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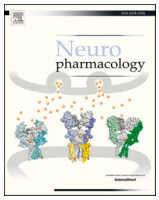

Channel-mediated ATP release in the nervous system

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A B S T R A C T

ATP is well established as a transmitter and modulator in the peripheral and central nervous system. While conventional exocytotic release of ATP at synapses occurs, this transmitter is unusual in also being released into the extracellular space via large-pored plasma membrane channels. This review considers the channels that are known to be permeable to ATP and some of the functions of channel-mediated ATP release. While the possibility of ATP release via channels mediating volume transmission has been known for some time, localised ATP release via channels at specialised synapses made by taste cells to the afferent nerve has recently been documented in taste buds. This raises the prospect that "channel synapses" may occur in other contexts. However, volume transmission and channel synapses are not necessarily mutually exclusive. We suggest that certain glial cells in the brain stem and hypothalamus, which possess long processes and are known to release ATP, may be candidates for both modes of ATP release -channel-mediated volume transmission in the region of their somata and more localised transmission possibly via either conventional or channel synapses from their processes at distal targets. Finally, we consider the different characteristics of vesicular and channel synapses and suggest that channel synapses may be advantageous in requiring less energy than their conventional vesicular counterparts.

This article is part of the Special Issue on "Purinergic Signaling: 50 years".

1. Purinergic signalling

ATP has been generally recognised as an intercellular signalling molecule since the classic review of Burnstock (1972). In the nervous system, ATP can act as a neurotransmitter and a gliotransmitter. While the release of ATP as a primary neurotransmitter at central synapses is rare, it is often coreleased with other neurotransmitters such as glutamate and GABA. ATP is perhaps the major gliotransmitter released by astrocytes via exocytosis and ion channels. Where ATP signalling differs from many other small molecule neurotransmitters, is that it is often released at the interface between the nervous system and the periphery. For example: release of ATP from bladder wall muscle on to nerve endings signals bladder distension; ATP is released from taste cells in the taste buds to signal sweet, umami, bitter and salt tastes (Roper, 2013); and ATP release at the ventral surface of the medulla oblongata contributes to the detection of systemic pH and CO_2 levels thereby regulating breathing to maintain these variables at physiological levels (Gourine et al., 2005). ATP signalling is also common in many other organs such as the liver, kidney and bone as well as playing key roles in the immune system and platelet aggregation. Perhaps this universality of ATP-signalling is to be expected. All cells make and use ATP as a central part of their metabolism and its supply is abundant, often reaching millimolar levels in cytosol. ATP is thus available for use to signal to neighbouring cells especially when, for the most part, the required concentration of ATP for signalling is 3-4 orders of magnitude lower than that present in cytosol.

Once released, ATP can act at a series of ligand-gated (P2X) and G-protein coupled receptors (P2Y) (Burnstock and Kennedy, 1985; Ralevic and Burnstock, 1998). The P2X receptors are trimeric non-selective cation channels; ATP thus mediates excitation. There are seven subtypes of P2X receptor each of which has somewhat different properties. The P2X7 receptor stand out as requiring high levels of ATP for activation. The P2X7, P2X2 and P2X4 receptors all have pores that are sufficiently large to allow the permeation of small molecules. Whereas the P2X receptors have an absolute requirement for ATP as the natural ligand, the P2Y receptors are much less selective. P2Y receptors can be activated by UTP, and some subtypes are preferentially activated by ADP and UDP.

A key part of chemical signalling is that there should be mechanisms for terminating the actions of the signalling agent. ATP signalling is no exception and there is a multiplicity of extracellular enzymes that degrade ATP to ADP, AMP and adenosine. These include the alkaline phosphatases, the ENTPDases, the ENPPases and ecto-5' nucleotidase. There are excellent reviews of this topic available (Zimmermann, 2000, 2006), but an important point is that these enzymes have different specificities; none are specific for adenine nucleotides, but some convert ATP to ADP whereas others effectively convert ATP to AMP or even adenosine. This is mechanistically significant as both ADP and adenosine can signal in their own right via specific receptors (P2Y1, P2Y12 and P2Y13 for ADP and A1, A2a, A2b and A3 for adenosine) (Ralevic

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and Burnstock, 1998).

The topic of this review is the process by which the entire purinergic signalling cascade is set in motion: the release of ATP. In his 1986 review, John Gordon observed that "ATP is a ubiquitous intracellular constituent and therefore any cell could potentially serve as a source of extracellular ATP" (Gordon, 1986). Gordon even speculated that channels might have pores large enough to serve as conduits for the release of ATP. While ATP had long been known to be released via exocytotic mechanisms, the discovery that Slc17A9 constitutes a vesicular nucleotide transporter (VNUT) capable of loading ATP into vesicles identified a key molecular component required for vesicular ATP release (Sawada et al., 2008). Nevertheless, Gordon's speculation of channel-mediated ATP release was highly prescient, and this is a major mechanism of ATP release both peripherally and within the nervous system. An encyclopaedic review of channel mediated release of ATP has been written by Taruno (2018); this review builds on this prior work by considering mechanistic aspects of this release process in the context of the peripheral and central nervous system.

2. Channels capable of releasing ATP

Demonstration that ATP permeates the pore of a channel (as opposed to being the downstream consequence via some other mechanism of activation of the channel) is difficult to achieve rigorously. The best evidence is electrophysiological where careful, preferably single channel, measurements can demonstrate permeation of ATP through the pore. This has been achieved for Cx43 (Kang et al., 2008), pannexin-1 (Bao et al., 2004; Narahari et al., 2021) and the maxi anion channel (Sabirov et al., 2001). An alternative approach is to directly examine ATP release and show its dependence on the expression of the channel in a model system. If this is also combined with pharmacology or genetic manipulations such as siRNA knockdown and careful controls this evidence also gives a high degree of assurance that ATP does indeed permeate the channel. This type of evidence has been gained for many connexins (Huckstepp et al., 2010a; Nualart-Marti et al., 2013a), pannexins (Chekeni et al., 2010), Volume-regulated anion channels -LRRC8 (Gaitan-Penas et al., 2016), CalHMs (Ma et al., 2018; Taruno et al., 2013) and P2X7R (Pellegatti et al., 2005). These findings are summarised in Table 1.

Except for the P2X7 receptor (McCarthy et al., 2019) and the maxi-anion channel, for which there is no experimentally determined structure, ATP releasing channels have hexameric or higher stoichiometry. While the connexins and pannexins are distinct gene families they share striking structural similarities. Connexin and pannexin subunits share the same overall topology, with a N-terminal helix forming part of the gate within the channel, and the C-terminus being cytoplasmic (Bennett et al., 2016; Brotherton et al., 2022; Deng et al., 2020; Flores et al., 2020; Lee et al., 2020; Maeda et al., 2009; Michalski et al., 2020; Myers et al., 2018; Ruan et al., 2020). Interestingly, VRAC is now known to be related to the pannexins: LRRC8a has a pannexin-like channel core with a very large cytoplasmic elaboration consisting of the leucine-rich repeats (Abascal and Zardoya, 2012; Jentsch et al., 2016; Kasuya et al., 2018; Kefauver et al., 2018; Qiu et al., 2014; Voss et al., 2014). The CalHMs, while having a variable stoichiometry of at least 7 subunits and as many as 11, also have an N-terminal helix that projects into the pore (Choi et al., 2019; Demura et al., 2020; Drożdżyk et al., 2020; Ren et al., 2022; Syrjanen et al., 2020; Yang et al., 2020).

The P2X7 receptor and its ability to release ATP requires further discussion. This was originally ascribed to a phenomenon of "pore dilation" (Chaumont and Khakh, 2008; Khakh et al., 1999; Surprenant et al., 1996). Some investigators suggested that the receptor complex required in some way to coassemble with pannexin-1 for this dilation to occur and allow passage of large molecules (Locovei et al., 2007; Pelegrin and Surprenant, 2006). This idea was comprehensively shown to be wrong when the same amino acid residues were demonstrated to control the selectivity for small ions and charged dyes i.e., the same permeation pathway regulated the passage of both ions and molecules (Browne et al., 2013). The concept of pore dilation itself was then shown to be an artefact of patch clamp recording via thorough biophysical analysis of the evidence for pore dilation (Li et al., 2015). Much of the electrophysiological evidence for pore dilation depended on gradual changes in reversal potential when extracellular Na^+ ions were substituted with $NMDG^+$ ions. This gradual change was interpreted as the pore dilating and allowing a progressive increase in the permeation of the $NMDG^+$ ions. However, this interpretation did not consider that under these conditions the intracellular concentrations of permeant ions could change (Li et al., 2015). This, in itself, would lead to a change in reversal potential without any change in pore diameter. Subsequent evidence has supported this analysis and shown that the P2X7R has an immediate low, but finite, permeability to small molecules leading to slow permeation through the channel (Harkat et al., 2017). However, the P2X7 receptor is primarily considered to be a cation selective channel, raising the question as to whether ATP (which is anionic) actually permeates the pore or occurs as a downstream consequence of P2X7 channel opening. It is certainly true that there is no direct electrophysiological evidence for ATP permeation through the channel. However, there is compelling evidence that a range of anionic molecules of sizes both bigger and smaller than ATP can indeed permeate the P2X7 pore (Browne et al., 2013; Duan et al., 2003) and that mutations of pore lining residues alter their permeation (Browne et al., 2013). On balance, we think that it is highly probable that the P2X7 receptor is a direct pathway of ATP release.

Table 1
Channels that are permeable to ATP. The 4-character PDB codes are given for experimentally determined structures to enable ready access via a database such as the RCSB Protein Data Bank (https://www.rcsb.org) or via viewing software such as PyMol or ChimaeraX.

Channel(s)	Stoichiometry	Structures	Evidence for ATP permeation
P2X7R	Trimeric	5u1v,6u9v	(Pellegatti et al., 2005; Suadicani et al., 2006)
Connexins (Cx43, 26, 32)	Hexameric	2zw3,7qeq,7f94, 6l3v,7jmd	(Huckstepp et al., 2010a; Kang et al., 2008; Nualart-Marti et al., 2013a; Pearson et al., 2005)
Pannexins	Heptameric	6vd7,6uzy, 7f8j	(Bao et al., 2004; Chekeni et al., 2010; Daneva et al., 2021; Lohman and Isakson, 2014)
VRAC (LRRC8a)	Hexameric	6nzw	Gaitan-Penas et al. (2016)
CalHM1-3	Variable but > heptameric	6lmt, 6lmv, 6lom	(Ma et al., 2018; Nomura et al., 2020; Taruno et al., 2013)
Maxi anion channels (Slco2a1)	Unknown	Predicted only: Alphafold2 AF-B4DJE6-F1	(Sabirov et al., 2001, 2017)

3. What gates ATP release channels?

There are many indirect mechanisms that can act on these channels -e.g. G-protein coupled receptor mediated signalling via phosphorylation (Alstrøm et al., 2015; Pogoda et al., 2016) or acetylation (Chiu et al., 2021), nitrosylation (Retamal et al., 2006), and even caspase cleavage of the C-terminus (Chiu et al., 2017). While these have the possibility of altering transmembrane ATP fluxes, we restrict our discussion to reversible gating mechanisms that act directly on, and are intrinsic to, at least some of the ATP release channels. Fig. 1 summarizes much of what we discuss below.

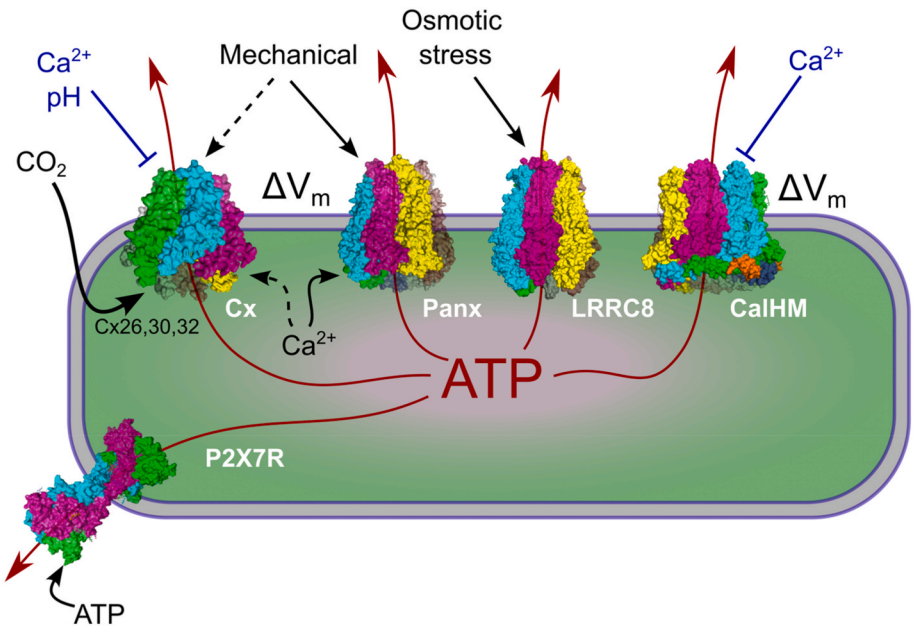

Fig. 1. The stimuli and channels that can release ATP. Molecular structures shown for connexins (Cx, PDB code: 2zw3), pannexins (Panx, PDB code: 6vd7), VRACs (LRRC8, PDB code: 6nzw), CalHMs (PDB code: 6lmt) and P2X7R (PDB code: 6u9v). The maxi anion channel is not shown as there is no molecular structure for this molelcule. Lines with arrows indicate a stimulatory action. Broken lines indicate an indirect mechanism. Blue lines terminating with bars indicate an inhibitory action. Currently, the opening action of CO_2 has only been documented for Cx26, 30 and 32. Not all connexins are necessarily opened by increases in intracellular Ca^{2+}. ΔV_m indicates that a change in transmembrane voltage is an effective gating stimulus. Note that in most cases V_m has to rise well above zero mV to attain significant channel opening.

3.1. Transmembrane voltage

Connexins, pannexins and CalHMs channels are intrinsically sensitive to transmembrane voltage. In general, very substantial depolarisation to very positive potentials is required to open these channels (Contreras et al., 2003; Gonzalez et al., 2007), although some opening at more modest positive potentials can be observed for pannexins (Bruzzone et al., 2003) and CalHMs (Ma et al., 2012). In excitable cells such as neurons or cardiac myocytes these big shifts in transmembrane potential are readily realisable. In other cells such as type 2 taste cells, the second messenger-mediated increase in intracellular Ca^{2+} following from activation of the taste receptors opens Trpm5, a non-specific cation channel, which then provides sufficient depolarisation to trigger CalHM opening. However, many connexins are expressed in non-excitable cells such as astrocytes or oligodendrocytes. In these cells it is questionable whether they experience sufficient depolarisation to trigger the opening of the channel. Most ATP release channels, as they are relatively non-selective for ions, have reversal potentials close to 0 mV. If the whole cell conductance of such a channel is sufficiently large, the membrane potential of the cell will tend towards this reversal potential. Although this could facilitate opening if gating was triggered by an alternative input, it is important to realize that the degree of opening at 0 mV for most of these channels is very modest. A further point to note is that there is an interaction between extracellular Ca^{2+} and the voltage sensitivity of CalHMs. If extracellular Ca^{2+} is lowered, then the voltage activation of the current shifts to lower potentials (Ma et al., 2012). However, as physiological concentrations of extracellular Ca^{2+} are closely regulated the significance of this remains uncertain.

3.2. Intracellular Ca^{2+}

Pannexins are gated by increases in intracellular Ca^{2+} although the mechanism of interaction has not been determined (Chiu et al., 2018; Locovei et al., 2006). Connexins such as Cx32 and Cx43 can be opened by an increase in intracellular Ca^{2+} (De Vuyst et al., 2006, 2009; Wang et al., 2012a). For Cx32 this depends on interaction of the C-terminus of the protein with Ca^{2+} calmodulin to promote channel opening, thus it is not an intrinsic property of the connexin. If the C-terminus is truncated in Cx32 (as occurs in the pathological mutation R220Stop), then Ca^{2+} dependent opening of the channel is prevented but can be restored by provision of a soluble peptide derived from the cytoplasmic loop (Carrer et al., 2018). The Ca^{2+} dependent opening of Cx43 is likewise not an intrinsic property of the channel but depends on other proteins such as calmodulin (De Vuyst et al., 2009). If intracellular Ca^{2+} rises above a certain level, it exerts an inhibitory effect on the gating of Cx43 hemichannels. It should be noted that most ATP-releasing channels will themselves be permeable to Ca^{2+} and their opening can also contribute to a Ca^{2+} influx.

3.3. Mechanical/osmotic stress

There are many instances reported of mechanically induced ATP release either by sheer stress (in endothelia), or osmotic stress (cell swelling) and other mechanical forces. With the exception of pannexins (Bao et al., 2004) and LRRC8 (VRAC) which is part of the pannexin gene family (Jentsch et al., 2016; Pedersen et al., 2016), ATP release channels are not intrinsically sensitive to mechanical forces. Instead, they are coupled to the mechanosensitivity of other channels such as those of the Piezo gene family (Coste et al., 2010, 2012; Nilius and Honoré, 2012) or TRPV4 (Redmon et al., 2021; Turovsky et al., 2020). TRPV4 seems essential for the mechanosensitivity of astrocytes and induces ATP fluxes via Cx43. The mechanism by which this occurs is unclear, but TRPV4 and Cx43 are in close proximity (Turovsky et al., 2020).

3.4. Gaseous CO_2

The beta connexins, Cx26, Cx30 and Cx32 are directly sensitive to CO_2: their hemichannels are opened by increases in the partial pressure of CO_2 (PCO_2) (Huckstepp et al., 2010a). Uniquely for Cx26, the gap junction is also closed by modest increases in PCO_2 (Nijjar et al., 2021). In air breathing vertebrates, where the resting PCO_2 in blood is around 30–40 mmHg, the modulation of beta connexin hemichannels is a physiological gating mechanism of great relevance particularly in non-excitable cells (Dale, 2021b; Dospinescu et al., 2019).

The mechanism of CO_2 sensitivity has been most thoroughly examined in Cx26 where both hemichannel opening and gap junction closing depends on a carbamylation motif (Meigh et al., 2013; Nijjar et al., 2021). CO_2 forms a transient covalent bond with the amine of a specific lysine residue within the motif (in Cx26 this is K125) via a carbamylation or carboxylation reaction (Meigh et al., 2013; Nijjar et al., 2021). Once formed the carbamylated lysine residue is hypothesized to interact with arginine 104 of the neighbouring subunit and the cross-subunit

interactions in the hexameric channel lead to conformational change. This has recently been extended to the purified protein and cryoEM structures for purified Cx26 gap junctions at different levels of PCO_2 clearly show conformational changes consistent with gating of the channel (Brotherton et al., 2022).

The carbamylation motif occurs across all the CO_2 sensitive beta connexins. Testing the CO_2 sensitivity of beta connexins from a variety of species, in combination with molecular phylogenetic analysis of the Cx32/Cx30/Cx26 clade, suggests that its presence is sufficient to endow a connexin with CO_2 sensitivity (Dospinescu et al., 2019). This is clearly illustrated by the observation that insertion of the motif into the non CO_2-sensitive beta connexin, Cx31, gave a gain of CO_2 sensitivity (Meigh et al., 2013).

3.5. Closing mechanisms

Physiological levels of extracellular Ca^{2+} are effective at blocking connexin hemichannels (Muller et al., 2002) and a common way to study connexin hemichannel currents is to remove extracellular divalents. CalHMs also open more readily if extracellular Ca^{2+} is lowered. As mentioned above, the physiological significance of this is unclear as extracellular Ca^{2+} levels are closely regulated. It is important to note that the Ca^{2+} binding site that closes hemichannels, is distinct from the intracellular opening action of Ca^{2+}, which is not a property intrinsic to the connexins. X-ray structures suggest that Ca^{2+} ions bind to a site near the extracellular surface to form an electrostatic barrier to ion permeation (Bennett et al., 2016; Gomez-Hernandez et al., 2003). Acidification closes connexin hemichannels, however this requires levels of acidic pH that are unlikely to occur during normal physiology (Khan et al., 2020; Wang et al., 2012b; Yu et al., 2007).

3.6. Combinatorial gating -interactions of mechanisms and release channels

This account shows that most channels capable of releasing ATP are controlled by multiple mechanisms intrinsic to the channel. An interesting question is to what degree these different gating mechanisms interact either cooperatively or antagonistically, and whether the conformational basis of each gating mechanism is the same or different. For connexins, most are voltage sensitive, and all are blocked by extracellular Ca^{2+}. But a sufficiently large voltage change is still able to open the channel even at physiological Ca^{2+} concentrations. Similarly, CO_2 can open the beta connexins in the presence of normal concentrations of extracellular Ca^{2+} (Dospinescu et al., 2019; Huckstepp et al., 2010a). However the CO_2 dependent opening of beta connexins is pH sensitive, probably due to the pH sensitivity of the carbamylation reaction itself, and modest acidification can reduce or occlude CO_2-induced channel opening (Huckstepp et al., 2010a). For the CalHMs we noted an interaction between extracellular Ca^{2+} concentrations and their voltage sensitivity (Ma et al., 2012).

A further related question is whether competition or interaction between different ATP-releasing channels occurs. There is some evidence, at least in model systems, for an interaction between the P2X7 receptor and pannexin 1 (Locovei et al., 2007; Pelegrin and Surprenant, 2006). Although the evidence for CalHMs being essential for ATP release from taste cells is rigorous and compelling, earlier evidence suggested a role for connexins and pannexins (Huang et al., 2007; Romanov et al., 2007, 2008). Subsequently, pannexin channels were found not to be required for ATP release from taste cells or normal behavioural taste sensation (Romanov et al., 2012; Vandenbeuch et al., 2015). Nevertheless, some residual taste sensation remains following genetic deletion of CalHMs (Ma et al., 2018; Taruno et al., 2013) and there is potential for an accessory role for these other ATP permeable channels in taste cell signalling (Vandenbeuch et al., 2015).

4. Selectivity of ATP release via channels

The connexins, pannexins, VRAC and CalHMs all possess a central pore that is sufficiently large in the open state to plausibly accommodate molecules such as ATP. However, the presence of a large pore does not necessarily indicate that any small molecule can permeate the channel. For example, Cx26 hemichannels are preferentially permeable to anionic fluorescent dyes (Zhao, 2005). Studies on metabolite permeation through connexins also suggest surprising selectivity (Hansen et al., 2014a, 2014b; Nielsen et al., 2017). For example: ATP can permeate all connexin hemichannels that have been studied, but smaller molecules such as lactate, glutamate and glucose do not permeate the Cx43 hemichannel (Hansen et al., 2014a). By contrast, glutamate and glucose permeate Cx30 hemichannels (Hansen et al., 2014a). This permeation profile suggests that there must be a selectivity filter -molecules presumably interact with pore lining residues and these interactions determine the degree to which they can permeate. Such considerations are likely to apply to all channels capable of releasing ATP and they may have preferential selectivity for this molecule over a variety of other small molecules present in cytosol (Gaete et al., 2020; Narahari et al., 2021; Nielsen et al., 2020). Indeed, the observation that dye permeation is saturable hints at a binding interaction with the residues of the permeation pathway that could impart selectivity (Gaete et al., 2020). With the advent of cryoEM techniques as the method of choice for resolving channel structures and excellent structures for many of the ATP-releasing channels (Table 1), a fundamental understanding of mechanisms of permeation of molecules through these channels may well be forthcoming.

In addition to the intrinsic properties of the various channels that can release ATP, there are other possible ways that selectivity can be imparted to the release process, even if the channels lack a high degree of selectivity. The most obvious is simply the concentration of the molecules in the cytosol. Here, ATP has an advantage in that it is present at millimolar concentrations (Rangaraju et al., 2014). However, molecules such as lactate or glutamate can also be present at similarly high concentrations.

ATP, glutamate and lactate carry net negative charge at physiological pH. As virtually all living cells have a resting transmembrane potential, the electrochemical driving force (rather than simply the transmembrane concentration differences) will determine the transmembrane fluxes of charged molecules via channels. Assuming that ATP is normally chelated with a Mg^{2+} ion, it will have a net negative charge of -2. The Nernstian equilibrium potential for ATP will thus be a massive $+145$ mV (assuming 5 orders of magnitude difference between the intracellular and extracellular concentrations of ATP). In a typical cell at rest, the driving force of ATP would thus be in excess of 200 mV. There is clear experimental evidence for the importance of transmembrane potential in determining the channel-mediated efflux of ATP. Following voltage dependent opening of Cx32 expressed in oocytes (by a step to $+40$ mV), far more ATP release (as measured by luciferase) occurs during the relaxation currents when the membrane potential is stepped back to -80mV than during the positive voltage step itself (Nualart-Marti et al., 2013a).

Glutamate is also likely to be highly asymmetric in concentration across the plasma membrane and could have a similarly large driving force to ATP, but at least some of the connexins do not permit permeation of glutamate. Lactate is more evenly distributed across the membrane and is likely to have an equilibrium potential closer to zero mV. This would still give a substantial driving force, but the evidence suggests that some connexins, at least, are relatively impermeable to lactate.

5. Two functional modes of channel mediated ATP release

In principle channel mediated ATP release could act in two ways (Fig. 2). The first possibility would be to mediate “volume transmission”.

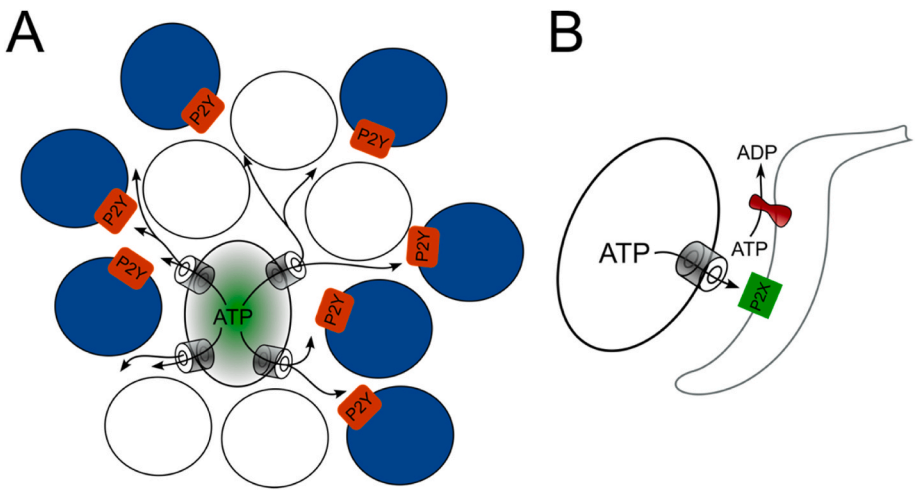

Fig. 2. Concept of volume transmission via channel mediated ATP release versus channel synapses. A) Volume transmission from a single cell (green) that releases ATP via channels. The ATP can diffuse to act on several cells within the diffusion radius (blue) that express suitable receptors (e.g., of the P2YR subtype). B) A channel synapse, where the presynaptic element can release ATP to act on a specialised postsynaptic element where suitable receptors are expressed, most likely of the P2XR subtype. In this scenario ATP diffuses only a short distance, achieves a high local concentration, and acts only a single cell.

In this model the ATP is released not at a specialised anatomical structure but instead diffuses in the extracellular space to allow actions of ATP over considerable distances and affecting any cells within the radius of diffusion that happen to have P2X or P2Y receptors (Fig. 1A). Often in this scenario multiple different cell types can respond to the released ATP. An example of this type of transmission is provided in the developing retina where Cx43-mediated ATP release from the retinal pigment epithelial cells regulates proliferation of the neural progenitor cells in the subventricular zone of the neural retina (Pearson et al., 2005). It is important to note that the distance over which ATP can diffuse will depend on expression and distribution of ectoATPases. Simulations based on realistic assumptions suggest that ectoATPases will limit ATP diffusion to a radius of only ~10 μm, whereas ADP (which can activate subtypes of P2Y receptors) can diffuse much further, 10–100 of μm (Dale, 2021a).

The second way for channel mediated ATP release to act would be a direct substitute for the conventional chemical synapse and would replace the exocytotic machinery with an ATP permeable channel gated by voltage or intracellular Ca^{2+} (Fig. 2B). In this scenario there would still be an anatomically defined synapse with a presynaptic and postsynaptic process in close apposition and the purpose of this type of transmission would be one to one communication between two specific cells. There is in fact a beautiful example of this type of synapse found in taste buds and it mediates the salty taste modality (Ma et al., 2018; Romanov et al., 2018).

These two scenarios are not mutually exclusive, for example it could be possible for a cell to release ATP via channels to mediate volume transmission at one location and form a channel or conventional synapse at another location. While this possibility has not been documented, we shall consider some examples of channel mediated ATP release where this might also occur.

6. Will depletion of intracellular ATP affect release?

As discussed above there is a very large electrochemical driving force on ATP. This raises the question to what extent depletion of intracellular ATP will affect the release of ATP via channels. Given that there are 5 orders of magnitude difference in the concentration of ATP either side of the membrane, it might be imagined that the release of ATP would be rather insensitive to variations of intracellular concentration -under most circumstances, even with substantial depletion there would still likely be at least 3 orders of magnitude concentration difference and thus a very large electrochemical driving force remaining.

Experimental evidence from channel synapses formed by type 2 taste cells does show the, perhaps unsurprising, requirement for continuous ATP production (Romanov et al., 2018). Treatment of acutely isolated taste cells with oligomycin for 5 min to block mitochondrial ATP generation resulted in tastant-evoked release of ADP rather than ATP. This does not show that channel-mediated ATP release *per se* causes significant depletion of ATP but may reflect the rates of baseline ATP consumption by the cell, and hence the need for continual synthesis.

This synthesis does not have to be very fast -it only has to compensate for the efflux of ATP through the channel, and the consumption of ATP required to restore ionic balances following channel opening. To provide some insight into this we have devised a very simple model in which there is gated release (by CO_2) of ATP via Cx32. Cx32 was present in a non-excitable model cell that had a K^+ leak conductance to create a resting potential (Fig. 3A). The cell was endowed with a rate of intracellular ATP synthesis (K_{ATP}) that was proportional to the difference between the concentration of intracellular ATP and some "target" concentration, in this case 3 mM. The currents flowing through Cx32 carried by ATP and small cations were calculated and used to determine the membrane potential which in turn altered the driving force on ATP. The permeability of Cx32 to ATP was set to be 10% of that of monovalent cations. The model also incorporated consumption of ATP related to the cationic current flow through Cx32 to enable restoration of ionic balance. Once released, ATP could be removed via a CD39-like ectoATPase with Michaelis Menten kinetics drawn from the literature. To give a further element of realism, the volume of the synaptic compartment was scaled to that of the presynaptic element (in effect the cell) and this scaling was taken from the channel synapse described in tase cells (Romanov et al., 2018). Three episodes of CO_2-dependent ATP release each lasting >200s were evoked.

There are several points of note: i) the presynaptic element depolarised close to the reversal potential for Cx32, implying the need for restoration of ionic balance via ATP consuming processes; ii) with K_{ATP} set to 0.0001 s^{-1} (equivalent to a maximum rate of ATP production of 0.3 μM s^{-1}), although intracellular ATP clearly depleted, there was little time dependent reduction of ATP release during the first and second episodes despite these episodes lasting >200s (Fig. 3B); and iii) as might be expected there was greater depletion at the third release episode compared to the second. However, if the rate constant for ATP production was at least 0.0064 s^{-1} then this depletion was almost non-existent (Fig. 3C). This rate constant is equivalent to a maximum rate of ATP production in the model of ~20 μM s^{-1}. It should be noted that the predictions of this model have not been experimentally tested.

7. Channel synapses

7.1. Taste receptor cells

One of the puzzles in understanding the perception of taste is that

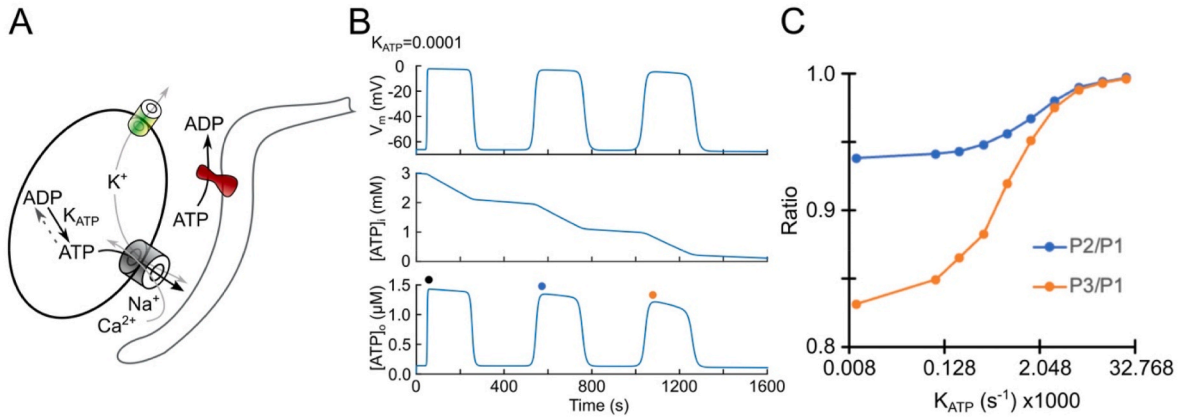

Fig. 3. Simplified model for channel mediated ATP release. A) Schematic representation of the model showing a leak K^+ selective channel (green) to allow generation of a resting potential and Cx32 (grey) allowing ATP release but also cation fluxes. ATP is synthesized so that it is maintained at a target concentration with a rate constant, K_{ATP}. ATP is also consumed by pumps that restore the flux of ions through Cx32 (dashed line). Once released ATP can be converted to ADP by ectoATPases (red). B) Example of model output for three episodes of CO_2 triggered opening of Cx32. Note that: the membrane potential reaches close to 0 mV the reversal potential for Cx32; the concentration of intracellular ATP ($[ATP]_i$) substantially depletes with this value of K_{ATP}, and extracellular ATP ($[ATP]_o$) reaches a peak concentration of about 1.4 μM during the CO_2 evoked release and shows only very slight depletion during the first two episodes. C) Systematic exploration of how depletion of the second and third release episodes (relative to the first) varies with the rate of intracellular ATP synthesis. A value of K_{ATP} of 0.064 s^{-1} virtually eliminates depletion. Matlab code for the simulation is provided (Cx32Model.m and Cx32Plot.m).

several different taste modalities (sweet, umami, bitter and salt) all rely on release of ATP and activation of P2X receptors to trigger afferent activity in the gustatory nerve. The evidence is very strong that all four modalities involve channel-mediated ATP release, yet the concept of keeping specific pathways of taste information separate would be undermined if ATP were to act as a volume transmitter in this case.

However, Romanov et al. provided a possible resolution to this conundrum by showing that the ATP release channels in taste cells (CalHM1/3) are arranged in a channel synapse (Romanov et al., 2018). This structure has the pre- (the taste cell) and postsynaptic membrane (the afferent nerve terminal) arranged only 10–15 nm apart with the CalHM channels present at this close membrane apposition, juxtaposed to P2X receptors in the membrane of the afferent nerve. They also documented the presence of an "atypical" mitochondrion close to (within 100 nm) the release sites in the presynaptic element of the channel synapse. This mitochondrion provides ATP for release via the synapse. Blockade of oxidative phosphorylation by oligomycin resulted in the release of ADP rather than ATP from the taste cell. The authors suggest a further advantage of the close location of the mitochondrion is to prevent accumulation and diffusion of Ca^{2+} into the bulk cytosol of the taste cell. If this were to occur, Trpm5 present in these cells would be activated, and as this did not happen it strongly suggests that a mechanism restricts the diffusion of Ca^{2+} entering via the CalHMs. This could happen via uptake of Ca^{2+} into the atypical mitochondrion and extrusion via the plasma membrane Ca^{2+} ATPase, with the closely juxtaposed atypical mitochondrion providing abundant ATP for this process. So far this is the only known example of ATP release at a channel synapse, but as Romanov et al. point out, this may only be because such synapses have not hitherto been recognised.

8. Volume transmission

8.1. RPE to SVZ in developing retina

The developed vertebrate retina is a highly organised structure, with well-defined layers that contain different cell types and synaptic connections. It forms a highly complex computational unit that provides several parallel streams of image related information to the brain. The innermost layer of the retina (next to the wall of the eyeball) is the retinal pigment epithelium (RPE). This contains dark pigment to reduce light scattering within the eyeball. The photoreceptors are found immediately next to the RPE, they communicate with the bipolar cells at the inner plexiform layer. The bipolar cells contact the ganglion cells at the outer plexiform layer. The ganglion cells provide the output from the retina and their axons comprise the optic nerve. In addition to this vertical pathway of communication from photoreceptor to ganglion cell there is also lateral communication via horizontal cells and amacrine cells.

The development of the neural retina must be tightly regulated to ensure the correct number of neurons of each type and ultimately the wiring within the retina itself. During development the RPE plays a key role. It is next to the subventricular zone (SVZ) of the neural retina which contains neural progenitor cells. These cells divide asymmetrically to generate a non-dividing daughter cell that goes on to differentiate into the various cell types found in the retina, and a second cell that retains its pluripotency and continues dividing to generate new progenitor cells. Close proximity of the RPE to the SVZ controls the rate of division of progenitor cells in the SZ suggesting that signals originating from the RPE act on the progenitor cells. One of these signals is ATP released via spontaneous gating of Cx43 hemichannels (Pearson et al., 2005). The progenitor cells express P2Y receptors, and their activation enhances the rate of division. Spontaneous waves of Ca^{2+} occur in cells of the RPE, and these trigger the opening of Cx43 hemichannels. Two consequences follow -a spreading wave of Ca^{2+} mobilization in the cells of the RPE, and also Ca^{2+} waves within the neural retina. If release of ATP from the RPE via Cx43 is prevented (via the Gap26 mimetic peptide) then cell division within the ventricular zone is slowed (Pearson et al., 2005).

This is a very clear example of channel-mediated volume transmission where extracellular diffusion of ATP not only promotes a travelling Ca^{2+} wave within the RPE, but also diffuses to another cell layer to trigger further receptor activation, Ca^{2+} waves and ultimately the rate of proliferation of progenitor cells. A similar connexin hemichannel mediated mechanism dependent on ATP signalling triggers Ca^{2+} waves in the radial glia of the developing cortex and regulates embryonic neurogenesis (Weissman et al., 2004).

8.2. ATP release in myelinated nerves

In all jawed vertebrates, the nervous system is fully myelinated (Zalc, 2006). The myelin sheath has long been known as an insulating mechanism vastly speeds action potential propagation, without increasing

axon diameter, via local current flow and saltatory conduction. The degree of myelination (the number of layers) and the internodal distances are key determinants of the speed of action potential propagation. To allow coordinated coherent communication in both the central and peripheral nervous system these processes must be tightly controlled both in their initial establishment during development but also in their maintenance during the life course.

ATP mediated signalling takes a central role in both the establishment of myelin (Fields, 2006; Stevens and Fields, 2000) and its maintenance. During action potential propagation there is swelling and displacement of the axon caused by mechanical stresses (Fields and Ni, 2010). By simultaneously measuring both this displacement, via an intrinsic optical signal, and the release of ATP, Fields and Ni demonstrated a close correlation between the two (Fields and Ni, 2010). They provided evidence that pharmacological blockers of VRACs were able to greatly reduce the ATP release but not affect axonal displacement, suggesting channel mediated release of ATP via VRACs. However, the unequivocal involvement of VRACs as the ATP releasing channel in axons remains to be determined, for instance by selectively knocking out LRRC8a or another subtype, since none of the pharmacological agents that target VRAC are completely selective.

Ino et al. developed these findings by demonstrating ATP release from developing sciatic nerve of young rat pups *in vivo* (Ino et al., 2015). They showed that this acted via P2Y2 receptors on myelinating Schwann cells to trigger Ca^{2+} signals both in the cytosol and in the mitochondria and that inhibiting this P2Y2 mediated pathway resulted in hypomyelination. This suggests that mechanically-triggered, channel-mediated ATP release from the axon acts on Schwann cells to help regulate the initial steps of myelination.

Once myelin is established it appears that its healthy maintenance may also require ATP release at least in the periphery. Axonal stimulation evokes Ca^{2+} transients in Schwann cells (Lev-Ram and Ellisman, 1995), showing that axon to Schwann cell communication still occurs in fully developed myelin. However, Cx32 is expressed in Schwann cells postnatally and may provide an additional source of activity-dependent ATP release (Nualart-Marti et al., 2013a, 2013b). The target of the ATP released from Schwann cells remains unknown, quite possibly this is an autocrine feedback loop that links axonal activity to the maintenance of myelin.

Mutations of Cx32 cause a progressive neuropathy known as X-linked Charcot Marie Tooth disease (CMTX) (Abrams, 1993; Abrams et al., 2003; Bergoffen et al., 1993; Cisterna et al., 2019; Kleopa and Sargiannidou, 2015; Nakagawa et al., 2001; Omori et al., 1996; Scherer and Kleopa, 2012; Wang and Yin, 2016). CMTX phenotypes can be recapitulated in mice through deletion of Cx32 and rescued by expression of WT Cx32 just in Schwann cells (Scherer et al., 2005). Overall, the evidence strongly suggests that CMTX is caused by loss of function (Abrams et al., 2000; Sargiannidou et al., 2009; Shy et al., 2007). Some CMTX mutations alter the ability of Cx32 to release ATP and other small molecules through hemichannels (Nualart-Marti et al., 2013a) and gap junctions (Oh et al., 1997), however the mechanistic link (what facet of altered channel function) between many Cx32 mutations and CMTX is incompletely understood (Karadima et al., 2014; Kleopa et al., 2012; Tsai et al., 2016). These data support the hypothesis that channel-mediated ATP release via Cx32 is important for retaining healthy function of myelinated nerves.

It should be noted that Cx32 has a dual role in myelin. In the Schmidt-Lanterman incisures, Cx32 forms “reflexive” gap junctions -gap junctions between different layers of myelin of the same internodal stretch (Balice-Gordon et al., 1998). These gap junctions provide a rapid radial pathway that greatly facilitates the transfer of metabolites across the myelin sheath. Potentially some CMTX mutations could affect this function too, but in Cx32 null mice transfer of dyes through this radial pathway still occurs, suggesting that other connexins may be involved (Balice-Gordon et al., 1998).

Overall, the actions of ATP in myelin are commonly regarded as volume transmission. In the developing nerve, where the initiation of myelination is a key event that requires coordinated activity between axon and oligodendrocyte or Schwann cell, this conforms to the paradigm. However, in established nerve fibres where myelination is complete, if ATP were only to act in an autocrine fashion to mediate some homeostatic feedback signal to the Schwann cells (Lyons et al., 1995; Mayer et al., 1997) that depended on activity in the axon they ensheathed, it might be acting as a local messenger rather than a volume transmitter. An interesting question, that would imply volume transmission even in established nerve, is whether ATP released from one Schwann cell paranode, via Cx32, can mediate cross talk between myelinated axons within a nerve bundle. This latter process might make sense if activity patterns of individual nerve fibres within a bundle were matched -e.g. within a motor nerve where firing rates of axons innervating different muscle groups are likely to be somewhat similar overall.

Although the role of Cx32 has primarily been studied in the peripheral nervous system, Cx32 is also expressed in oligodendrocytes. There is mounting evidence that some forms of CMTX present with cognitive symptoms and alterations in central white matter (Hardy et al., 2019; Hu et al., 2019; Kasselimis et al., 2020; Wen et al., 2018). Cx32 mediated signalling may therefore also be important for myelinated axons in the central nervous system.

8.3. Other examples

From the examples above, channel mediated volume transmission via ATP plays a role in developmental processes. A further example of this is evidence of connexin-mediated ATP release during the development of the organ of Corti, which may help to establish the tonotopic map (Piazza et al., 2007; Tritsch et al., 2007). However, ATP-dependent volume transmission participates in diverse regulatory processes in the fully developed nervous system particularly from glial cells. Cultured astrocytes respond to mechanical distortion by releasing ATP through Cx43 hemichannels (Turovsky et al., 2020). While this might be thought to contribute to detection of intracranial pressure, mice with glial-specific deletion of Cx43 respond normally to changes in intracranial pressure. However, the resting heart rate of these mice is lower than in wild type animals, suggesting that Cx43-mediated release of ATP and possibly other molecules influences sympathetic drive to the heart via activation of receptors on neurons in the ventral medulla (Turovsky et al., 2020). Astroglial Cx43 hemichannels in the hippocampus appear to be at least partially open under basal conditions and permit continual release of ATP which modulates the strength of the main excitatory synaptic pathways in this region (Chever et al., 2014). Channel-mediated ATP release has a role in the neurovascular control of blood flow (Thakore et al., 2021). Activation of TRPA1 on brain parenchyma capillaries causes a Ca^{2+} influx into the capillary endothelia that triggers opening of Pannexin-1. ATP release via Pannexin-1 elicits propagating Ca^{2+} waves in capillary endothelia that travel towards the arterioles and eventually result in arteriole dilation (Thakore et al., 2021).

9. Volume transmission with potential for synapses

9.1. CO_2 sensing in the medulla oblongata

Breathing is regulated by the partial pressure of CO_2 (PCO_2) in arterial blood. If this increases, so does the rate (respiratory frequency) and depth (tidal volume) of breathing. Chemoreceptors exist both peripherally (in the carotid body) and centrally in the medulla oblongata. About 70% of the total chemosensory response is mediated by the central chemoreceptors. As CO_2 combines with water to produce bicarbonate and hydrogen ions, an increase in PCO_2 will result in an acidification of arterial blood and cerebrospinal fluid. Although certain central chemosensory cells respond to changes in pH, notably those of the RTN (Guyenet et al., 2019) and Raphe (Ray et al., 2011; Richerson,

2004), there is also evidence that CO_2 is detected directly and independently of changes in pH by the central chemosensors (Shams, 1985).

The molecular basis of this direct CO_2 detection has now been elucidated (Dale, 2021b, 2021van de Wiel et al., 2020). It depends on the direct interaction of CO_2 with Cx26 via carbamylation of a specific lysine residue (Meigh et al., 2013). By designing a dominant negative Cx26 subunit that could coassemble with the wild type subunits *in vivo* and remove CO_2 sensitivity from the heteromeric assembly, the link between CO_2 binding to Cx26 and the chemosensory regulation of breathing has been established (van de Wiel et al., 2020). The key cells for direct CO_2 sensing via Cx26 are glial cells found in the caudal parapyramidal area (cPPy). They account for nearly half of the adaptive ventilatory response at moderate levels of hypercapnia (6% inspired CO_2) (van de Wiel et al., 2020). They have a cell body at the ventral surface and long processes that project rostrally and medially. Cx26 is present in the cell body and Cx26-mediated ATP release can be detected at the ventral medullary surface (Huckstepp et al., 2010b). This suggests that signalling from these cells conforms to the volume transmission paradigm. However, the presence of long processes in the glial cells is most easily explained if they serve to mediate targeted communication with distant cells. While this has not been demonstrated it cannot be excluded that ATP signalling from these cells could also occur via Cx26 mediated channel synapses or via conventional exocytosis from the processes of these cells.

9.2. Tanycyte nutrient sensing

There are striking parallels between nutrient sensing in tanycytes and taste sensation in taste buds. Tanycytes have a cell body that forms part of the wall of the 3rd ventricle. They also have a single process that emerges and projects through the hypothalamic parenchyma and can at least in some cases form a terminal process that wraps around blood vessels (Bolborea and Dale, 2013). Tanycytes are sensitive to a number of nutrients in CSF including glucose (Benford et al., 2017; Frayling et al., 2011), a range of amino acids (Lazutkaite et al., 2017) and free fatty acids (Geller et al., 2019). The detection of glucose occurs via two pathways: a Glut2/glucokinase dependent mechanism reminiscent of the way pancreatic beta cells sense glucose (Orellana et al., 2012); and also via the sweet taste receptor (Tas1r2/Tas1R3) heterodimer (Benford et al., 2017). Amino acid sensing occurs via two different "umami" receptors -the Tas1R1/Tas1R3 heterodimer and the mGluR4 receptor (Lazutkaite et al., 2017). In both cases nutrient detection involves ATP release which has been detected with biosensors at the surface of the 3rd ventricle (Frayling et al., 2011) and as a wave that travels from the ventricular surface deeper into the parenchyma (Lazutkaite et al., 2017). This ATP release is thought to be channel mediated: Cx43 for glucose (Orellana et al., 2012); and through pannexin1 and CalHM1 for amino acids (Lazutkaite et al., 2017). This release of ATP conforms to the volume transmission paradigm as it is able to trigger Ca^{2+} waves that travel through the tanycyte cell body layer (Benford et al., 2017; Frayling et al., 2011; Lazutkaite et al., 2017) and effectively amplify the response of a single tanycyte into a population response (Bolborea et al., 2020). However, tanycytes are also capable of activating neurons of the arcuate nucleus including both the orexigenic (NPY/AgRP containing) neurons and the anorexigenic (POMC-containing) neurons (Bolborea et al., 2020). At least part of this communication is via ATP and this once again raises the possibility, currently untested, that it could be via channel synapses. Of course, this release could also occur via an exocytotic pathway. But it seems likely that tanycytes, like the glial cells of the cPPy involved in CO_2 sensing, have a mixed mode of signalling -volume transmission at the site of CO_2/nutrient detection and targeted signalling at a more distal location.

Table 2
Characteristics of channel synapses and conventional exocytotic synapses relevant to this review.

Feature/ characteristic	Channel Synapse	Conventional synapse
Complexity	Simple -just the channel and the transmitter needed.	Complex, requires many components related to vesicles, vesicular transporters, vesicular fusion, and membrane recycling through endocytosis.
Transmitter localisation	Transmitter is free to diffuse in cytosol. Requires a high concentration in cytosol.	Transmitter is compartmentalised to vesicle. Cytosolic concentration can be low.
Transmitter release across the membrane	Release will be sensitive to V_m and if the transmitter is charged sensitive to the electrochemical driving force.	Dependent only on the concentration in the vesicles.
Transmitter specificity and range	Likely to be less specific and only be suitable for a few transmitters.	High potential for specificity -but dependent on properties of vesicular transporters expressed.
Energetics	Likely to require restoration of ionic gradients and extrusion of Ca^{2+} via ATPases, but relatively low amounts of ATP synthesis required.	High ATP consumption.
Plasticity	Unknown	Many short and long term mechanisms of plasticity.

10. The pros and cons of channel synapses versus conventional synapses

Table 2 summarizes a comparison between conventional exocytotic synapses and channel synapses. A channel synapse perhaps has two advantages over a conventional one: simplicity of the required molecular machinery; and lower energy consumption. Conventional synapses are complex structures, and many different proteins are required for loading and trafficking of vesicles, Ca^{2+} regulated vesicular fusion, and endocytotic membrane recycling. Conventional synapses consume large amounts of ATP to power the vesicle cycle (fusion and recycling) (Rangaraju et al., 2014). While channel synapses still require some ATP synthesis and consumption this is only to compensate for the release of ATP and the restoration of transmembrane ionic gradients (Fig. 2) and is therefore probably much lower than a conventional synapse.

However, channel synapses carry considerable disadvantages too. Firstly, the permeant molecule needs to be present in cytosol at high concentrations. There is potential for this high concentration to interfere with metabolic processes. This is not a problem with respect to ATP, as this is normally present in millimolar concentrations and cellular metabolism depends on a high ATP:ADP ratio. The specificity of release via channel synapses may be less than a conventional synapse. That said, VNUT, the vesicular nucleotide transporter has relatively poor selectivity for ATP and can load many different nucleotides into vesicles (Sawada et al., 2008). Conventional synapses are well known for their capacity for plasticity -variations in transmitter release and postsynaptic sensitivity underlie many adaptive processes in the nervous system and are also fundamental to learning and memory. Whether channel mediated synapses have the same potential for plasticity remains unknown.

Perhaps in sensory pathways, where accurate transmission from the primary sensory cell to follower cells is required, channel synapses are advantageous -the lower energetics and highly reliable release (without a need for plasticity) makes them ideal in this context. Nevertheless, sensory pathways that are continually active such as those for hearing and vision use exocytotic transmitter release (of glutamate), suggesting a high rate of ATP consumption may occur in some sensory pathways.

Interestingly, in the cochlea, evolution has chanced upon an alternative solution to this energetic requirement. The mechanosensory channels on the stereocilia of the hair cells are in contact with the K^+-rich endolymph. Mechanotransduction in these cells is thus accompanied by an influx of K^+ rather than Na^+. This avoids the energetic need to pump out Na^+ ions to maintain transmembrane ionic gradients within the hair cells themselves. The energetic requirement of creating the K^+ rich endolymph and the endocochlear potential (which provides the inward driving force on K^+ and is essential for sound perception) has, in effect, been exported to the stria vascularis (Wangemann, 2006).

11. Potential advantages of channel mediated volume transmission

Channel mediated release may be highly advantageous for volume transmission mediated by ATP. Here the functional requirement is to influence as many of the surrounding cells as possible (Fig. 1A). The simplicity of channel mediated release means that a cell involved in volume transmission could have ATP release channels located over its entire surface. A single release channel may provide a flux of ATP release that would require a very high vesicular fusion rate (Dale, 2008), thus lessening the energy expenditure needed to maintain this rate of release. Furthermore, as this mode of ATP release is likely to act at P2Y receptors which have a high affinity for ATP, there is not a need to generate very high concentrations of extracellular ATP, thus depletion of intracellular ATP and hence release is likely to be negligible.

12. Concluding remarks

From the first speculation that ATP might be released via channels (Gordon, 1986), many of the channels that can mediate this release have been identified (Table 1). Indeed, the field has progressed so far that high resolution experimentally determined structures now exist for most channels capable of releasing ATP. A common feature of many of these channels, in particular the pannexins, connexins and CalHMs, is that their gating is modified by many different mechanisms (e.g., mechanical, transmembrane voltage and intracellular and extracellular Ca^{2+}). These channels therefore have the potential to act as integrative hubs for ATP release that can be controlled by several stimuli. It will be interesting to test in future to what degree the gating of these channels by these multiple stimuli is synergistic. Most examples of channel mediated release can be considered as volume transmission, but ATP-mediated communication from taste cells to the afferent sensory nerve is a notable exception to this and provides the only currently known example of a channel synapse. However, there is potential for mixed modes of ATP signalling -volume transmission in one region and directed communication via synapses (conventional or channel mediated) at a distant location. Investigation of this possibility may provide new insights into the roles of ATP signalling.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2023.109435.

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