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Introductory overview of purinergic signalling

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1. ABSTRACT

Purinergic neurotransmission was proposed in 1972 following identification of adenosine 5'-triphosphate (ATP) as the transmitter in non-adrenergic, non-cholinergic inhibitory nerves in guinea-pig taenia coli. Subsequently ATP was identified as a co-transmitter in sympathetic, parasympathetic and most nerves in the peripheral and central nervous systems. ATP acts as a short-term signalling molecule in neurotransmission, neuromodulation and secretion and has long-term (trophic) roles in cell proliferation, differentiation and death in development and regeneration. Three subclasses of purine and pyrimidine receptors have been identified, P1 adenosine (4 subtypes), P2X ionotropic nucleotide (7 subtypes) and P2Y metabotropic receptors (8 subtypes). ATP is released physiologically by many cell types by mechanical deformation and, after release, ATP undergoes ectonucleotidase degradation. Purinergic receptors appeared early in evolution and have a widespread distribution on many non-neuronal cell and neurons. Purinergic signalling is involved in embryonic and stem cell development. There is a rapidly growing literature about the pathophysiology of purinergic signalling including therapeutic developments for diseases, including stroke, thrombosis, osteoporosis, kidney failure, bladder incontinence, cystic fibrosis, dry eye, cancer and brain disorders.

2. INTRODUCTION: ATP AS A TRANSMITTER IN NON-ADRENERGIC, NON-CHOLINERGIC NERVES

The existence of transmitters released from autonomic nerves that were neither of the classical neurotransmitters, acetylcholine (ACh) or noradrenaline (NA), was discovered in 1963 when inhibitory junction potentials were recorded in the guinea-pig taenia coli in response to nerve stimulation in the presence of atropine and guanethidine (1). These non-adrenergic, non-cholinergic (NANC) responses were shown to be present in intrinsic enteric neurons controlled by vagal and sacral parasympathetic nerves and NANC transmission was later shown in the urinary bladder and vascular system.

The next step was to try to identify the transmitter released during NANC inhibitory transmission in the gut and excitatory transmission in the urinary bladder. Several criteria needed to be satisfied to establish a neurotransmitter: synthesis and storage in nerve terminals; release by a Ca^{2+}-dependent mechanism; mimicry of the nerve-mediated responses by the exogenously applied transmitter; inactivation by ectoenzymes and/or neuronal uptake; and parallel block or potentiation of responses to stimulation by nerves and exogenously applied transmitter. Many different substances were examined in the late 1960s, including amino acids, monoamines, neuropeptides, but

none satisfied the criteria. However, we were influenced by a seminal paper by Drury and Szent-Györgyi (2) showing powerful extracellular actions of purines on heart and blood vessels, papers by Feldberg showing extracellular actions of ATP on autonomic ganglia (3) and a paper by Pamela Holton in 1959, which showed release of ATP during antidromic stimulation of sensory nerves supplying the rabbit ear artery (4). To our surprise ATP beautifully satisfied all the criteria needed to establish it as a transmitter involved in NANC neurotransmission (5). In 1972, an article was published in *Pharmacological Reviews* formulating the purinergic neurotransmission hypothesis (6). Unfortunately, few believed this hypothesis over the next 25 years and it was often ridiculed at meetings and workshops. Resistance to this concept was perhaps understandable because ATP was well established as an intracellular energy source involved in the Krebs cycle and other biochemical pathways and it seemed unlikely that such a ubiquitous molecule would also act as an extracellular messenger. However, ATP, an early biological molecule, probably evolved both as an intracellular energy source and an extracellular signalling molecule. It was not until receptors for ATP were cloned and characterized in the early 1990's and neuron-neuron synaptic transmission identified in sympathetic ganglia and in the brain in 1972 that the purinergic hypothesis began to be widely accepted (see 7).

For many years our understanding of neurotransmission was dominated by the concept that one neuron releases only a single transmitter, known as 'Dale's Principle'. This idea arose from a widely adopted misinterpretation of Dale's suggestion in 1935 that the same neurotransmitter was stored in and released from all terminals of a single neuron, a suggestion which did not specifically preclude the possibility that more than one transmitter may be associated with the same neuron. Based on experiments that showed release of ATP with NA from sympathetic nerves (8) and many hints in the literature, the cotransmission hypothesis was formulated in 1976 (9). Purinergic cotransmission is now well established, not only in sympathetic nerves, but also in parasympathetic, sensory-motor, enteric nerves and developing motor nerves to skeletal muscle. More recently ATP has been shown to be co-released with glutamate, γ-aminobutyric acid, dopamine, NA, 5-hydroxytryptamine and ACh in different populations of nerve fibres in the central nervous system (CNS) (see 10).

3. RECEPTORS TO PURINES AND PYRIMIDINES

Implicit in purinergic transmission is the existence of specific receptors. In 1978, a basis for distinguishing two types of purinergic receptors was proposed, one selective to adenosine (called P1), which was antagonized by methylxanthines and the other selective for ATP/ADP (called P2) (11). This was a useful step forward, explaining some of the early confusion in the literature resulting from the rapid extracellular breakdown of ATP to adenosine and extended our concept of purinergic neurotransmission, by identifying postjunctional receptors as P2, while prejunctional P1 receptors mediated neuromodulatory negative feedback responses or auto-regulation of transmitter release. A pharmacological basis for distinguishing two types of P2-purinoceptors, defined as P2X and P2Y was proposed in 1985 (12) and when P2 receptors were cloned in the early 1990s (13-16) and second messenger mechanisms examined, this subclassification was consistent with P2X ion channel receptors and P2Y G protein-coupled receptors (17). Currently, 4 subtypes of P1 receptors are recognized, 7 subtypes of P2X receptors and 8 subtypes of P2Y receptors, including some responsive to the pyrimidines UTP and UDP (18). It was shown that three of the P2X receptor subtypes combine to form cation pores (19) either as homomultimers and heteromultimers, and more recently heterodimerization has been shown between P2Y receptor subtypes. Many non-neural as well as neuronal cells express multiple receptors (20) and this poses problems about how they mediate interacting physiological events. It is becoming clear that the purinergic signalling system has an early evolutionary basis (21) with fascinating recent studies showing cloned receptors in two primitive invertebrates, *Dictyostelium* and *Schistosoma*, that resemble mammalian P2X receptors (22, 23) and ATP signalling in plants has also been described (24-26).

4. PHYSIOLOGY AND PATHOPHYSIOLOGY OF PURINERGIC SIGNALLING

While early studies were largely focused on short-term purinergic signalling in such events as neurotransmission, neuromodulation, secretion, platelet aggregation, chemoattraction and acute inflammation, there has been increasing interest in long-term (trophic) purinergic signalling involving cell proliferation, differentiation, motility and death in development, regeneration, wound healing, restenosis, epithelial cell turnover, cancer and ageing (27, 28). For example, in blood vessels, there is dual short-term control of vascular tone by ATP released as an excitatory cotransmitter from perivascular sympathetic nerves to act on P2X receptors on smooth muscle, while ATP released from endothelial cells during changes in blood flow (shear stress) and hypoxia acts on P2X and P2Y receptors on endothelial cells leading to production of nitric oxide and relaxation (29, 30). In addition, there is long-term control of cell proliferation and differentiation, migration and death involved in neovascularization, restenosis following angioplasty and atherosclerosis (31). For many years, the source of ATP acting on receptors was considered to be damaged or dying cells, except for exocytotic vesicular release from nerves. However, it is now known that many cell types release ATP physiologically in response to mechanical distortion, hypoxia or to some agents (32). The mechanism of ATP transport is currently being debated and includes in addition to vesicular release, ABC transporters, connexin or pannexin hemi-channels, maxi-ion channels and even $P2X_7$ receptors (7). There is now much known about the extracellular breakdown of released ATP by various types of ectonucleotidases, including: E-NTPDases, E-NPPS, alkaline phosphatase and ecto-5'-nucleotidose (33). There is current interest in the roles of purinergic signalling in neuron-glial interactions in the CNS (34, 35).

It is well known that the autonomic nervous system shows high plasticity compared to the CNS. For example, substantial changes in cotransmitter and receptor expression occur during development and ageing, in the nerves that remain following trauma or surgery and in disease situations (36). For example, a P2Y-like receptor was identified in *Xenopus* that was transiently expressed in the neural plate and again later in secondary neuralation in the tail bud, suggesting involvement of purinergic signalling in the development of the nervous system (37). There is transient expression of $P2X_5$ and $P2X_6$ receptors during development of myotubules and of $P2X_2$ receptors during development of the neuromuscular junction (38). In the rat brain, $P2X_3$ receptors are expressed first at E11, $P2X_2$ and $P2X_7$ receptors appear at E14, $P2X_4$, $P2X_5$, and $P2X_6$ receptors at P1 and $P2X_1$ receptors at P16 (39).

Primitive sprouting of central neurons was shown in experiments in which the enteric nervous system was transplanted in the striatum of the brain (40). It was later shown that a growth factor released from enteric glial cells acting synergistically with ATP (and its breakdown product, adenosine) and nitric oxide, were involved (41). It is suggested that similar synergistic activity of purines and growth factors might be involved in stem cell activity (42).

It was established early that ATP was a major cotransmitter with ACh in parasympathetic nerves mediating contraction of the urinary bladder of rodents (43). In healthy human bladder, the role of ATP as a cotransmitter is minor. However, in pathological conditions, such as interstitial cystitis, outflow obstruction and most types of neurogenic bladder, the purinergic component is increased to about 40% (36, 44). Similarly, in spontaneously hypertensive rats, there is a significantly greater cotransmitter role for ATP in sympathetic nerves (45).

Clopidogrel, a $P2Y_{12}$ receptor antagonist, which inhibits platelet aggregation, is a highly successful drug against thrombosis and stroke (46). Purinergic compounds are also being developed for the treatment of hypertension and atherosclerosis (31), inflammatory bowel disease (47), dry eye and cystic fibrosis (48), cancer (49, 50) and for a number of other diseases (36).

$P2X_3$ receptors were cloned in 1995 and shown to be largely located in small nociceptive sensory nerves that label with isolectin B4 (51, 52). Central projections are located in inner lamina 2 of the dorsal horn of the spinal cord and peripheral extensions in skin, tongue and visceral organs. A unifying purinergic hypothesis for the initiation of pain was published (53) and a hypothesis describing purinergic mechanosensory transduction in visceral organs in 1999, where ATP, released from lining epithelial cells during distension, acts on $P2X_3$ and $P2X_{2/3}$ receptors in subepithelial sensory nerve endings to send nociceptive messages via sensory ganglia to the pain centres in the brain (54, 55). Supporting evidence including epithelial release of ATP, immuno-localization of $P2X_3$ receptors on subepithelial nerves and activity recorded in sensory nerves during distension that is mimicked by ATP and reduced by $P2X_3$ receptor antagonists has been reported in the bladder (56), ureter (57) and gut (58). Purinergic mechanosensory transduction is also involved in urine voiding as evidenced in $P2X_3$ knockout mice (59). For neuropathic and inflammatory pain $P2X_4$, $P2X_7$ and $P2Y_{12}$ receptors on microglia have been implicated and antagonists to these receptors are very effective in abolishing allodynia (60, 61) and there is also strong interest in the potential roles of purinergic signalling in trauma and ischemia, neurodegenerative conditions including Alzheimer's, Parkinson's and Huntington's diseases and in multiple sclerosis and amyotrophic lateral sclerosis. There are also studies in progress of purinergic signalling in neuropsychiatric diseases, including depression, anxiety and schizophrenia and in epileptic seizures (62).

5. CONCLUSIONS

Purinergic signalling is widespread in neural and non-neural cells and is now well established to play pivotal roles in many physiological and pathophysiological activities. The focus of this Special Issue is on purinergic signalling in bone and inflammation.

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