ATP receptors in sickness, pain and death

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Extracellular ATP elicits biological responses ranging from cell death to synaptic transmission. Recent gene-cloning efforts have uncovered a family of cell-surface ATP receptors, which are potential targets for the development of novel drugs to treat airway and cardiovascular diseases, inflammation and pain.

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Adenosine triphosphate (ATP) is best known as the molecular currency of intracellular energy stores. Less well appreciated is its multifaceted role as an extracellular signaling molecule acting through cell-surface receptors. Within the cardiovascular, immune, and nervous systems, ATP modulates a plethora of physiological states and cellular responses, including vascular tone, electrolyte transport, mast cell degranulation, and synaptic transmission in the central nervous system and periphery. Physiological and pharmacological studies have convincingly shown that ATP exerts its actions by binding to a family of functionally distinct cell-surface receptors, known as purinergic type 2, or P_2, receptors [1]. This is a notable achievement given that there are almost no receptor-subtype-selective antagonists or radioligands available to aid in the characterization of ATP-mediated responses or ATP-binding sites. Despite the limitations imposed by this lack of pharmacological tools, it was proposed several years ago [2,3] that ATP resembles certain other neurotransmitters in its ability to activate both G-protein-coupled receptors (the P_{2U}, P_{2Y}, and P_{2T} subtypes) and ligand-gated ion channels (P_{2X} and P_{2Z} subtypes), and that these receptor types could be distinguished by their agonist sensitivities and signal-transduction mechanisms. These predictions have been borne out by the recent characterization of functional cDNA clones encoding numerous members of the ATP-receptor family.

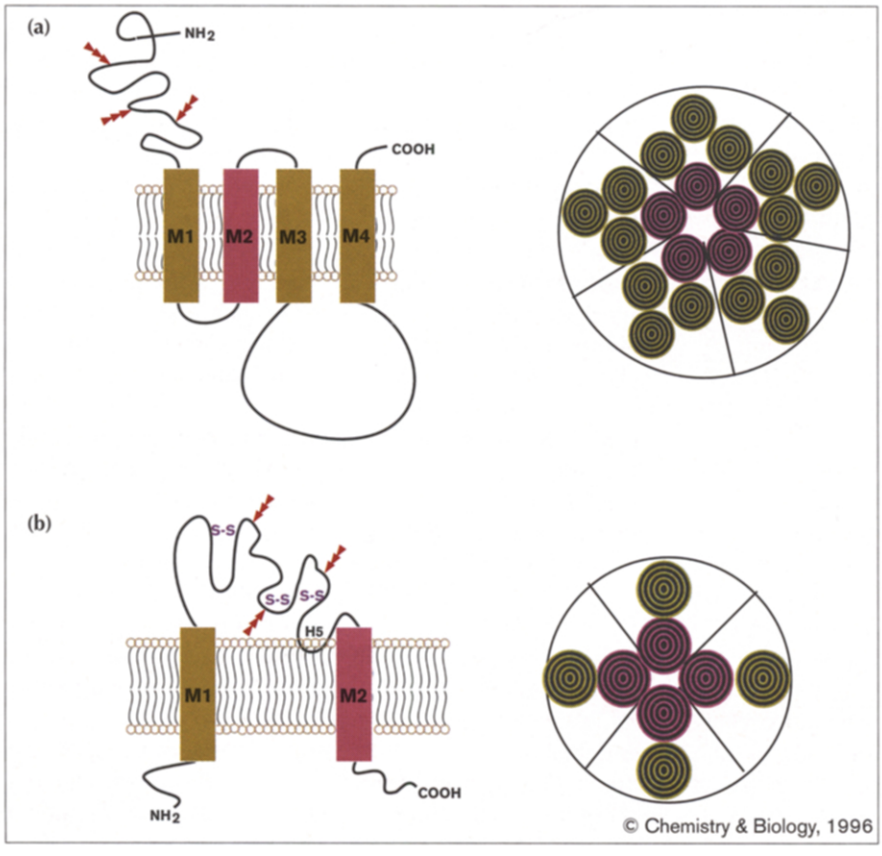
Molecular characterization of ATP receptors

The first members of the P_2-receptor family to be cloned corresponded to the P_{2U} and P_{2Y} subtypes, as evidenced by their pharmacological properties when expressed in *Xenopus* oocytes [4,5]. Examination of their deduced protein sequences showed them to be members of the superfamily of G-protein-coupled receptors, forming a distinct branch of the family that is more closely related to receptors for platelet-activating factor, angiotensin II, interleukin-8A and thrombin than to receptors for adenosine and cAMP. Other members of this gene family have now been cloned from a variety of tissues and organisms. Functional analysis of these cloned receptors reveals a diversity of subtypes beyond that previously detected using available pharmacological tools. Although these cloned receptor subtypes all share the ability to transduce signals via the activation of phospholipase C and the mobilization of intracellular calcium, they are differentially sensitive to a variety of nucleotide agonists. To accommodate this new molecular information, a revised nomenclature has been proposed [6] for purinergic receptors, in which G-protein-coupled purinergic receptors are designated P_{2Y1}, P_{2Y2}, etc., as a function of their sequence similarity (an updated compendium of purinergic receptors is currently maintained at a site on the World Wide Web (http://mgddk1.niddk.nih.gov:8000/)).

Perhaps the most surprising outcome to arise from the molecular cloning of ATP receptors has been the recent discovery of a family of P_{2X} receptors that defines a novel structural motif for ligand-gated ion channels. Many excitatory and inhibitory ligand-gated ion channels share a common subunit structure in which a large amino-terminal, extracellular, ligand-binding domain is followed by four transmembrane segments. For the best characterized member of this channel superfamily, the nicotinic acetylcholine receptor (nAChR), five subunits assemble to delineate a central water-filled ion pore. Because the biophysical properties of native P_{2X} receptors resemble those of nAChRs, it was expected that these proteins would have a common structural design. But the deduced protein sequences of the first two ATP-gated channel subtypes to be cloned (P_{2X1} from rat vas deferens smooth muscle and P_{2X2} from the PC-12 rat pheochromocytoma cell line) suggest a subunit and receptor structure that is quite different from that of the nAChR [7,8] (Fig. 1). Each of these P_{2X} subunits is predicted to have a topology in which two transmembrane segments are separated by a large extracellular ligand-binding loop, with amino and carboxyl termini residing within the cytoplasm.

Although the P_{2X} receptors bear no sequence similarity to other signal transduction proteins, their predicted topological organization is reminiscent of that proposed for the family of voltage-insensitive ion channels [9], which include inwardly-rectifying and pH-sensitive potassium channels, amiloride-sensitive sodium channels and presumptive mechanosensory ion channels from worms. Recent studies suggest that these channel complexes may be composed of four subunits, but the stoichiometry of functional ATP-gated channels has yet to be determined.

Figure 1



Predicted structures of **(a)** a nAChR and **(b)** an ionotropic ATP receptor. In both cases the M2 transmembrane segment is predicted to form the ion-conducting pore. The subunit stoichiometry of the nAChR is well established, while that of the ionotropic ATP receptor remains speculative. Arrows represent glycosylation sites.

When the first two P_{2X} receptors were cloned, the only entry in the sequence databases showing any sequence similarity was a partial cDNA clone called RP-2; this clone was isolated in a screen designed to identify genes whose expression is induced during programmed cell death in rat thymocytes [10]. As it turns out, RP-2 encodes a protein corresponding to P_{2X1}. In the light of previous observations that extracellular ATP can elicit programmed cell death in thymocytes and other cell types (e.g., see [11]), this cloning convergence provides additional evidence to support the intriguing notion that ATP might act as a paracrine or autocrine signaling molecule during apoptotic death in some physiological systems.

Cloned cDNAs representing at least six different P_{2X} subtypes have now been reported (see [12] and references therein) and all are capable of forming functional homomeric channel complexes when expressed in *Xenopus* oocytes or transfected mammalian cells. In general, these channels resemble native P_{2X} receptors in showing a lack of selectivity among small monovalent cations, a reversal potential near zero millivolts, and an inward rectification of their current–voltage relationships. Some channels also exhibit a relatively high permeability to calcium ions and a marked potentiation of ATP-elicited current responses in the presence of extracellular zinc. These properties may be important for the function of P_{2X} receptors in the long-term modulation of cellular processes such as cell death (apoptosis) or synaptic plasticity.

Properties of these heterologously expressed cDNAs appear to account for many, but by no means all, of the diversity of ATP-gated channels studied in various tissue preparations. This suggests that there are still more subtypes to be cloned and/or that some of the native receptors are heteromeric complexes formed by association of different subunits. The formation of heteromers between P_{2X2} and P_{2X3} receptors has been observed; co-expression of these two subunits results in the formation of ATP-gated channels with properties different from those of either of the homomeric channels [13,14]. The properties of these heteromeric channels resemble those reported for channels found in sensory neurons of the nodose ganglion and dorsal root ganglion (namely a slowly desensitizing response to α,β-methylene ATP) [15]. As discussed below, the sensory nervous system represents a particularly interesting opportunity for molecular biology to clarify the subunit composition of native ATP-gated channel complexes within the pain signaling pathway. Defining the structure and expression of P_{2X} receptors will provide a

basis for the rational design of novel therapeutic agents for the control of pain.

ATP and pain

It has been over forty years since Holton and Holton [16] suggested that ATP released from sensory neurons is important in synaptic transmission. Building on this early observation, other investigators sought to establish that ATP acts as a neurotransmitter, directly activating the dorsal horn neurons that are responsible for pain transduction in the spinal cord. Early studies were limited by the fact that ATP is actively metabolized to adenosine in the extracellular space. Adenosine is now recognized as an inhibitory transmitter in the dorsal horn of the spinal cord, acting through its own family of purinergic (P_1) receptors [17,18]. With the development of neuronal cell culture preparations and patch-clamp recording methods, ATP has been unambiguously identified as a direct activator of ion channels on spinal cord dorsal horn neurons and primary afferent neurons from a variety of sources, including both somatic and visceral sensory ganglia [19].

What might be the role of these channels in the sensory transduction of pain, both at the site of tissue damage and at spinal cord synapses that relay sensory information to the brain? ATP released from damaged cells into the extracellular space could activate primary afferent nociceptors, specialized sensory neurons that transduce intense thermal, mechanical, and noxious chemical stimuli into the electrochemical signals of pain. This hypothesis is supported by the observation that ATP induces pain in a human blister-based model [20]. Direct evidence for the selective expression of ATP-gated channels in sensory neurons has been provided by the cloning of several P_{2X} subtypes. As mentioned above, the P_{2X3} subtype, whose expression is restricted to sensory neurons [14], can assemble with the more widely expressed P_{2X2} subunit. Together, these subtypes form ATP-gated channels whose properties resemble native receptors found on sensory neurons [13]. As these cloned receptor subunits have just become available for study, their role in signal transduction in nociceptors and other sensory neurons can now be more fully explored.

The notion that ATP has a role in the spinal cord pain pathway is supported by observations that the P_2 antagonist suramin can reduce pain transduction in rats when placed in the cerebrospinal fluid at the level of the spinal cord. Similar administration of the P_{2X} receptor agonists α,β-methylene ATP or 2-methylthio ATP reduces the latency of a pain response to a heat stimulus applied to the tail, which is interpreted as enhancing pain signal transduction [21]. At the molecular level, most P_{2X} receptor subtypes cloned to date show significant expression within the spinal cord [12], including superficial layers of the dorsal horn, where primary afferent nociceptors make synaptic connections with spinal interneurons. These interneurons connect with both local reflex loops and ascending pathways that transmit sensory signals to the brain, where they are ultimately perceived as pain. Whole-cell electrophysiological recordings from a subset of dorsal horn neurons suggest that ATP is an enhancer of the excitatory actions of the neurotransmitter glutamate, but it remains possible that ATP is itself a principal excitatory agent within the spinal cord [22].

A new frontier for drug design

ATP is fast being recognized as an important extracellular signaling molecule in a vast array of biological processes. Nevertheless, the pharmacopœia available for the study and manipulation of ATP receptors is still rather primitive. As molecular studies show, the ATP-receptor family is more complex than previously appreciated, and the need for receptor-subtype-selective drugs has become all the more apparent. For example, the P_{2X4} receptor subtype is relatively insensitive to many compounds commonly used to define P_{2X} sites [23]. Thus, identifying fast, excitatory responses to ATP *in vivo* will require the development of new compounds that can distinguish synaptic events mediated by ATP from those elicited by other excitatory transmitters. Further motivation for the development of specific and potent ATP-receptor agonists and antagonists is provided by the possibility that these drugs may be useful in the management of common physiologic disorder, including pulmonary and cardiovascular disease, urologic dysfunction, inflammation and pain syndromes.

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