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Review

Nonsynaptic and nonvesicular ATP release from neurons and relevance to neuron–glia signaling

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ABSTRACT

Studies on the release of ATP from neurons began with the earliest investigations of quantal neurotransmitter release in the 1950s, but in contrast to ATP release from other cells, studies of ATP release from neurons have been narrowly constrained to one mechanism, vesicular release. This is a consequence of the prominence of synaptic transmission in neuronal communication, but nonvesicular mechanisms for ATP release from neurons are likely to have a broader range of functions than synaptic release. Investigations of activity-dependent communication between axons and myelinating glia have stimulated a search for mechanisms that could release ATP from axons and other nonsynaptic regions in response to action potential firing. This has identified volume-activated anion channels as an important mechanism in activity-dependent ATP release from axons, and renewed interest in micromechanical changes in axons that accompany action potential firing.

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1. Introduction

1.1. A brief history of ATP release

In 1927 Cyrus Fiske and Yerlagadda Subbarow, working at Harvard Medical School, applied a new colorimetric assay they had developed for determination of inorganic phosphate to an extract of freshly dissected cat muscle. They observed a curious delay in color production. "The time required to reach a constant reading was about thirty minutes, whereas ordinarily the full color (relative to the standard) has developed within four minutes or less [1]." Either something in the extract was inhibiting the reaction or something in muscle was generating inorganic phosphate. They concluded from further experiments that an organic molecule in muscle must be generating phosphate. Furthermore, they reported that electrical stimulation of muscle or depriving the tissue of

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oxygen depleted the "labile phosphorus", from the tissue. "The outcome appears likely to throw light on a field of biochemistry never before suspected of being in any way related to phosphoric acid." Two years later, in 1929, they identified the compound as adenosine 5'-triphosphate (ATP), which generates inorganic phosphorus by hydrolysis of its three phosphate atoms [2].

That same year, Albert Szent-Györgyi and Alan Drury found that substances they isolated from muscle and injected intravenously into dogs and other animals disturbed the cardiac rhythm, dilated the coronary artery, and inhibited smooth muscle contraction in the intestine [3]. They concluded that the molecule in muscle extract responsible for the effects was "adenylic acid." In a footnote they reported, "Through the kindness of C. H. Fiske we have been able to test the action of the pyrophosphoric or diphosphoric ester of adenylic acid. Its action is similar to that of adenylic acid and adenosine." (p. 218).

Thus in the same year, the two fundamental actions of this versatile biological molecule were discovered: as the energy source for metabolism and biochemical reactions, and as an extracellular signaling molecule. In 1930, Subbarow, was awarded the PhD degree. He later discovered the antibiotic tetracycline, the anticancer drug methotrexate, and isolated the vitamin folic acid, among many other important pharmacological discoveries [4]. Szent-Györgyi would receive the Nobel Prize in 1937 for discovering vitamin C.

Research on the extracellular signaling function of ATP lagged considerably behind research on ATP as a cellular energy source, because the biology of cell signaling could not be elucidated by biochemistry alone, but also because release of ATP from a cell seemed incongruous with its vital role as the cellular energy source. In his review paper published in 1982, titled "Does ATP Cross the Cell Plasma Membrane?" [5], Irshad Chaudry concluded that experimental evidence clearly established this fact, but the mechanisms were unclear. He argued that the critical role of ATP in cellular respiration and energy required that ATP must be able to cross membranes of intracellular organelles, so in principle, regulated movement of ATP across the plasma membrane seemed highly plausible.

1.2. Release of ATP from non-neuronal cells

The discovery of ATP from muscle extracts and its action in stimulating heart rate and dilating arteries suggested a possible signaling role for the molecule in increasing blood supply to muscle tissue during exertion. Boyd and Forrester studied several substances that were released by muscle into a bathing solution, which when applied to frog heart would increase the heart rate. In 1968 they identified ATP as one of these molecules liberated from muscle without tissue damage [6]. Later studies showed that ATP was released into the bloodstream of humans after exercise [7–9]. In the 1970s and 1980s fluid shear force [10], and hypoxia [11,12] and various ligands [13] were shown to stimulate release of ATP from muscle or endothelial cells, but whether this was a specific secretory mechanism or a non-specific increase in membrane permeability was unclear.

Today many mechanisms for ATP release from cells have been identified and they are activated by a wide range of stimuli [for reviews 14–18]. These ATP release mechanisms include damage to the cell membrane, mechanical stress, hypoxia, inflammation, several agonists, and electrical excitation of neural tissue. In non-neuronal cells, several cellular mechanisms for ATP release have been identified and studied extensively, including exocytosis, gap junction hemichannels, ion channels, the cystic fibrosis transmembrane conductance regulator, P2X7 receptors, and nucleoside transporters. This is in marked contrast to ATP release from neurons where essentially only one mechanism of ATP release has been investigated.

1.3. Release of ATP from neurons

The earliest studies showed that electrical stimulation of axons liberated ATP [19,20]. These studies were contemporaneous with the first suggestion that neurotransmitter release was quantal, first proposed by Del Castillo and Katz in 1957 [21], and the discovery of the synaptic vesicle in 1954 [22,23]. Studies on synaptosomes showed that ATP was present in high concentrations inside synaptic vesicles [24–26], and chromatography [27,28], radioactive tracer studies [29–31], and firefly chemiluminescence assay [32,33], showed that ATP was released by neurons in the CNS and PNS. Electrophysiological studies in the 1980s and 1990s, too numerous to list comprehensively here, firmly established ATP as a neurotransmitter throughout the nervous system [34–37 and Lalo et al., this issue]. As late as 1996, however, Hamann and Attwell disputed the evidence that ATP was released from synaptic vesicles [38]. This was based on their studies failing to detect ATP release from brain slice unless the stimulus voltage was raised to extreme levels that damaged cell membranes. Although they used methods similar to those used previously (detecting firefly chemiluminescence with a photomultiplier tube), the sensitivity of earlier studies showing release of ATP from hippocampal slice [32] was far greater than that of Hamann and Attwell (6.2×10^{-11} M vs. 1×10^{-6} M ATP). Recently the ATP transporter that concentrates ATP into vesicles has been identified [39], removing any doubt that ATP can be released by vesicular mechanisms. There was some challenge in the 1970s and 1980s to the vesicular mechanism of neurotransmitter release in general [40–42], but exocytosis was and remains almost universally accepted as the mechanism whereby neurotransmitters and neuromodulators are release from neurons.

1.4. ATP release from axons and extra-synaptic regions

As the defining feature of the neuron doctrine, the synapse has long been the focus of research on neurotransmission and neuronal plasticity. Synaptic release of neurotransmitter has also guided thinking about many other aspects of cell signaling in the nervous system, such as the effects of neural activity on development of neurons and glia. However, many developmental events regulated by neural activity, for example, axon outgrowth, cell proliferation, migration, and differentiation, operate before synaptogenesis. (See for example [43].) When calcium imaging revealed responses in glia (Schwann cells and astrocytes) that were initiated by electrical stimulation of neurons, spillover of transmitter released into the synaptic cleft was understood to be the basis for activity-dependent neuron–glia communication [44–46]. Likewise, regulation of synaptic transmission by glia was considered the focus of glial influence on information processing in neural circuits [47–49]. ATP quickly emerged early as one of the primary intercellular signaling molecules activating calcium responses in glia, and in communication between all types of glial cells [50,51]. However, calcium imaging showed that glia situated along axon segments could also respond to neurotransmitters and ATP released by axons during electrical stimulation [52–54], and that impulse activity, by releasing ATP from axons, could affect proliferation, differentiation, and myelination of Schwann cells [52,55] and oligodendrocytes [56,57]. Although synapses are not present in nerve segments, release of ATP and other neurotransmitters from axons was assumed to be by exocytosis of vesicles with the axolemma [54], or, in the case of glutamate, by reversal of neurotransmitter reuptake mechanisms [53]. In support of the vesicular release of ATP from axons, removal of extracellular calcium was found to inhibit the glial calcium responses to axon stimulation [54].