Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut

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Commentary by

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The classical view that autonomic control of smooth muscle consisted of antagonistic sympathetic noradrenergic and parasympathetic cholinergic nerves was challenged in the early 1960's by Burnstock and his colleagues Max Bennett, Graham Campbell, Mollie Holman and Mike Rand in Melbourne, and also by Martinson in Göteborg. Stimulation of the guinea-pig taenia coli in the presence of adrenergic and cholinergic blocking agents produced fast inhibitory junction potentials which were blocked by tetrodotoxin. The search for the transmitter responsible for these non-adrenergic, non-cholinergic (later termed NANC) nerve responses was pursued in the late 1960's with my colleagues Graham Campbell, Dave Satchell, Brian Dumsday and Anne Smythe and led to the surprising proposal in the paper under discussion that adenosine triphosphate or a related nucleotide might be the transmitter involved.

This 'purinergic' hypothesis, as it was later termed in the Pharmacological Review by Burnstock in 1972, met considerable resistance, partly perhaps because ATP was regarded solely as an intracellular molecule contained in all cells and of particular importance as an energy source, and it was concluded that such a ubiquitous molecule was unlikely to act as a neurotransmitter, even though the presence of powerful ectoenzymes for the breakdown of ATP were already well known. A good friend and colleague of mine, Austin Doyle, Professor of Medicine in Melbourne, who was noted for his caustic wit, exclaimed to the audience during the farewell party for my move from Australia to England in 1975 that the transmitter in purinergic nerves appeared to be 'pure-imagine'

In our 1970 paper, we set out to see what substances could satisfy the criteria set out by Eccles and others for establishing the identity of a neurotransmitter for the NANC inhibitory nerves in the guinea-pig taenia coli (a preparation I had learnt in Edith Bülbring's laboratory in Oxford before going to Melbourne) and for NANC nerves in both the toad stomach and turkey gizzard.

Firstly, a putative transmitter must be synthesised and stored within the nerve terminals from which it is released. Once released it must be mimicked by the exogenous application of the transmitter substance. Also, enzymes which inactivate the transmitter and/or uptake systems for the neurotransmitter or its derivatives must also be present, and, finally, drugs which affect the nerve-mediated response must be shown to modify the response to exogenous transmitter in a similar manner. Many substances were examined as putative transmitters in the NANC nerves of the gastrointestinal tract and bladder, but the substance that best satisfied the above criteria was the purine nucleotide, ATP. A tentative model of storage, release, receptor activation by and inactivation of ATP during purinergic transmission in the gut and urinary bladder was proposed in the 1972 Pharmacological Review and in recent years seems to be generally accepted. A recent Volume of 'Seminars in the Neurosciences' (August 1996) is devoted entirely to purinergic neurotransmission.

Another concept, namely that each nerve cell can synthesise, store and release only one neurotransmitter, was challenged by Burnstock in 1976 and the existence of nerves that can synthesize, store and release more than one pharmacologically active substance is now widely accepted. While most of the experiments demonstrating purinergic cotransmission in sympathetic nerves were carried

out in the vas deferens in the early 1980's, the first evidence for sympathetic cotransmission involving ATP together with noradrenaline came from studies I made with Che Su and John Bevan on the guinea-pig taenia coli, whilst on sabbatical leave in California in 1971. We showed that stimulation of the periarterial sympathetic nerves led to release of tritium from guinea-pig taenia coli pre-incubated in [^{3}H]-adenosine (which is taken up and converted largely to [^{3}H]ATP) and that the release of both tritium and noradrenaline was blocked by guanethidine.

The 1970 paper has provoked debate through the years and, particularly at the present time, not only about purinergic signalling but also about the identity and roles of the cotransmitters in nerves in the gastrointestinal tract and the concept of 'chemical coding' (*i.e.* identified combinations of transmitters in nerves whose targets and central connections are known) that was introduced by Furness and Costa. When the technique of immunohistochemistry for neuropeptides was used widely in the mid 1970's, the idea that vasoactive intestinal polypeptide (VIP) was the neurotransmitter, rather than ATP in the NANC nerves (the 'third nervous system' as it was sometimes called in those days) in the gut gained ground and papers entitled 'Peptidergic rather than purinergic' were published. However, the pharmacological experiments were not entirely supportive, partly because in most gut preparations the response to VIP was very slow and sustained after a long latency, in contrast to the fast relaxations and inhibitory junction potentials produced by nerve stimulation and ATP. After earlier hints from the laboratory of John Gillespie (particularly Anne Bowman and Billy Martin) in the early 1980's, a new contender for the NANC inhibitory transmitter emerged when nitric oxide (NO) was recognised in 1989/1990 by Rand, Snyder, Garthwaite, Boeckxystaens and others to be a neurotransmitter in the nervous system as well as the endothelial-derived relaxing factor of Bob Furchgott. Most laboratories now support the view that ATP, NO and VIP are cotransmitters in NANC inhibitory nerves, although the proportions vary markedly in different regions of the gut and in different species. Thus, while ATP remains a strong contender in the guinea-pig taenia coli as first proposed in the 1970 paper, in some preparations, particularly sphincters, NO or VIP is the dominant transmitter utilized.

The concept of purinergic signalling has broadened through the years to include not only purinergic cotransmission in different nerve types in both peripheral and central nervous systems, but also other roles for ATP including: control of secretion, immune cell activity, endothelial release of nitric oxide, and long-term ('trophic') control of cell proliferation, growth, differentiation and apoptosis. A useful advance in the field was initiated in 1978 when I proposed that purinoceptors could be divided into P_1 (adenosine) and P_2 (ATP and ADP) types and later in 1985, together with Charles Kennedy, into P_{2X} and P_{2Y} subtypes. A strong boost to the interest in purinergic mechanisms came in 1992 when purinergic transmission was demonstrated between neurones in coeliac ganglia by AnneMarie Surprenant and colleagues, by Eugene Silinsky and, in the brain, by Frances Edwards and Alasdair Gibb at University College London, and again in 1993/1994 when P_2 purinoceptors were first cloned. We now recognise that P_2-purinoceptors belong to two major families, a P_{2Y} family of G-protein coupled receptors (cloned in 1993 by us in collaboration with Eric Barnard and by Kevin Lustig in Dave Julius's laboratory) and a P_{2X} family of ligand-gated ion channel receptors cloned in 1994 by Valera, North and colleagues in Geneva and by Anthony Brake, again in the laboratory of David Julius). Currently, 7 subtypes of the P_{2X} family and 8 of the P_{2Y} family have been cloned and functionally characterised; both clinicians and the drug industry are turning their attention to the clinical implications of purines and possible therapeutic targets of selective agonists and antagonists.

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