THE BIOLOGIC ACTIONS OF EXTRACELLULAR ADENOSINE TRIPHOSPHATE

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■ In addition to its well-known involvement in intracellular energy and intermediary metabolism, adenosine triphosphate (ATP), when present in extracellular domains, can elicit functional reponses in a large number of types of tissue and cells.[1,2] While exogenous ATP can be rapidly catabolized to adenosine by a number of ectophosphohydrolases, many of the observed actions of extracellular ATP can be distinguished from those triggered by occupation of the well-characterized A_1- and A_2-receptors for extracellular adenosine. What is currently emerging is the view that extracellular ATP per se may be of physiological significance as a signaling agent in many biologic systems. Many of these functional effects of ATP, furthermore, can be observed at extracellular concentrations (nanomolar to low-micromolar) that are generally well below the Michaelis constant (K_m) values for most intracellular adenosine triphosphatases (ATPases) and other ATP-utilizing enzymes. These observations have suggested that there exist specific cell surface receptors for extracellular ATP.

Until fairly recently, most studies involving the actions of extracellular ATP and adenine nucleotides were performed on preparations of smooth muscle. This work was prompted by Burnstock and colleagues[3] who, in 1970, proposed that ATP is released as a excitatory neurotransmitter in smooth muscles, and that the nucleotide is the primary transmitter substance responsible for neurogenic inhibitory responses mediated by what has been referred to as "nonadrenergic, noncholinergic" inhibitory nerves. It has turned out that Burnstock's original hypothesis has been difficult to prove, but it nevertheless initiated many experiments to support or disprove it. The outcome of this work has been somewhat different than envisaged, in that ATP has been shown to be a cotransmitter from nerves in which it is stored. In these studies of Burnstock and colleagues, the use of the term *P_2-purinergic* was introduced to designate the putative receptor(s) for ATP and to distinguish them from so-called *P_1-purinergic* receptors for adenosine.

In addition to initiating either excitatory or inhibitory responses in every smooth muscle preparation examined, ATP or related adenine nucleotides were also reported to stimulate or modify contractile, electric, metabolic, or secretory responses in heart, liver, brain, vascular endothelium, platelets, and several endocrine/exocrine tissue types.[1,2] Among the tissues in

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this listing that receive innervation, ATP and/or its breakdown product, adenosine, have been shown to modulate the release of other neurotransmitters from nerves.[4] The ATP-induced vasorelaxation characteristic of certain blood vessels is now known to be mediated by the ATP-triggered release of prostacyclin and endothelium-derived relaxing factor from endothelial cells.[5] Another recent development is the demonstration that extracellular ATP can markedly potentiate the inflammatory response of neutrophils to conventional inflammatory stimuli such as immune complexes and chemotactic peptides.[6] Ward et al., moreover, have demonstrated that activated platelets (at cytocrits found in blood) can release enough ATP to elicit this potentiation in circulating phagocytes.[6]

In parallel with these functional, pharmacological studies, additional investigations have been directed towards identifying potential sources of extracellular ATP. While ATP is present in millimolar concentrations in the cytosol of all eukaryotic cell types, extracellular levels of the nucleotide are normally maintained at extremely low levels by ubiquitous ectoATPases and ectophosphohydrolases, which rapidly and efficiently hydrolize extracellular nucleotides.[7] Thus, as is the case for any putative signaling agent, appreciable levels of extracellular ATP should occur only transiently and in response to specific physiological and/or pathological conditions. In this regard, several major sources of extracellular ATP have been identified: (1) ATP is copackaged in both adrenergic and cholinergic neurotransmitter granules and thus can be released during neurotransmission into synaptic spaces.[8] (2) ATP, which is also copackaged with serotonin in platelet granules, can be locally released in significant amounts during platelet activation.[8] (3) Cytosolic ATP stores can be released by the sudden breakage of intact cells, as might occur during the rupture of blood vessels and other tissue injury. These latter two sources suggest that significant amounts of extracellular ATP may locally accumulate at vascular sites of thrombus formation and infection or inflammation.

CURRENT PERSPECTIVES AND FUTURE DIRECTIONS

The bulk of previous studies on the actions of extracellular ATP have been descriptive in nature. Only within the past 3 to 5 years have questions been asked about the coupling of the stimulus to the intracellular response. There are some very fundamental aspects concerning extracellular ATP as a signaling agent that require, in many cases, novel experimental approaches. Such approaches are needed to characterize the two major elements underlying transmembrane signaling by any extracellular ligand: (1) the nature of the ligand–receptor interaction; and (2) the coupling of the ligand-occupied receptor to the various receptor-activated effector systems.

ATP Receptor Interactions.

Structurally, ATP is a complex molecule; the conformation of ATP in physiological solutions containing divalent cations is, in itself, an area of study. For example, are the physiological effects of extracellular ATP mediated by ATP complexed with magnesium (or calcium), or do the putative ATP receptors recognize only the free tetrabasic form, ATP^{4-}? Data from a number of studies support the latter possibility.[1,2] In general, the understanding of how the physicochemical properties of the ATP molecule relate to the induction of responses is much less developed than that pertaining to most biologic signaling ligands. In dealing with the biologic actions of ATP, an additional complication is the fact that information on the metabolism of adenine nucleotides by ectophosphohydrolases and other ectoenzymes that may recognize ATP has been virtually nonexistent until very recently. A very unusual aspect of some ATP-induced responses is that the response may actually involve a mechanism whereby the ATP is hydrolyzed, possibly by ectoprotein kinases, to trigger cellular coupling. This is perhaps the only example of a biologic ligand being transformed chemically in the process of stimulating a cell.

While the effects of ATP per se on many cell types are known, it is only now being recognized that different receptors may mediate various types of ATP-induced responses, i.e., that there are multiple P_2-purinergic receptor subtypes.[1,2] This has been primarily facilitated by the use of various "nonhydrolyzable" ATP analogs and other nucleotide triphosphates. For example, $\alpha\beta$-methylene- and $\beta\gamma$-methylene-ATP are equipo-

tent to, or more potent than, ATP itself in triggering the contraction of certain smooth muscles and in activating changes in plasma membrane Ca^{2+} conductance in neuronal and smooth muscle cells, i.e., in excitatory systems. Conversely, these analogs are unable to mimic the ability of extracellular ATP to elicit the rapid mobilization, in many cell types, of intracellular Ca^{2+} stores, which are released by 1,4,5-inositol triphosphate. The existence of different receptors in these latter cell types is also suggested by the fact that the potency of adenine-substituted analogs, such as 2-methylthio-ATP or uridine triphosphate, is greater than ATP itself. Thus, there are at least two ATP-receptor subtypes that are characterized by adenine nucleotides of differing rank orders of potency. Furthermore, these ATP-receptor subtypes appear to be coupled to different signal-transduction cascades. This situation is, of course, analogous to that which characterizes the multiple receptor subtypes of many other signaling systems involving neurotransmitters, drugs, hormones, and other modulatory ligands. The classification of purinergic receptors for ATP has been limited by the lack of subtype-specific antagonists that bind reversibly, but with high affinity. This lack of selective, high-affinity, radiolabeled antagonists (or agonists) has also limited the application of classic ligand–receptor binding methodologies to the study of ATP receptors. The use of nucleotide affinity probes for the study of extracellular, ATP-induced signaling has been successfully initiated.[9] The further development of high-affinity ligands suitable for the detailed characterization of ATP receptors is a high priority for future research. In this regard, Harden and his colleagues have reported recent success in the use of ^{35}S-ADPβS as a high-affinity probe for a P_2-purinergic receptor expressed by avian erythrocytes and several mammalian cell types.[10]

Coupling of ATP-receptors to Transmembrane Effector Systems

The last few years have witnessed a proliferation of studies detailing the effects of extracellular ATP on specific effector systems that are activated by occupation of cell surface receptors. Extracellular ATP has now been shown to activate the inositol-phospholipid-specific phospholipase C, with consequent mobilization of intracellular Ca^{2+} stores in a wide variety of cell types including hepatocytes, endothelial cells, vascular smooth muscle cells, and human phagocytic leukocytes. In some of these cell types, this activation of an inositol-phospholipid breakdown can be substantially inhibited by pertussis toxin, indicating a possible involvement of a pertussis-toxin-sensitive guanosine triphosphate-binding regulatory (G-) protein in coupling the ATP receptors to the phospholipase.[11] In rat hepatocytes, however, ATP receptors can activate not only the polyphosphoinositide-specific phospholipase C, but also a phospholipase that utilizes phosphatidylcholine (PC) as a preferred substrate.[12] Pretreatment of the cells with pertussis toxin has no marked inhibitory effect on the ability of ATP to activate either phospholipase. Other studies have suggested that P_2-purinergic effects in hepatocytes may be mediated by two ATP receptor subtypes that are coupled to distinct G-proteins. Most importantly, however, these studies demonstrate that by virtue of being functionally coupled to one of the major transmembrane signaling systems (inositol phospholipid phospholipase C), this subtype of ATP receptor may mediate many of the aforementioned actions of extracellular ATP in diverse nonexcitable cell types.

The other major effector systems that have been characterized in some detail are ATP-activated cation conductance pathway(s) in the plasma membranes of cardiac myocytes, vascular and visceral smooth muscle cells, and sensory neurons.[13,14] The ability of nanomolar to micromolar concentrations of extracellular ATP to activate these conductance pathways has been documented by state-of-the-art patch-clamp recording of both whole-cell currents and single-channel currents. While there are some differences in the ATP-activated conductance pathways observed in these various cell types, there are salient common features. These include: (1) cation specificity with little selectivity for monovalent cations; (2) very substantial activity even at hyperpolarized membrane potentials; (3) subsecond activation and rapid inactivation; (4) an apparent lack of involvement of diffusible second-messengers in generating the increased ionic conductances; (5) a substantial Ca^{2+} permeability in some cell types. These

data strongly suggest that an ATP-receptor-activated cation channel is a common feature of many excitable cells and may be a likely candidate for explaining at least some aspects of "non-adrenergic, noncholinergic" neurotransmission.

Finally, there is a long-standing observation that high concentrations of ATP^{4-} can make certain cell types permeable to molecules up to 1000 d in size, including Ca^{2+} and other ions.[15] Recent studies have indicated that this response can be distinguished (using mutant cell lines) from the ATP-induced mobilization of intracellular Ca^{2+} stores discussed above. The physiological significance of this "permeabilizing" response is unknown. It will be important to determine whether the ATP is inducing the formation of large channels or pores. It is intriguing to note that the size of these presumed pores is similar to the gap junction channels that mediate cell-cell coupling. The ATP-induced permeabilization response, moreover, is at least superficially similar to permeabilization or lytic responses triggered by various insect toxins as well as that initiated by killer T cells.

Mechanisms Underlying ATP Storage in and Release From Secretory Elements

Investigations into the mechanisms of ATP storage in, and release from, specific secretory organelles have been facilitated by the improved preparations of synaptosomes and a variety of secretory granules and vesicles. In particular, the use of highly purified cholinergic synaptosomes prepared from the electric organ of the Torpedo eel has allowed detailed comparison of the regulation of ATP versus acetylcholine release.[1] Left unresolved, however, are the mechanisms underlying the uptake and concentrative storage of very large amounts (10^{-2} to 10^{-1} mol, if free in solution) of ATP within cholinergic, adrenergic, adrenal chromaffin, or platelet serotonin granules. It should also be pointed out that some cell types (e.g., vascular endothelial cells) that are not normally thought of as classic neurosecretory types have been shown to release ATP under defined in vitro conditions by as yet unexplained mechanisms.

Mechanisms Underlying the Catabolism or Utilization of Extracellular ATP

Recent studies in this area have concentrated on (1) characterizing the kinetic details of extracellular ATP metabolism in various cell systems;[7] and (2) identifying and isolating the enzymes that catalyze these reactions. Studies of the latter kind will be essential for determining whether ATP receptors per se can be physically distinguished from the ectoATPases and other cell surface proteins that bind ATP.

SUMMARY

The study of the biologic effects of extracellular ATP is rapidly progressing from cataloging the integrated biologic responses of isolated tissues to characterizing the biochemical basis of signal transduction in isolated cells. Clearly, many fundamental issues remain; these include the classification of receptor subtypes, identification of defined or new signal-transduction pathways, generation of specific high-affinity ligands for the various ATP receptor subtypes, and ultimately, the development of protocols for the identification, isolation, cloning, and physicochemical characterization of the receptor moities per se. Such work will be essential for developing selective ATP-receptor antagonists. Such antagonists, in turn, will facilitate evaluation of the role of these receptors in physiological and/or pathological processes. A possible therapeutic role for drugs selective for these receptors is suggested by the critical pathophysiological roles of the human cell types (phagocytic leukocytes, vascular endothelial cells) known to express such receptors.

The earlier, seminal work on the biologic actions of extracellular ATP was largely performed by smooth-muscle physiologists and pharmacologists. With the expanding range of, and interest in, this topic, recent studies have been carried out largely by diverse groups of pharmacologists, neurobiologists, physiologists, and biochemists. Not surprisingly, these various scientists often investigate certain aspects of extracellular ATP action from different perspectives, using the specialized tools and methods of their respective disciplines. Hopefully, by integrating these interdisciplinary perspectives on the biologic actions of extracellular ATP, there will be rapid progress in this field during the next few years. ▲

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