved in the gut and in the periphery was often with very elegant, surgical procedures where you isolated particular pathways. This is, of course, much more difficult in the brain. To do the experiments that proved co-transmission wasn't nearly so easy, although, in my opinion, there were clear hints because if ATP is coming out, let's say, with glutamate in the hippocampus, and it breaks down to adenosine - and it was well known that adenosine was a prejunctional modulator - where did it come from? It could have easily come as a co-transmitter, and I always try to persuade the hippocampal people to do another experiment because when you get co-transmission, you often get synergism between the two major neurotransmitters - co-transmitters.

Well, synergistic means that when two agents are applied, that they don't just add to each other - it's not an equal response which is added when you put the two together. You get an enhanced response, and that means synergism is when one of the agents enhances the response of the other by a mechanism, again, which is not terribly clear yet, although there are some papers about this. We've certainly shown synergism in the vas deferens between noradrenaline and ATP. A tiny amount of noradrenaline enormously potentiates the response of ATP on the smooth muscle of the vas deferens.

The Chair of Anatomy at UCL, 1975 – the secret of a successful department

Maybe when people are in their mid-forties, this is the time when they're head hunted. A number of jobs came up all over the place - in North America, in Australia, and in England - but this one (at UCL) appealed to me. I think it was … I much admired J Z Young, University College is a great place, and I felt it would be a wonderful challenge, and I thought about it a lot. In fact, I brought my family over for three months and said we would take the vote at the end of three months as to whether we would stay or not.

I believe that a head of department should not only give administrative leadership, but academic leadership. He must be passionate himself for science, because if he doesn't do it, he doesn't. He starts doing the logical thing, and not looking at irrational, awkward characters who are brilliant scientists and, just like artists, want to get on with it. And I never wanted to lose sight of that, and I wanted *my* passion and enthusiasm to rub off on everybody, so I wasn't just an administrator, and I think that's a terrible mistake when departments are run by administrators. I used to tell my staff, 'Anything short of anarchy is okay with me. If you disturb other people, I'll stop you, but you work like artists, the way you want to work.' And that kind of freedom was very attractive to a lot of the great scientists we had in the department, and I was pleased about that. And we were of course, very multidisciplinary. You wouldn't know what department it was. It had molecular biology, biochemistry, everything.

Discovering receptor antagonists – theophylline

There are many different receptor subtypes, and if you want to find out which physiological response is mediated by what receptor, if you don't have the tools to do it, which are selective agonists and antagonists, you can't find it out. So its absolutely crucial because, in the long run, in terms of therapeutic developments, we must know what selective receptors are on what selective cells if we're going to attack it, and we have to know where they are.

In the beginning there were no clear-cut antagonists for anything - for ATP or adenosine - and it's implicit, if ATP is a signalling molecule, acting extracellularly, there must be receptors for it. So I started thinking about this and reading the literature, which I always do, and again, I found hints and ideas, and then did some experiments myself, and in 1978 - this is pretty early on - I realised that a lot of the ambiguous results that were in the literature - ATP and adenosine doing different things, and different time courses - very weird. I realised that if ATP is broken down rapidly to adenosine, there are, in fact, two families of receptors - one for ATP and ADP, which I call P2 receptors - and one for adenosine, the breakdown product,

which I call P1. Now, it turned out that the P1 receptors - the adenosine receptors - were beautifully antagonised by theophylline.

Discovering receptor antagonists – how caffeine works

Caffeine was known to block the adenosine effects for quite a long time. Before, in fact, I came up with this sub-classification in ’78, and it’s very interesting about caffeine because - I won’t go into the explanations about how it works on the brain - coffee makes people excited. It simply blocks the prejunctional modulators for adenosine on excitatory nerves. It blocks that, and then more excitatory transmitter comes out. So that’s how caffeine works.

Adenosine receptors prove therapeutically disappointing

Bertil Fredholm, who’s made really important contributions in the purinergic signalling field, but his main interest has always been adenosine, so we run parallel paths. It’s not that there’s a huge conflict, and personally, I think it’s a big mistake to separate discussion of the actions of adenosine with that of ATP, because wherever ATP is released, it does rapidly break down to adenosine, and both receptors are usually involved. In the case of transmission postsynaptically, it’s the ATP receptor. Presynaptically, as a neuromodulator, it’s the P1 receptor - the adenosine receptor. And sometimes, adenosine and ATP, like in the taenia coli, act synergistically. The breakdown product, in fact, enhances the effect of ATP. You can’t really divorce these two signalling molecules in my opinion, but there has been, fortunately now, in the latest purine meetings, which are held every two years - in the beginning it was nearly all ninety-five per cent adenosine, then it switched in the nineties to more like eighty per cent ATP, and now the two tend to be treated together at these meetings, which pleases me a great deal.

Although I, myself, have not worked nearly so much on adenosine receptors as ATP receptors, they are undoubtedly extremely important. In the beginning, the drug companies were much more interested in the adenosine P1 receptors than ATP. They found, the chemists found developing antagonists for P2 receptors daunting, and adenosine was a G protein coupled receptor, the kind of thing which they were more familiar with. And they did develop very good agonists and antagonists, but on the whole, the therapeutic developments with the adenosine receptors have not been that successful because they’re both, they’re all very ubiquitously distributed. So, to get localised effect, it has been successful for superventricular tachycardia, and there are very interesting developments in the brain - especially Parkinson’s Disease - with A2A receptors interacting with dopamine; in sleep; in various central nervous system maladies, adenosine receptors are important.

Breakthrough in purinergic signalling concept, 1985

Then the next step didn't take place until 1985 when one of my PhD students, Charles Kennedy, who's a very good purine worker today, we published a paper in which we felt we could subdivide the P2 ATP receptor into two subtypes, which we called P2X and P2Y, on the basis of pharmacology. When I say, we divided into P2X and P2Y, on a pharmacological basis, for example, we found that alpha, beta -methylene ATP seemed to be effective on P2X receptors, whereas 2-methyl-thio ATP was selective for P2Y receptors. It hasn't held up altogether, but nevertheless, this was the basis and that's what we proposed. Now, the exciting thing for us is that when we cloned the receptors, we and others in the early nineties, it turned out that cloned - and also looked at second messenger systems - that P2X and P2Y was validated because it worked out the molecules were totally different for P2X, and it was an ion channel receptor, and P2Y was a G protein coupled receptor. We were lucky, as I've often been in my science, I must admit.

Discovering the first ATP receptors, 1990s

I think that a major turning point in the acceptance of the purinergic signalling idea concept really happened in the early nineties. First of all, people – not our lab – cloned the P1 receptors and found that there were four subtypes - A1, A2A, A2B, and A3. - and they characterised them and they developed, since then, very good selective agonists and antagonists. But it wasn't until '93 when I persuaded my good old colleague – we were students together at King's College (London) many years ago – Eric Barnard, who had a very fine record for cloning nicotinic receptors. And I persuaded him – it wasn't easy – to see if he could clone and characterise the ATP receptor, and he and Webb, his student, and others, worked on this and came up with the first ATP receptor, which was P2Y1, a G protein coupled receptor typically with 7 transmembrane domains, intracellular C, extracellular end terminals.

Almost simultaneously, people in San Francisco came up with a P2Y2 receptor which was different in that UTP as well as ATP was an agonist on this receptor. So they were the first two G protein coupled receptors, and a year later, in '94, Alan North, and again the people in San Francisco came up with the P2X receptor and showed that it was an ion channel receptor, and had a very interesting structure - two transmembrane domains, intracellular short terminals, C and N terminals, and an extracellular loop, which had ten conserved cystines in it. Later on, in the following years, various labs including our and North's lab, and others, cloned other subtypes, and so there are now seven subtypes of the ion channel P2X receptor and currently eight subtypes have been defined and characterised of the P2Y G protein coupled receptor.

Fast response receptors and the mysteries of P2X7

So the ion channel P2X receptors, by and large, because of their molecular structure, mediate fast, very fast responses like neurotransmission. And the P2Y receptors tend to mediate, which are G protein coupled, 7 transmembrane domains, very common receptors - there are many G protein coupled receptors - and these tend to mediate slower responses. But I want to stress that all of them that we've been discussing so far, whether it's neurotransmission, neuromodulation, platelet aggregation, secretion, these are all fast responses. There's a bit of difference between X and Y, yes, but they're fast.

P2X7 is fascinating for several reasons. If you put low doses of ATP you just open cation channel, like the other receptors, but if you put high doses on, several amazing things happen. First of all, the cells bleb - nobody knows what that's about - and then they shed little microvesicles which contain inflammatory cytokines, like interleukin and others, and this is very important in inflammatory reactions. And the other thing it does is, suddenly, a very large pore opens, which leads to cell death by apoptotic mechanisms, which is very important especially in pathological conditions. Although, I have to say, that there are some papers emerging where P2X7 also seems to stimulate cell proliferation, and so there are mysteries still to be solved about P2X7, but its importance is undeniable.

Interaction between purino and other receptors

One of the problems we face in the purinergic field is that many cells have multiple receptors on them. Not only multiple purinoreceptors - P1, P2X subtypes, P2Y - but also sometimes muscarinic, catecholamine receptors, glutamate receptors. How do they interact? How does it work? We have to solve this? Interestingly, in microglia, we have a hint already because there are about six different receptors that do different things. One receptor, P2Y12, mediates the migrations of these microglia to sites of damage. Another one, P2Y6 – another type - changes the phenotype from the resting form, which is very elaborate, to the amoeboid form at the site, and that amoeboid form then starts expressing a P2Y6 receptor which shows macrophage activity. And then P2X4 and P2X7 are involved in neuropathic pain. So we already have a hint that sometimes, some receptors are mediating fast responses, other receptors are mediating slow trophic events like proliferation or differentiation, and others are dormant except in pathological situations, and then they're doing something. But we need to analyse this in much greater detail. And there are some cell types, like lung epithelial cells and kidney cells, where there are different receptors, vasolaterally, from those that are found on the internal lumen, and they do different things. All this has to be resolved. It's still a young field.

The next challenge – linking purinoreceptors and behaviour

If we turn to the brain, which is a formidable challenge as far as purinoreceptors go, I have to say that, for the first thirty years or so, people recognised that there were adenosine receptors which were prejunctional modulators of the release of excitatory transmitter, and they thought that was probably the main role of purinoreceptors in the brain. It was only much later, starting in '92, when synaptic purinergic transmission was first proposed in the *Nature* paper, and since then there's been a *huge* literature showing P2X and P2Y receptors all over the brain. And it's very interesting that, at one level, we therefore know it's involved in neurotransmission and neuromodulation, and some trophic events. But the real question is, what are they doing behaviourally? And there's hardly any experiments on this at all. There's just the beginning - there's a little bit on food appetite, appetitic behaviour, various … there's a bit of stuff on central control by the brain stem of autonomic function, which involves purinergic signalling and the NTS, and various other regions of the brain stem, RVLM and so on. But, behaviourally, there's a huge gap, and if I had to say which area I would hope would be for future study, I would long to have behavioural studies. One of the reasons that this has been held up is because most of the even selective antagonists that we have, don't work *in vivo*. So - and some of them don't go through the blood brain barrier - so it's very interesting that Roche have just come up with a P2X3 antagonist which will be very important for pain and various other problems, which is what you desire for the therapeutic development - a small molecule, orally bio-available, stable *in vivo*. So this is going … it's in clinical trials, and this might be one of the first really important agents that can be used to study behavioural questions.

ATP and evolution

I was, after all, professor of zoology for a while, and I'm interested in evolution. I think it's very fascinating, and I think it's a great approach. And I actually felt intuitively, for what it's worth, that ATP was really a very primitive signalling molecule, so I started reading thoroughly the literature and doing a lot of comparative physiology experiments and there's no doubt at all that, certainly lower vertebrates, but also nearly all the invertebrate groups - whether you're looking at coelenterares or annelids or insects or crustacea, whatever - there are huge responses to ATP, to GTP, to adenosine. And pharmacologically, there's even a hint that there's both P1, P2X and P2Y in different systems, but nobody, until recently, had cloned any receptors in these primitive animals. But marvellously, in the last year or so, there have been two *very* important papers. One showing what's called a social amoeba, *Dictyostelium.* They cloned a receptor which is very similar in its molecular structure to P2X2 receptors in the mammal, and then, just before this, *Schistosome mansoni*, which is a

primitive parasite, they cloned a receptor in this and showed that it's rather like the P2X4 receptor in the mammalian system. This is remarkable. My own feeling and speculation was that I thought the adenosine receptor would probably have appeared first. I thought the P2Y receptor – another G protein - coupled with probably slow responses, be next. And then P2X would be next. But in insects, with their very fast responses, P2X is important, and in some of the other systems where there's a nervous system it looks as though P2X emerged early too. But this is all very speculative at this stage. I'm hoping this is another area where people - in the growing number of people in the purinergic signalling field - will attack it and find out more about the evolution of this system. My own view is that the way evolution works, if this was a dominant molecule very early on, why should it not be simultaneously developed as an intracellular energy source, and an extracellular signalling molecule? I mean, there's another amazing development. In the last year, there have been ten papers showing that ATP acts on receptors in plants and this is … they're involved, apparently, in the mechanism of regeneration of damaged plants. Intriguing.

Purinoreceptors and embryological development

One ought to mention, perhaps, the old phrase that 'ontogeny repeats phylogeny', and that's why I've also been looking at development - embryological development - and it turns out that purinoreceptors play a very dominant role in development. You get a transient appearance of different subtypes, which suggest that they're involved in a particular differentiation process and then they're eliminated, and then the next phase comes up. And we've shown this in skeletal muscle and in various models that we've been looking at. I think it's intriguing. And the latest thing - my only last experiment I really would love to do is stem cells. We've been looking at purinoreceptors in embryonic stem cells and they shine like beacons, and my guess is, because they're so important in development - in embryological development - that they're probably important in stem cells. And what we're going to look – again we come back to synergism – there's other evidence that growth factors work synergistically with purinoreceptors. My guess is that if we put them with the right growth factors, we'll see which cell types they develop into. I think this is an intriguing area, connected somehow to development.

Therapeutic outcomes – clopidogrel

The current emphasis of the many people now working in many countries in the purinergic signalling field is the pathophysiology, and of course, drug companies are interested in the therapeutic potential of this signalling system. The first drug, which has been developed from the purinergic signalling story is a drug called clopidogrel, and this is used against stroke and thrombosis. Platelets have three receptors on them - P2Y12 receptors, which mediate

platelet aggregation; P2Y1 receptors which mediate aggregation but also cell shape; and thirdly there's a P2X1 receptor, which there are still speculations about what it does. But the antagonist to P2Y-12 – clopidogrel - blocks platelet aggregation, and this has developed into what *The Economist* called, a couple of months ago, a 'blockbuster drug' - making millions now, against stroke and thrombosis. It's sometimes used together with aspirin, and there were huge clinical trials first before this came on the market.

Therapeutic outcomes - ATP and pain mechanisms

Pain is another very exciting area of current interest and development because in '95 we and another lab cloned a P2X3 receptor, which seems to be almost exclusively localised on sensory nocioceptive fibres. And, when we developed this story, it turns out that it's a new approach to pain, because instead of, like, morphine [which] interrupts the pain pathway in the spinal cord level and so on, ATP is the *initiator* of pain on the nerve endings, and it's released locally. We started by looking at the tongue. We knew the tongue is very … it hurts if it's hot, it's burnt, and it hurts if you bite it. We took out the tongue with the sensory nerves attached, and we recorded from the sensory nerves when we heated the tongue, and there was a big electrical discharge in these sensory fibres, and this response was mimicked by ATP applied to the tongue, and largely blocked by ATP antagonists. So, this was the first hint that this might be a mechanism. And then we moved to the bladder, and to the ureter - one of the most painful things you can get is a stone in the ureter. And in the gut, if you get colic gut. These are all very painful things, and it's always distension. During distension, ATP is released from these lining ureterelial cells, epithelial cells in the gut, and there's always been a mysterious sub-epithelial sensory nerve plexus, and we've shown with immunochemistry that it's loaded with P2X3 and P2X2-3 hetero multimer receptors. So, the ATP released, acts on these receptors on sensory nerves, which send the message through the central nervous system to the brain areas and record pain.

The evidence is powerful, and this is very exciting. Of course, the search is on now for a P2X2-3 antagonist, which might open up a whole new way of dealing with certain kinds of pain … some kinds of pain [which] morphine is not effective against. And this looks very interesting, I think, particularly for visceral pain.

Therapeutic outcomes - incontinence

There's another company, which are very interested in bladder incontinence, because we've used P2X3 knockout mice – we get rid of the pain, but amazingly we also interfere with normal bladder voiding - and so, the whole question of whether this can be used for

incontinence of bladder and obstructed bladder, neurogenic bladder, is being explored by several companies at the present time.

The dynamics of purinergic transmission

For many years, drug companies used young healthy male volunteers to develop their drugs, and then they were surprised that it didn't work in old people. Of course, there's a huge change in the proportions of co-transmitters and the expression of receptors. In surgery, the surgeons don't realise that when you cut a nerve, or when you've got trauma of a nerve, the other nerve changes – it's not a control at all – changes its composition of co-transmitters. There are lots of examples of this now, and of receptors for these transmitters. So it's a very plastic system, and that's very important to realise that. In development and in surgery and trauma, and pathology in particular, and even between the sexes, with the hormones, changes the proportions of the neurotransmitters.

It's not a problem to work out which co-transmitter is the principle one and which are minor ones in any given situation, because they change with time. Let me give you some important examples - and the way you do it is with selective antagonists - you stimulate the nerve, you record a response, and you find out which proportion of it is blocked by noradrenaline-blocking agents - if it's sympathetic co-transmission - and which is blocked by purinergic, P2 purinoreceptor antagonists - in most cases P2X1 - if it's a smooth muscle. Now, the interesting thing. For example, we talked about sympathetic co-transmission and I said that the proportions vary. There have been descriptions of sympathetic innervation of arterioles in the gut, where ATP is the *sole* transmitter, and the noradrenaline that comes out acts as the prejunctional modulator. Do you call that a purinergic nerve? Well, I guess nerves anyway. But that's … there are other places like renal arteries where the dominant transmitter is noradrenaline, and ATP a minor effect. And then there are interesting things in pathological situations. For example, in spontaneously hypertensive rats there are now four or five papers showing a hugely increased ATP component of co-transmission in hypertension. Then there's another example in the parasympathetic innervation of the bladder. I think I mentioned in the experimental animal bladder, it can be fifty to sixty per cent purinergic, and the rest cholinergic. But in the human bladder – the normal, healthy, human bladder - we were disappointed to find that only two or three per cent was purinergic, so it didn't look as though it was going to be very interesting as a therapeutic development. But it turns out, in the pathological bladder - in the interstitial cystitis, in the obstructed bladder, in the urogenic bladder - suddenly up to forty per cent of the parasympathetic response is purinergic. So the proportions change in development, in old age, and in pathological conditions.

Directed research or nurturing the creative spirit

I'm not really happy about what's happening in universities at the present time. There's a lot of talk about corporate plans and about directed research. In my view, unlike industry, which has to be directed, I understand that, their objectives are different. I think universities should be about nurturing the creative spirit of individuals and they shouldn't be directed. They should be free to develop. You never know what, in basic science, is going to turn out to be terribly important, even if it isn't obvious at the beginning. So, I don't like this trend. I'm for individuality and I'm for creative nurturing of gifted people and giving them, like artists, a free hand. You wouldn't tell artists to all work on a particular problem - the poets and painters and sculptors. You wouldn't expect to do that with artists and you shouldn't with scientists, because they're closely related, in my opinion. Creative people express themselves in science or art.

Ideas for future research and an offer to young scientists

There are a lot of exciting new areas I think that young people could get on to. Certainly, behaviour studies - effect of purinergic signalling on behaviour - and many more pathological studies. I think stem cells are a gold mine. I think evolutionary studies are really worth doing - cloning some more receptors in invertebrates and lower vertebrates. I think embryology. I think a whole field of the interaction of purinoreceptors with growth factors and various genes is going to be terribly important and interesting. The literature is exploding and I keep seeing wonderful new things that I wish I could go on and do, but I've got to slow down, I guess.

I think that what's exciting is the question. I often ask, when I'm examining a PhD, 'Tell me, in a nutshell, what question you were asking, what approach you took that could produce original work and if there's a takeaway original finding.' And sometimes they say to me, 'What do you mean by "what question"? What I did was this and that.' And this is terrible. You must have a question in science. You *must*. And you must try to answer … make sure that the techniques you use can answer that question, because some very bright people ask a good question but the techniques are not available to answer it, and they spend years doing stuff which later on is shown to be obsolete.

You know, science is made up of clubs. When we started with the idea that purinoreceptors might be involved in pain, the 'pain club' was very resistant to this to start with. The embryologists were resistant to the role of purines. It took a while. Each one of these things takes a while, but I don't mind going into new areas. I start in ignorance, I build in knowledge, I make good collaborations with leading figures, which is important these days – you can't be the best in the world at every technique - and we stumble along, you know. I'm not going to

stop. I don’t want to stop. I’m in the thick of it. I love seeing young people at meetings and I’m inspired by their enthusiasm and reactions, and I wish I could on. I mean, I have had 106 PhD and MD students that I’ve personally supervised – that was a great pleasure - keeps you young, working with passionate young people. This is what its all about, and I don’t want that to stop. Can’t! Look, I’m only seventy-eight-years-old. I’m ready to go on, so if any of you youngsters out there feel like working with me, give me a call. I’d find that very, very attractive.