Calcium and ATP control multiple vital functions

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Introduction

Life on Planet Earth, as we know it, revolves around adenosine triphosphate (ATP) as a universal energy storing molecule. The metabolism of ATP requires a low cytosolic Ca\(^{2+}\) concentration, and hence tethers these two molecules together. The exceedingly low cytosolic Ca\(^{2+}\) concentration (which in all life forms is kept around 50–100 nM) forms the basis for a universal intracellular signalling system in which Ca\(^{2+}\) acts as a second messenger. Maintenance of transmembrane Ca\(^{2+}\) gradients, in turn, requires ATP-dependent Ca\(^{2+}\) transport, thus further emphasizing the inseparable links between these two substances. Ca\(^{2+}\) signalling controls the most fundamental processes in the living organism, from heartbeat and neurotransmission to cell energetics and secretion. The versatility and plasticity of Ca\(^{2+}\) signalling relies on cell specific Ca\(^{2+}\) signalling toolkits, remodelling of which underlies adaptive cellular responses. Alterations of these Ca\(^{2+}\) signalling toolkits lead to aberrant Ca\(^{2+}\) signalling which is fundamental for the pathophysiology of numerous diseases from acute pancreatitis to neurodegeneration. This paper introduces a theme issue on this topic, which arose from a Royal Society Theo Murphy scientific meeting held in March 2016.

This article is part of the themed issue ‘Evolution brings Ca\(^{2+}\) and ATP together to control life and death’.

1. ATP and Ca\(^{2+}\) link cellular energetics and signalling

Calcium ions (Ca\(^{2+}\)) and adenosine triphosphate (ATP) are those fateful molecules that defined the form of life that emerged and evolved on Planet Earth. Indeed, ATP is a universal substrate for energy storage, whereas Ca\(^{2+}\) is a ubiquitous intracellular signalling molecule; moreover, ATP-based energetics and Ca\(^{2+}\)-based signalling are profoundly interdependent. By some unaccounted chance, the primordial alkaline ocean provided an environment specifically suitable for ATP metabolism, which can proceed only at a very low (sub-micromolar) concentrations of ionized Ca\(^{2+}\). As a result, the cytosol of the most primitive cellular ancestors contained exceedingly low levels of cytosolic free Ca\(^{2+}\). When the Ca\(^{2+}\) concentration in the ocean started to increase (due to acidification and wash-out of Ca\(^{2+}\) from the rocks) the development of a system controlling Ca\(^{2+}\) movements across the cellular membrane became of vital importance, and provided evolutionary pressure for emergence of cellular Ca\(^{2+}\) homeostatic and Ca\(^{2+}\)-based signalling systems [1,2]. This rapid increase in the environmental [Ca\(^{2+}\)] instigated an explosive evolution of eukaryotes and then of multicellular life forms [3]. The Ca\(^{2+}\) signalling system, which uses the immense transmembrane concentration gradient for Ca\(^{2+}\), utterly depends on ATP, as maintenance of a low cytosolic free [Ca\(^{2+}\)] ultimately requires energy-dependent Ca\(^{2+}\) trans-
Ca\(^{2+}\) sensors) controlling cellular responses. The Ca\(^{2+}\) signals are necessary attributes of life from its very beginning to its very end. A spermatozoid penetrating an oocyte instigates a specific form of Ca\(^{2+}\) signal that seals the oocyte membrane thus allowing conception, whereas uncontrolled Ca\(^{2+}\) entry, which occurs in stress and trauma, signals cell death. In between lies the realm of Ca\(^{2+}\) regulated cell functions. In muscle, Ca\(^{2+}\) signals trigger contraction, in secretory cells cytosolic \([\text{Ca}^{2+}]\) rises evoke secretion, in the nervous system intracellular Ca\(^{2+}\) fluctuations underlie neurotransmission, plasticity and integration, while long-lasting Ca\(^{2+}\) signals regulate expression of genes in all types of cells. The universality of these functions reflects the versatile ensemble of Ca\(^{2+}\) regulating molecular cascades, which, by being assembled into specific and highly plastic toolkits [6], ensure maximal adaptability of the functional outputs. Aberrant remodelling of these toolkits, however, leads to pathology, and abnormal Ca\(^{2+}\) signalling contributes to numerous diseases, from heart failure and pancreatitis to neurodegeneration [7–9].

2. Calcium signalling and purinergic transmission in the nervous system

The unparalleled computing power of the human brain is defined by in excess of 15 trillion connections that integrate approximately 300 billion neural cells into a cellular network optimized for parallel information processing. Operation of these connections, which are defined as chemical and electrical synapses, is ultimately controlled by ATP and Ca\(^{2+}\). Conceptually, neuronal cells are represented by electrically excitable (i.e. capable of generating propagating action potentials) neurons and electrically non-excitable neuroglial cells. Both types of neural cells communicate through chemical messengers, which are either secreted into the extracellular space or directly propagated via gap junctions in cellular syncytia. Neuronal connectivity is mainly mediated through chemical synapses, in which neurotransmitters released from axonal terminals diffuse to the target cell and activate receptors, which control excitability of postsynaptic cells. Neurotransmitters are localized in the synaptic vesicles concentrated in axonal terminals; concentration of neurotransmitters in these vesicles occurs through specific vesicular transporters (for glutamate, GABA or ATP) that use H\(^{+}\) gradients established by vesicular ATPases. Exocytosis of synaptic vesicles is governed by local microdomains of high ionized \([\text{Ca}^{2+}]\) that rapidly evolve following opening of voltage-dependent Ca\(^{2+}\) channels [10]; this being the fundamental mechanism of excitation–secretion coupling [11]. At the postsynaptic site, activation of neurotransmitter receptors triggers depolarization and produces complex cytosolic Ca\(^{2+}\) signals which in turn regulate synaptic plasticity and postsynaptic integration. Pathological remodelling of postsynaptic Ca\(^{2+}\) signalling is a leading mechanism in synaptic dysfunction which translates into cognitive deficiency, for example, in neurodegenerative diseases [12]. Preservation of physiological Ca\(^{2+}\) signalling, and hence of a healthy Ca\(^{2+}\) signalling toolkit, seems to be imperative for lifelong adaptive plasticity. Several lines of evidence point to a particular role for vitamin D in long-term regulation of Ca\(^{2+}\) homeostasis (together with redox homeostasis, which is again inseparable from ATP metabolism); incidentally, vitamin D deficiency leads to an increased prevalence of age-dependent neurodegeneration and senile dementia, which may, at least in part, be associated with impaired Ca\(^{2+}\) signalling [13]. Several Ca\(^{2+}\)-dependent signalling cascades are fundamental for the integration of neuronal networks with their supportive neuroglial environment. Evolution of the nervous system went through a deep specialization and separation of function between different classes of neural cells: neurons perfected their electrical excitability for fast synaptic transmission, whereas all homeostatic and defensive tasks were shifted to glia. Astroglia, in particular, became principal homeostatic cells of the CNS, being responsible for maintaining the controlled environment that permits proper operation of neuronal ensembles [14]. Astrocytes are integrated into multicellular syncytia through gap junctions; these permit intercellular diffusion of various molecules including second messengers. Diffusion of inositol 1,4,5-trisphosphate (IP\(_3\)) underlies long-range propagating Ca\(^{2+}\) waves that serve as a substrate for astroglial excitability. Astroglial Ca\(^{2+}\) signals control multiple vital functions, from secretion to mounting defensive reactive responses [8,15].

The roles of ATP in synaptic transmissions are many; apart from excitation–secretion coupling, ATP controls multiple stages in vesicular formation and maturation, and it is indispensable for restoring ionic gradients. In the context of synaptic physiology, however, ATP plays another fundamental role, being a major neurotransmitter. ATP as a signalling molecule for intercellular communications is unique, being almost ubiquitous: purinergic transmission is present in all living forms, from protists and fungi to plants and animals [16]. In the nervous system, purinergic transmission is similarly ubiquitous without any apparent anatomical segregation that is characteristic for other neurotransmitters (for example, dopamine) and ATP is present mainly in the midbrain and nigro-striatum and acetylcholine (ACh) in the brainstem, hippocampus and parts of the cortex as well as being the main peripheral neurotransmitter). ATP released from axonal terminals as well as from astroglia acts as a co-transmitter in many central synapses and as a transmitter in the periphery and contributes to both electrical excitability and numerous trophic effects, being involved in regulation of development, metabolism, structural plasticity and ageing [17].

Purinergic signalling is mediated through an extended family of purinoceptors, classified into four major groups of P1/A1 adenosine receptors, P2X ionotropic ATP receptors, P2Y metabotropic purinoceptors and P0 adenosine receptors [18]. Metabotropic P2Y receptors, which are activated by ATP, ADP and UTP, mainly exert trophic effects; in particular, these receptors control neurogenesis in development and adulthood [19]. The P2X ionotropic receptors are trimeric cationic (i.e. Na\(^{+}\)/K\(^{+}\)/Ca\(^{2+}\)) ligand-gated channels assembled (in homo- or heteromeric fashion) from seven subunits known as P2X\(_1\) to P2X\(_7\) according to the historic order of cloning [20]. These P2X receptors are widely distributed through neurons and neuroglia and also contribute to many aspects of synaptic plasticity. The P2X\(_7\) receptor, in particular, is widespread in microglial cells and its activation controls microglial defensive and immune capabilities. All P2X receptors are permeable to Ca\(^{2+}\) and their activation triggers complex Ca\(^{2+}\) signals, which regulate synaptic transmission. Ionotropic purinoceptors share their topology with the acid-sensitive ion channel (ASIC), which is also widely distributed through neural cells. Proton ATPases are invariably present in synaptic vesicles and their lumen is therefore rich in protons and hence intensely acidic (pH approximately 5). As a result, vesicular release of
3. Roles of intracellular Ca\(^{2+}\) and ATP in the control of exocytosis

The classical work of Baker & Knight [22,23] established the critical importance of intracellular Ca\(^{2+}\) and ATP for the control of exocytotic secretion from permeabilized adrenal chromaffin cells (figure 1a). It is now clear that a rise in the cytosolic Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) is the trigger for initiating exocytosis in a wide range of cell types, but that this only happens in the presence of millimolar levels of intracellular ATP. The molecular mechanism by which a local rise in [Ca\(^{2+}\)]\(_i\) triggers release of neurotransmitters from nerve endings has been elucidated in considerable detail by the work of Sudhof and his collaborators [25]. It is interesting to compare the Ca\(^{2+}\)-sensitivity of exocytosis to that of another secretory function, namely fluid secretion in exocrine glands, which depends on Ca\(^{2+}\) activation of high-conductance K\(^+\) channels (see later section). As seen in figure 1b, an increase in [Ca\(^{2+}\)]\(_i\) from 0.1 to 1 \(\mu\)M at the physiological membrane potential of \(-40\) mV causes a very substantial increase in the open-state probability of the channel and, indeed, such a change in [Ca\(^{2+}\)]\(_i\) causes a doubling of exocytotic secretion, as seen in figure 1a.

Although both Ca\(^{2+}\) and ATP are needed for secretion, the relationship between the control of intracellular Ca\(^{2+}\) and ATP levels varies markedly between cell types. As an example of this, it is instructive to compare the sequence of events in two different cell types present in the same organ, namely the pancreas (figure 2). The quantitatively dominant exocrine acinar cells secrete digestive (pro)enzymes, which are required for the digestion of food products, whereas the endocrine beta-cells secrete insulin, required for the control of blood sugar levels. In both cases, there is a need for Ca\(^{2+}\) signals to stimulate exocytosis (stimulus–secretion coupling) and a requirement for adjusting metabolism to the increased energy demand of the secretion process (stimulus–metabolism coupling).

In the pancreatic acinar cells, ACh—released from periacinar nerve endings—activates receptors on the basolateral membrane generating IP\(_3\) inside the cells which, in turn, releases Ca\(^{2+}\) from the endoplasmic reticulum (ER) [26,27]. The subsequent rise in [Ca\(^{2+}\)]\(_i\) in the apical (secretory) region, where the IP\(_3\) receptors are localized, causes immediate uptake of Ca\(^{2+}\) into the peri-granular mitochondria via the mitochondrial Ca\(^{2+}\) uniporter in the inner mitochondrial membrane. Recent work suggests that the gating of this pathway is controlled by a Ca\(^{2+}\) sensor in the mitochondrial matrix [28]. The rise in the intra-mitochondrial [Ca\(^{2+}\)]\(_i\), in turn, activates the three Ca\(^{2+}\)-sensitive dehydrogenases in the Krebs cycle, stimulating ATP production [26]. In spite of the enhanced ATP utilization, due to the energy requirement for exocytosis, the overall result of this regulation is that the intracellular ATP level increases. As shown in figure 2a, the sequence of events in the pancreatic acinar cells is such that the initial rise in [Ca\(^{2+}\)]\(_i\) triggers mitochondrial ATP generation. The rise in [Ca\(^{2+}\)]\(_i\) also opens Ca\(^{2+}\)-sensitive ion channels in the plasma membrane which is important for fluid secretion (not shown in situ).
in figure 2, but see next section). The initial release of Ca$^{2+}$ from intracellular stores, particularly from the ER, also triggers opening of store-operated Ca$^{2+}$ channels (not included in figure 2, but see later section), which enable store refilling [29,30].

In the neighbouring insulin-secreting beta-cells, a very different relationship exists between regulation of Ca$^{2+}$ and ATP (figure 2b). The increase in the plasma glucose concentration after a meal results in glucose uptake into the beta-cells and its metabolism then leads to an increased mitochondrial ATP production. The resulting rise in the ratio of ATP/ADP closes ATP/ADP-sensitive K$^+$ channels (figure 2), and that in vivo is linked to AMPA receptor-independent elevations of external [K$^+$] and concomitant decreases in the external concentrations of Ca$^{2+}$, Mg$^{2+}$ and H$^+$ [36]. Importantly, local cortical activity of sleeping mice could be converted to the stereotypical EEG pattern of wakefulness by imposing a change in the extracellular ion composition [36]. Thus, the kind of ion changes seen in glandular tissues (figure 3) not only can be observed in the brain (figure 3b), but appear to play an extremely important role in the transition between different behavioural states.

4. Roles of intracellular Ca$^{2+}$ and ATP in controlling fluid and electrolyte movements

Cytosolic Ca$^{2+}$ signals, in addition to stimulating exocytotic secretion of enzymes, hormones or neurotransmitters, also activate fluid secretion in exocrine glands, such as the salivary glands, the lacrimal gland, the sweat glands and the pancreas [27,33,34]. In the resting (unstimulated) condition, the salivary glands, for example, secrete little or no fluid, but nerve stimulation—via release of ACh or noradrenal in from parasympathetic or sympathetic nerve endings, respectively, in the gland tissue—will induce an immediate and substantial fluid flow. For the salivary glands, as well as the lacrimal and sweat glands, fluid secretion is the principal organ function, whereas in the pancreas it is the necessary vehicle for the washout of secreted (pro)enzymes into the gut [34].

The mechanism by which cytosolic Ca$^{2+}$ signals activate exocrine fluid secretion is now well established [33] and involves opening of Ca$^{2+}$-activated K$^+$ channels (figure 1b) in the baso-lateral and Ca$^{2+}$-activated Cl$^-$ channels in the apical acinar membrane, allowing operation of the Na$^+$, K$^+$, 2Cl$^-$ co-transporter in the baso-lateral membrane. Thus Cl$^-$ is taken up across the baso-lateral membrane by a combination of three transport proteins, namely the Ca$^{2+}$-activated K$^+$ channels, the Na$^+$, K$^+$, 2Cl$^-$ co-transporters and the Na$^+$/K$^+$/2Cl$^-$ pumps, and released into the glandular lumen via the apical Cl$^-$ channels. K$^+$ recirculates across the baso-lateral membrane via Na$^+$/K$^+$ pumps, K$^+$ channels and the Na$^+$, K$^+$, 2Cl$^-$ co-transporters. Na$^+$ moves into the glandular lumen via para-cellular pathways, through leaky tight junctions, attracted by the lumen negativity created by the opening of the apical Ca$^{2+}$-activated Cl$^-$ channels [33].

Ca$^{2+}$ activation of the Cl$^-$ and K$^+$ channels causes a very marked rise in the K$^+$ concentration in the extracellular (interstitial) fluid of the glands, as seen, for example, by the sharp rise in [K$^+$] in the venous outflow from the perfused submandibular gland upon intra-arterial injection of ACh or noradrenal (figure 3e). In the exocrine glands, the substantial change in the interstitial fluid [K$^+$] associated with activation of fluid secretion is not in itself of major importance, but simply reflects necessary ionic shifts required for fluid formation. It is, however, very interesting that Nedergaard and her collaborators [36] have very recently shown that neuromodulators, including catecholamines, induce increases in the extracellular [K$^+$] in brain cortical slices electrically silenced by tetrodotoxin (figure 3b) and that in vivo arousal is linked to AMPA receptor-independent elevations of external [K$^+$], and concomitant decreases in the external concentrations of Ca$^{2+}$, Mg$^{2+}$ and H$^+$ [36]. Importantly, local cortical activity of sleeping mice could be converted to the stereotypical EEG pattern of wakefulness by imposing a change in the extracellular ion composition [36]. Thus, the kind of ion changes seen in glandular tissues (figure 3) not only can be observed in the brain (figure 3b), but appear to play an extremely important role in the transition between different behavioural states.

5. Abnormal Ca$^{2+}$–ATP relationships drive pathological processes

The precise pattern (spatial extension and timing) of physiological Ca$^{2+}$ signals varies enormously between different cell types but, generally, such signals consist of repetitive [Ca$^{2+}$] spikes and often these are confined to a specific sub-cellular space where local Ca$^{2+}$ regulation is required. Localized Ca$^{2+}$ signaling is possible because of the restricted diffusion of Ca$^{2+}$ in comparison with, for example, Mg$^{2+}$ [37]. However, localized Ca$^{2+}$ signals can only be transient; sustained [Ca$^{2+}$], elevations will always become global and this, usually, leads to pathological effects. Examples of such pathology are described by Maleth & Hegyi [34] and Peng et al. [27]. Sustained global [Ca$^{2+}$], elevations are toxic, partly because the Ca$^{2+}$ signal reaches parts of the cell that under normal physiological circumstances should not have been invaded and partly because overloading of mitochondria with Ca$^{2+}$ causes a decrease in ATP formation due to depolarization of the inner mitochondrial membrane caused by opening of the permeability transition pores [38]. Cytosolic Ca$^{2+}$ overloading initiates a ‘circulus vitiosus’ in which the markedly reduced mitochondrial ATP generation will prevent Ca$^{2+}$ pumps, both in the plasma membrane and in the ER, from clearing the cytosol of the excessive Ca$^{2+}$ content, resulting in a further [Ca$^{2+}$], elevation [26].

6. Store-operated Ca$^{2+}$ entry, its role in pathology and how it can be inhibited

In electrically non-excitable cells, such as epithelial and immune cells, which do not possess voltage-gated Ca$^{2+}$ channels, Ca$^{2+}$-release activated Ca$^{2+}$ (CRAC) channels constitute the main Ca$^{2+}$ entry pathway and these channels, as the name implies, are opened as a direct consequence of Ca$^{2+}$ release from intracellular stores, via a now well characterized molecular machinery [30,39]. By monitoring simultaneously the emptying of the ER Ca$^{2+}$ store (after inhibition of the ER Ca$^{2+}$ pumps by thapsigargin) and the CRAC current, it can be seen that the rise of the current follows closely the decrease in the intra-store [Ca$^{2+}$] [29]. Excessive primary Ca$^{2+}$ release from intracellular stores is generally the initiating event in the actions of toxic agents or excessive concentrations of neurotransmitters and hormones [27,29], but cytosolic Ca$^{2+}$ overload
requires Ca\(^{2+}\) entry through the CRAC pathway and this is therefore increasingly seen as an attractive molecular target for drug therapy [27,29,30].

The principal argument against CRAC inhibition as a potential treatment against, for example, asthma [30] or pancreatitis [27,29,34] is that CRAC channels are widely distributed in the body, so that unintended side-effects would be likely to occur. Nevertheless, it has recently been shown in three different in vivo mouse models of experimental pancreatitis that CRAC inhibition is a remarkably effective treatment [40]. With regard to this disease, there is a ‘window of opportunity’ in the acute stage [41], where inhibition of not only pancreatic Ca\(^{2+}\) entry but also Ca\(^{2+}\) entry into immune cells would be beneficial to combat this inflammatory disease. Unfortunately, at this stage, there are no CRAC channel blockers approved for clinical use, but this could soon change [30].

Interplay of Ca\(^{2+}\) signalling between different, but neighbouring, cell types can amplify the effects of toxic agents but, if CRAC channels are involved in both types of cells, pharmacological inhibition of this Ca\(^{2+}\) entry pathway can be particularly effective. This seems to be the case in pancreatitis. In the quantitatively dominant acinar cells, toxic agents, such as fatty acid ethyl esters or bile acids, cause excessive intracellular Ca\(^{2+}\) release followed by excessive CRAC-mediated Ca\(^{2+}\) entry [27,29,34]. If sustained, this would activate trypsin inside the cells and lead to necrosis with leakage of activated proteases into the interstitial fluid. Here, one of the proteases, kallikrein, would liberate bradykinin (BK) from kininogen and BK would act on type 2 BK receptors in the neighbouring stellate cells. This, in turn, would cause intracellular Ca\(^{2+}\) release and subsequent Ca\(^{2+}\) entry into the stellate cells via CRAC channels [42,43]. Ca\(^{2+}\) signal generation in the stellate cells, via an unknown mechanism, exacerbates the toxic effects of fatty acid ethyl esters and bile acids on the acinar cells [42]. CRAC channel inhibition would diminish excessive Ca\(^{2+}\) signal generation in both acinar and stellate cells [27,34,42,43], and this may

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**Figure 3.** Stimulant-evoked changes in the extracellular [K\(^{+}\)] in a salivary gland and the brain. (a) Results from an experiment on an isolated perfused submandibular gland in which changes in the [K\(^{+}\)] in the venous effluent from the gland, evoked by brief intra-arterial injections of ACh or adrenalin (Adr), are monitored. [K\(^{+}\)] in the arterial inflow was 4 mM. (b) Traces of [K\(^{+}\)]\(_e\) (extracellular fluid) shifts in cortical brain slices after administration of a neuromodulator cocktail (noradrenalin, ACh, dopamine, orexin and histamine). Scale bars (lower right corner), horizontal, 5 min; vertical, 0.2 mM [K\(^{+}\)]\(_e\). For further explanation, see text. (a) Adapted from Petersen [35] and (b) adapted from Ding et al. [36].
be the reason for the remarkable success of such inhibition in treating pancreatitis in the mouse in vivo [40].

7. Conclusion
Precise regulation of cellular functions is vitally important for virtually all aspects of the life of multicellular organisms. Ca$^{2+}$ has emerged through evolution as the most important regulator of a vast range of different cellular functions. Very subtle and precise mechanisms exist for controlling the handling of Ca$^{2+}$, even in very specific sub-cellular compartments, through an impressive array of Ca$^{2+}$ channels, transporters and pumps precisely localized and tuned to the specific needs of cells in different tissues. Enormous progress has been made in identifying these mechanisms and although many aspects still require more studies, we now have a good working knowledge of the basic control mechanisms of the major cellular functions. As most of such functions require energy in the form of ATP, it is essential that Ca$^{2+}$ and ATP work together, so that when Ca$^{2+}$ activates a particular function, for example, muscle contraction or secretion, ATP is made available. Although these links are relatively well understood at the single-cell level, they are less well understood at the integrated organ level and particularly in relation to overall brain function. In recent years, increasing attention has been paid to the role of Ca$^{2+}$ and ATP in various disease states, where dysregulation of the normal relationship between Ca$^{2+}$ signalling and mitochondrial ATP production occurs. Cytosolic Ca$^{2+}$ overloading is a major element of many diseases and much work has been, and continues to be, done on how to prevent this. Some promising avenues have opened up recently. The translation of new insights gained at the single-cell level into a full understanding of integrated organ function in vivo and application of our knowledge of how to interfere with specific critical pathways to real clinical scenarios are far from trivial and still pose significant challenges.

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