ATP-mediated signalling in the central synapses

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Abstract

ATP released from the synaptic terminals and astrocytes can activate neuronal P2 receptors at a variety of locations across the CNS. Although the postsynaptic ATP-mediated signalling does not bring a major contribution into the excitatory transmission, it is instrumental for slow and diffuse modulation of synaptic dynamics and neuronal firing in many CNS areas. Neuronal P2X and P2Y receptors can be activated by ATP released from the synaptic terminals, astrocytes and microglia and thereby can participate in the regulation of synaptic homeostasis and plasticity. There is growing evidence of importance of purinergic regulation of synaptic transmission in different physiological and pathological contexts. Here, we review the main mechanisms underlying the complexity and diversity of purinergic signalling and purinergic modulation in central neurons.

1.Introduction

An ability of extracellular ATP to act as neurotransmitter in the CNS has been anticipated from yearly days of research into purinergic signalling. This notion was inspired by the observations of the bulk release of purines from brain tissue and release of ATP from whole-brain synaptosomes (Potter and White, 1980; Sulakhe and Phillis, 1975; White, 1978). Later, ATP-induced membrane depolarisation and ATP-evoked excitatory transmembrane currents were recorded in the peripheral and central neurons (Jahr and Jessell, 1983; Krishtal et al., 1983) inspiring a search for ATP-gated ion channels and ATP-mediated excitatory synaptic transmission in the CNS. During following two decades, the fast ATP-mediated synaptic currents were observed *in situ* in the central neurons of several CNS regions, including medial habenula (Edwards et al., 1992; Robertson and Edwards, 1998), spinal cord (Bardoni et al., 1997), locus coeruleus (Nieber et al., 1997), hippocampus (Pankratov et al., 1998), and neocortex (Pankratov et al., 2002a; 2003). These findings were paralleled (and, to some extent, overshadowed) by considerable progress in the understanding of structure and function of the P2X and P2Y receptors to ATP and roles for these receptors in various brain pathologies (Burnstock, 2015; 2018; Burnstock et al., 2011; Khakh and North, 2006). Recent data also highlighted an importance of neuronal ATP receptors in communications between glial cells and neurons (Boue-Grabot and Pankratov, 2017; Franke et al., 2012; Illes et al., 2019; Lalo et al., 2018; Lalo et al., 2014a; Rivera et al., 2016). Nowadays, ATP-mediated signalling is widely recognised as essential component of CNS processes, including cellular differentiation and development, programmed cell death, circadian clocks, and, of course, synaptic transmission and plasticity (Abbracchio et al., 2009; Burnstock and Dale, 2015; Burnstock *et al.*, 2011; Pankratov et al., 2009).
Yet, while reading reviews on the purinergic signalling in the CNS, one cannot avoid noticing a gap between large bulk of data on the expression of P2 purinoreceptors in the central neurons and rather scarce reports of ATP-mediated synaptic events. This mismatch can be explained by some peculiar functional properties of ATP-mediated synaptic transmission which will be the main focus of this review. We will also review important implications of these properties for physiological role of purinergic neurotransmission. We will look at central synapses in a broader sense, taking into account putative participation of extrasynaptic purinoreceptors activated by the glia-derived ATP.

2. Purinoreceptors in central synapses

Action of ATP as synaptic transmitter is mediated by the P2 family of purinoreceptors which consist of metabotropic P2Y and ionotropic P2X subclasses (Abbracchio et al., 2006; Burnstock *et al.*, 2011; Jarvis and Khakh, 2009). By now, a great deal of experimental data on structure, localisation and pharmacological and functional properties of these receptors have been obtained (for review, (Abbracchio *et al.*, 2006; Burnstock *et al.*, 2011; Illes et al., 2021; Jarvis and Khakh, 2009; von Kugelgen and Hoffmann, 2016)). In last two decades, several transgenic lines of P2X and P2Y knock-out and reporter mice have been developed and used to

explore the expression and function of these receptors in the central neurons (Grohmann et al., 2021; Khoja et al., 2016; Marin-Garcia et al., 2008; Sim et al., 2006; Xu et al., 2016; Zhang et al., 2022).

2.1 P2Y receptors

The P2Y receptor subclass belongs to the conventional 7-transmembrane domain GPCRs and consists of a number of subtypes (P2Y1,2,4,6,11-14) most of which have been reported to be abundantly expressed in the CNS, both in neurons and glia. The predominant subtype expressed in the central neurons is P2Y1 which has been found both at the pre- and postsynaptic *loci*; expression of P2Y2,4,12,13 has also been reported (Burnstock, 2018; Burnstock *et al.*, 2011; Zarrinmayeh and Territo, 2020). In particular, the P2Y1 and P2Y2 receptors have been localised in the hippocampal and cortical pyramidal neurons (Grohmann *et al.*, 2021; Koch et al., 2015; Lommen et al., 2021). Interestingly, surface expression of almost all types of P2Y receptors in hippocampus has been reported to have a diurnal rhythm, at least in rodents (Lommen *et al.*, 2021).

The effect of P2Y receptors in central synapses are mediated by several intracellular signalling cascades, including activation of phospholipase C, adenylate cyclases, protein kinases, and direct interaction with plasmalemmal ion channels (Abbracchio *et al.*, 2006; von Kugelgen and Hoffmann, 2016). Due to a complexity of downstream mechanisms, neuronal P2Y receptors can exert opposing effects on synaptic transmission. In particular, one of the widely reported effects of pre-synaptic P2Y receptors, is reduction of release of glutamate and noradrenaline in the spinal cord, hippocampus, and cerebral cortex (Csolle et al., 2008; Heinrich et al., 2008; Koizumi et al., 2003; Rodrigues et al., 2005). One should note that most of P2Y receptors (except P2Y12-14 subtypes coupled to Gαi) are coupled to the G$\alpha q_{/11}$-subunit activating the phospholipase C which main downstream effect is IP3-mediated release of Ca^{2+} from intracellular stores. So the mechanisms of P2Y-mediated presynaptic inhibition are rather complex. The most likely mechanism is down-regulation of presynaptic Ca^{2+}-influx via up-regulation of K^{+}-channels and down-regulation of voltage-gated Ca^{2+}-channels (Abbracchio *et al.*, 2006; Guzman and Gerevich, 2016; von Kugelgen and Hoffmann, 2016). Molecular cascades underlying these effects relay mainly on direct interactions of ion channels with $\beta\gamma$ subunits of G-proteins coupled to P2YRs (Guzman and Gerevich, 2016; von Kugelgen and Hoffmann, 2016). Alternative explanations for apparent P2Y- and ATP-dependent inhibition of transmitter release could be multi-synaptic interactions (Koch *et al.*, 2015) or indirect negative modulation by adenosine formed after rapid breakdown of ATP (Cieslak and Wojtczak, 2018; Rodrigues et al., 2015; Zarrinmayeh and Territo, 2020).

However, facilitatory effects of P2Y1 and P2Y2 on synaptic release of glutamate were also observed, in particular in the medial habenula (Price et al., 2003) and olfactory bulb (Fischer et al., 2012). The P2Y-mediated presynaptic modulation of glutamate release has been implicated in several types of long-term synaptic plasticity, in particular astrocyte-mediated heterosynaptic depression (Chen et al., 2013; Guzman and Gerevich, 2016; Price *et al.*, 2003). At the same time, P2Y1 receptors were shown to enhance the release of dopamine in the *nucleus accumbens*, midbrain and prefrontal cortex (Koch *et al.*, 2015; Krugel et al., 2001a; Krugel et al., 2001b). As for the release of GABA, action of P2Y receptors appears to be context-dependent, for instance both positive (very likely, by P2Y1 and P2Y2) and negative (by P2Y4) modulation of GABA release from the synapses between various cerebellar interneurons and Purkinje cells have been observed (Brockhaus et al., 2004; Donato et al., 2008; Saitow et al., 2005). Also, P2Y1 receptors have been reported to activate the release of GABA from hippocampal interneurons in Ca^{2+}-dependent manner (Bowser and Khakh, 2004; Kawamura et al., 2004).

At a postsynaptic site, the main pathway downstream of P2Y receptors is phospholipase C-mediated release of Ca^{2+} from the endoplasmic reticulum, which can subsequently activate various intracellular Ca^{2+}-dependent cascades, including regulation of phosphorylation and modulation of activity and trafficking of membrane receptors and ion channels (Abbracchio *et al.*, 2009; Burnstock, 2007; Gerevich et al., 2007; Guzman and Gerevich, 2016; Guzman et al., 2010; Saitow *et al.*, 2005). For instance, P2Y1 receptors have been reported to modulate activity of the voltage gated Ca^{2+}-channels (Guzman *et al.*, 2010) and AMPA (Maiolino et al., 2019) and GABA receptors (Saitow *et al.*, 2005) and trigger desensitization of P2X3 receptors (Gerevich *et al.*, 2007) and internalisation of mGluR1 receptors in Ca^{2+}- and phosphorylation-dependent manner (Mundell et al., 2004). Also, early data indicated a possibility of interactions between postsynaptic P2Y and NMDA receptors (Cavaliere et al., 2004; Luthardt et al., 2003). However, result of further research suggests that P2Y-induced changes in the NMDA receptor activity can be mediated by astrocytes, where P2Y1

receptors trigger Ca^{2+}-elevations and release of glutamate or D-Serine (Lee et al., 2007; Shen et al., 2017; Wirkner et al., 2007; Zeng et al., 2009).

Thus, participation of P2Y receptors in the fast synaptic transmission is manifested mostly in modulation of neurotransmitter release and activity of neurotransmitter receptors (Table 1) which, in turn, contributes to regulation of short- and long-term synaptic plasticity (Abbracchio *et al.*, 2009; Burnstock *et al.*, 2011; Guzman and Gerevich, 2016; Khakh and North, 2012; Rivera *et al.*, 2016).

Other widely reported physiological effects of P2Y receptors in central neurons include regulation of cell differentiation, proliferation, and migration, they also have been recently implicated in neuroinflammation and neurodegeneration (Alves et al., 2018; Burnstock, 2015; 2018; Burnstock and Dale, 2015; Burnstock *et al.*, 2011; Rodrigues *et al.*, 2015). The bulk of evidence, emerged in the last decade, suggests that main roles for P2Y receptors in the CNS relate to glia-neuron interactions and slow neuromodulation (usually in pathological context) rather than direct participation in the synaptic transmission (Alves *et al.*, 2018; Burnstock, 2018; Guzman and Gerevich, 2016; Illes *et al.*, 2019; Zarrinmayeh and Territo, 2020).

2.2 P2X receptors

The fast component of purinergic synaptic transmission is mediated by ionotropic P2X receptors. In contrast to neuronal P2Y receptors whose synaptic action is context-specific leading either to decrease or increase in synaptic strength and neuronal firing, P2X receptors exert mostly facilitatory effects on synaptic signalling contributing to postsynaptic depolarisation and pre- and post-synaptic elevation of cytosolic Ca^{2+} (Bhattacharya et al., 2013; Heinrich *et al.*, 2008; Khakh and North, 2012; Pankratov and Lalo, 2014; Rodrigues *et al.*, 2005) (Table 1). The ionotropic P2X receptors are trimeric ATP-gated cationic (Na^+, K^+ and Ca^{2+}) channels which are formed from subunits containing two transmembrane domains and extracellular ligand-binding loop; seven subunit subtypes (P2X1– P2X7) has been found out so far (Illes and Alexandre Ribeiro, 2004; Illes *et al.*, 2021; Jarvis and Khakh, 2009; North, 2002). The P2X1–5 subunits have been widely reported to assemble in both homo- and heteromeric fashion, whereas P2X6 subunit seems to be present on the cell surface only in heteromeric receptors and P2X7 subunit assembles predominantly to form homomeric receptors (Illes *et al.*, 2021; Jarvis and Khakh, 2009; North, 2002). The study of P2X receptors structure at high atomic resolution has begun rather recently, so molecular mechanisms underlying their function, in particular ion permeability, pore opening and desensitisation are not fully explored and understood (Browne et al., 2010; Hattori and Gouaux, 2012). Although P2X subunits exhibit rather high sequence homology and share a common topology, they vary considerably by their main functional properties, such as kinetics of activation and desensitisation, permeability and affinity to agonists and antagonists (Browne *et al.*, 2010; Illes and Alexandre Ribeiro, 2004; Jarvis and Khakh, 2009; North, 2002).

Ability of different subunits to form various heteromeric receptors (putatively at different stoichiometric ratio) renders the surface expression of wide palette of functional P2X receptors which vary by their kinetics, pharmacological profile and Ca^{2+}-permeability receptors (Illes *et al.*, 2021; Jarvis and Khakh, 2009; North, 2002). For instance, responses of homomeric P2X1 and P2X3 receptors exhibit rapid kinetic of activation and very rapid desensitization with slow recovery (Khmyz et al., 2008; Lalo et al., 2010; North, 2002). In contrast, of the presence of P2X2, P2X4 or P2X5 subunits, either in homo- or heterotrimeric receptors (e.g., P2X1/5, P2X2/3) makes them much less prone to desensitization in the presence of agonist. Further, receptors of different subunit composition exhibit different pharmacological profiles (Illes *et al.*, 2021; Jarvis and Khakh, 2009; Lalo et al., 2008; Lalo et al., 2001). For example, P2X4 receptors are distinct by their high sensitivity to the positive modulator ivermectin and lack of sensitivity to the canonical P2 antagonist PPADS, instead they show high specificity and sensitivity to the blocker 5-BDBD (Illes *et al.*, 2021). The P2X1 and P2X3 subunits have high affinity to the non-hydrolysable ATP-analog α,β-methylene ATP, which does not activated P2Y receptors, and render their heteromers with other receptors sensitive to this compound (Illes and Alexandre Ribeiro, 2004; North, 2002).

In relation to the putative participation in the synaptic transmission in central neurons, the 7th subtype of P2X receptors stands apart due to its peculiar functional properties. First, P2X7 receptors have very low affinity and need to be exposed to very high (hundreds of micromoles) ATP concentration for activation (Illes and Alexandre Ribeiro, 2004; North, 2002). This property is somewhat compensated by the slow but sustained facilitation of P2X7 receptor-mediated ion currents during long-lasting or repetitive application of agonist. The molecular mechanisms underlying this effect remain controversial, since the once widely-accepted

concept of pore "dilation" has been recently scrutinized and several alternative mechanisms have been suggested (Illes *et al.*, 2021). Also, the P2X7 receptors can be strongly inhibited by the divalent cations (Ca^{2+}, Mg^{2+}) at physiological concentrations (Anderson and Nedergaard, 2006; Illes and Alexandre Ribeiro, 2004; North, 2002).

These properties render P2X7 receptors much more suitable for the slow modulation of neuronal activity, especially in the pathological context of brain tissue damage or inflammation when the local extracellular ATP concentration can rise to substantial levels, rather than for direct contribution into the fast synaptic signaling. The early data of expression of functional participation of the P2X7 receptors in central neurons and the notion of their putative contribution in the postsynaptic signaling have been scrutinized (Anderson and Nedergaard, 2006; Zhang *et al.*, 2022) since they were based mainly on immunostaining with P2X7 antibodies which appeared to have poor specificity and neuronal responses to P2X7-preferring ATP analog BzATP, which can also activate other P2X subtypes, albeit with smaller affinity (Anderson and Nedergaard, 2006; Zhang *et al.*, 2022). There is also a growing evidence of abundant expression of P2X7Rs in the glia and immune cells across various brain regions (Burnstock, 2018; Illes *et al.*, 2021; Rivera *et al.*, 2016; Zhang *et al.*, 2022). Furthermore, the recent data (Zhang *et al.*, 2022) obtained in the novel cell type- specific P2X7R knockout mice provided new insights in the mechanisms of changes in the whole-cell transmembrane current, membrane potential or neuronal firing caused by P2X7 receptors agonists and antagonists. Such changes in the neuronal activity, traditionally attributed to the putative postsynaptic P2X7Rs, were shown to originate from indirect modulation mediated by the astrocytic and oligodendrocytic P2X7Rs which, in turn, regulate release of glial signalling molecules (Zhang *et al.*, 2022).

Importantly, all combinations of P2X subunits display significant permeability to Ca^{2+} which is larger than that of Ca^{2+}-permeable AMPA receptors (Burnstock, 2007; Jarvis and Khakh, 2009; Pankratov and Lalo, 2014). Furthermore, Ca^{2+}-permeability of some P2X receptor subtypes expressed by the central neurons, is comparable to that one of NMDA receptors (Pankratov and Lalo, 2014). One should emphasize that, in contrast to NMDARs, Ca^{2+}-influx via P2X receptor channel does not require membrane depolarisation, so Ca^{2+}-permeability of P2XRs can underly their specific roles in modulation of synaptic strengths which will be discussed below.

The abundant expression of P2X receptors, both at the mRNA and protein levels, was widely reported for various CNS regions, apart from neurons, they were detected in the immune and glial cells (Burnstock, 2015; 2018; Illes *et al.*, 2019; Illes et al., 2012; Khakh and North, 2006; Lalo *et al.*, 2008). In particular, expression of P2X2 and P2X4 receptors was widely reported and supported by electrophysiological and Ca^{2+}-imaging characterization of ATP-mediated neuronal activity in various CNS regions, including spinal cord, hippocampus, locus coeruleus, hypothalamus and somatosensory cortex (Illes *et al.*, 2012; Khakh and North, 2012; Pankratov *et al.*, 2009). Also, presence of P2X1 and P2X3 subunits in the central synapses, in particular as a part of P2X1/4 and P2X2/3 heteromeric receptors, was suggested by the electrophysiological and pharmacological data and co-localisation with synaptic markers (Lalo et al., 2016; Pankratov *et al.*, 2002a; Pankratov et al., 2007; Rodrigues *et al.*, 2005). For a long time, the P2X4 receptors were believed to be a dominant P2X subtype in the central neurons, in particular in the hippocampus and cortex; this notion was based mostly on the mRNA expression and immunocytochemistry data (Burnstock, 2015; Cavaliere et al., 2003; Illes and Alexandre Ribeiro, 2004; Kang et al., 2003; Khakh and North, 2006; Rubio and Soto, 2001; Watano et al., 2004). However, more recent work using BAC transgenic *P2rx4* – reporter mice (Xu *et al.*, 2016) reported rather sparse P2X4 labelling across CA1 pyramidal neurons and dense clustering of P2X4Rs in the cortical neurons of layers II and III. The latter result is in good agreement with functional data obtained in the P2X4-knockout mice, showing the deficit in the purinergic synaptic currents and long-term synaptic plasticity (Lalo *et al.*, 2014a; Lalo *et al.*, 2016).

Discrepancies between the data on the expression of P2X receptors, obtained mainly by in situ hybridisation, immunocytochemistry and transcriptomic approaches, and the data obtained using electrophysiology and transgenic mice, can, most likely, be explained by two specific functional properties of P2XRs – desensitization (Jarvis and Khakh, 2009; Khmyz *et al.*, 2008; Lalo *et al.*, 2010; North, 2002) and high lateral and intracellular mobility (Lalo *et al.*, 2010; Li et al., 2000; Richler et al., 2011; Robinson and Murrell-Lagnado, 2013; Toulme and Khakh, 2012; Toulme et al., 2006). These two properties are entangled, since often apparent desensitization and recovery of P2X-mediated responses is associated with their internalisation (Fois et al., 2018; Gerevich *et al.*, 2007; Khmyz *et al.*, 2008; Stokes, 2013).

Although mechanisms regulating the surface density of P2X receptors are not fully understood yet, several important molecular pathways have been identified so far. In particular, surface density of the P2X1 and P2X4 subunits is regulated via dynamin-dependent endocytosis (Bobanovic et al., 2002; Jo et al., 2011; Lalo *et al.*, 2010). An opposing process (membrane insertion) is slower and has been reported to rely on molecular chaperons (Lalo et al., 2012). In the result of continual internalisation, the P2X4-containing receptors are retained mainly in intracellular pools, limiting their availability for the immediate response to ATP release from synapses or glial cells. This may explain a lack of detectable P2X4 receptor mediated currents reported is some experimental studies (Khakh and North, 2012). At the same time, internalized P2X4 receptors are not subjected to the fast lysosomal degradation which creates a certain "readily-deliverable pool" of receptors which can be rapidly trafficked to the plasma membrane (Toulme et al., 2010).

The lateral mobility of the P2X receptors also exhibits some peculiar (if not mysterious) features (Robinson and Murrell-Lagnado, 2013; Toulme and Khakh, 2012). As shown by single-particle tracking of surface P2X2 and P2X4 subunits, their lateral diffusion is rather fast and could be facilitated further upon activation with agonist. At the same time, the diffusion of P2X receptors expressed in central neurons turned out to be confined within extra- and perisynaptic areas of dendritic membrane (Rivera *et al.*, 2016; Toulme and Khakh, 2012). This agrees closely to the results of electron microscopy studies which reported the perisynaptic location of P2X4 and P2X6 subunits on the hippocampal CA1 pyramidal and cerebellar Purkinje neurons (Rubio and Soto, 2001). The mechanisms underlying these specific constrains on lateral mobility of P2X receptors remains elusive; they may be related to the interaction of P2X receptors with lipid rafts or cytoskeleton (Allsopp et al., 2010; Lalo *et al.*, 2010; Marchenkova et al., 2016; Robinson et al., 2014; Vacca et al., 2004).

So, it is conceivable that the P2X receptors remain excluded from the central excitatory synapses, at least at baseline levels of synaptic activity. As a result, recording of notable P2X-mediated synaptic responses often requires a repetitive stimulation or higher stimulus strength (Khakh and North, 2012; Lalo *et al.*, 2016; Pankratov *et al.*, 1998; Pankratov *et al.*, 2007; Pankratov et al., 2002b), which is not just a simple obstacle to overcome in experiment. On one hand, repetitive release of ATP from synaptic terminals or glial cells (either by exogenous stimulation or due to physiological activity in the network) can drive more P2X receptors to the surface. In particular, the study of P2X receptor-mediated signalling in individual cortical synapses showed that ATP released from synaptic terminals can up-regulate the trafficking of P2X receptors into the synapses manifesting in marked increase in the quantal size of purinergic synaptic currents (Lalo *et al.*, 2016). On another hand, long-term exposure of P2X1 and P2X3 receptors to ATP could lead to their desensitisation and promote their internalisation (Burnstock, 2007; Robinson and Murrell-Lagnado, 2013; Vacca et al., 2009). So, the subtype composition and overall density of P2X receptors at given synapse may undergo significant variations, very likely in the activity- and Ca^{2+}-dependent manner. Such bi-directional alterations in the P2XR-mediated component of synaptic transmission can contribute to the mechanisms of acute and homeostatic synaptic plasticity (Boue-Grabot and Pankratov, 2017; Lalo et al., 2019; Lalo et al., 2011b; Pankratov *et al.*, 2009). The "fluidity" of membrane trafficking of P2X receptors can also be related to the age-related plasticity in P2X-mediated signalling in CNS. Changes in the expression of different P2X subunits and in the P2X-mediated responses during development and aging have been reported for central neurons and glial cells (Burnstock, 2007; Lalo *et al.*, 2019; Lalo et al., 2011a). Also, the P2Y and P2X receptor-mediated signaling turned out to be responsive to the environmental enrichment and calorie restriction diet (Lalo *et al.*, 2019).

Thus, there is accumulating data implicating neuronal P2X and P2Y receptors into the function of central synapse. Still, the mechanistic details of the activation of ATP receptors and molecular cascades underlying their roles in the brain are not fully understood. We will discuss the details of most important candidate mechanisms below.

3. Release of ATP in CNS

ATP is a ubiquitous biological molecule present in all cells and intracellular organelles, including secretory vesicles. Therefor it is not surprising that role of ATP as a *bona fide* neurotransmitter is universally acknowledged nowadays. Indeed, exocytosis of ATP was detected in a variety of secretory and non-secretory cells such as oocytes (Aleu et al., 2003), PC12 cells (Fabbro et al., 2004), osteoblasts (Romanello et al., 2005), pancreatic β-cells and peritoneal mast cells (Burnstock, 2007) , peripheral and central neurons (Abbracchio *et al.*, 2009; Burnstock, 2007; Pankratov *et al.*, 2009) and glial cells (Illes *et al.*, 2019; Lalo *et al.*, 2014a). Apart

from vesicular exocytosis, there are several mechanisms of non-vesicular release of ATP from undamaged cells such as release from lysosomes fusing with plasmalemma and concentration gradient-driven diffusion through the gap-junction hemichannels, large conductance anion channels and dilated P2X7 receptors (Fields and Burnstock, 2006; Johnsen et al., 2019; Montero and Orellana, 2015; Rivera *et al.*, 2016; Taruno, 2018). The release of ATP from different cells and synaptosome preparations was detected and verified using a variety of approaches, including biochemical and fluorescent sensors (Frenguelli et al., 2007; Johnsen *et al.*, 2019; Kitajima et al., 2020; Ollivier et al., 2021; Vessey et al., 2020), inhibition of release with Ca^{2+}-chelators and inhibitors of exocytosis and vesicular nucleotide transporters (Bal-Price et al., 2002; Pankratov and Lalo, 2015; Sawada et al., 2008), direct visualisation by live cell imaging (Pangrsic et al., 2007; Vessey *et al.*, 2020) and registration of neuronal quantal responses to individual vesicles (Jo and Role, 2002; Lalo *et al.*, 2014a; Lalo *et al.*, 2016). Several specific features of release ATP as signalling molecule, like co-storage of ATP with other neurotransmitters, existence of vesicular and non-vesicular pathways of release, fast breakdown to other transmitters (AMP and adenosine) and possibility of release both from neurons and glial cells can confer a great complexity to the spatial and temporal features of purinergic signalling. We will look at these mechanisms in the context of signal transmission in the CNS synapses.

3.1 Release of ATP from synaptic terminals

The vesicular release of ATP from synaptic terminals is widespread and rather well documented. Early observations of exocytosis of ATP from peripheral nerves, where ATP is co co-stored with acetylcholine (Burnstock, 2007; Reigada et al., 2003; Stadler and Fenwick, 1983; Stjarne, 2001), inspired an extensive research of ATP release in the CNS and the first evidence of calcium- and membrane-depolarisation-dependent release of ATP was obtained for whole brain synaptosomes (Potter and White, 1980; White, 1977). This was followed by the detection of ATP storage and release from synaptosomes from specific brain regions such as hippocampus (Terrian et al., 1989), spinal cord (Sawynok et al., 1993) and neocortex (Salgado et al., 1997). Finally, the evidence of ATP release elicited by synaptic stimulation *in situ* was obtained in several CNS regions, including hippocampus (Pankratov *et al.*, 1998; Wieraszko and Seyfried, 1989), medial habenula (Edwards *et al.*, 1992), spinal cord (Jo and Schlichter, 1999) and hypothalamus (Jo and Role, 2002; Sperlagh et al., 1998). This evidence was based mainly on the detection of the stimulation-evoked purinergic currents which exhibited kinetics and quantal behaviour expected from the currents of synaptic origin.

From the early days of research into the release of ATP, one its peculiar feature, namely release as a co-transmitter accompanying some principal transmitter, become evident. Co-storage and co-release of ATP with other neurotransmitters, e.g., ACh and noradrenaline, was initially observed in the peripheral nerve terminals and then was expanded, almost as a universal principle of purinergic signalling, to the central neurons (Burnstock, 2007; Cunha and Ribeiro, 2000; Potter and White, 1980). The notion of ATP as co-transmitter was substantiated by observation that ATP acts as sole transmitter only in the medial habenula neurons (Burnstock, 2007; Khakh and North, 2006; 2012). Still the mechanistic details of ATP co-storage and co-release in the central synapses remains elusive.

The biochemical mechanisms of vesicular storage do not entirely agree with ATP being a universal co-transmitter. Accumulation of any transmitters in synaptic vesicle requires a proton pump (like vH^+-ATPase), to create high concentration of protons and positive charge in the vesicular lumen. This electrochemical gradient drives the uptake of neurotransmitters mediated by specific transporters. Accumulation of positively charged amines and ACh is driven mostly by H^+ gradient, whereas accumulation of negatively charged amino acids (glutamate, glycine, and GABA) is driven largely by membrane potential difference (Kelly, 1993). Hence, it is more likely that negatively charged ATP is accumulated in vesicles storing ACh or noradrenaline-containing rather than vesicles containing negatively charged glutamate or GABA.

Indeed, the best-studied examples of action of ATP as a co-transmitter are related to the co-storage of ATP and ACh in the synaptic vesicles in the *Torpedo* ray electric organ and the neuromuscular junctions (Stadler and Fenwick, 1983; Van der Kloot, 2003). On the other hand, detailed investigation of the quantal behaviour of postsynaptic purinergic currents revealed that spontaneous ATP-mediated synaptic events occurred asynchronously of GABAergic mIPSCs or glutamatergic mEPSCs (Lalo *et al.*, 2016; Pankratov *et al.*, 2007) whereas quantal purinergic EPSCs could be evoked by the stimulation of the same individual axons or synaptic boutons as glutamatergic EPSCs. These results suggest that ATP can indeed be co-released with glutamate or GABA but from the separate pool of synaptic vesicles. Interestingly, individual cortical synapses

showed high heterogeneity in regard of co-release of ATP and glutamate with some synapses showing a substantial purinergic component and some synapse lacking it completely (Lalo *et al.*, 2016; Pankratov *et al.*, 2007). The notion of heterogeneity is also supported by the data of (Jo and Role, 2002) who observed spontaneous purinergic currents in the hypothalamic neurons occurring synchronously with GABAergic mIPSCs which was consistent with co-storage of ATP and GABA in the same vesicles.

There is also substantial evidence of distinct pharmacological and functional features of exocytosis of ATP and "main" transmitters in various central synapses. The neurochemical studies of release of ATP and noradrenaline in hypothalamus (Sperlagh *et al.*, 1998) highlighted the differences in their dependence on the stimulation strength and presynaptic modulation. Other studies reported the lack of correlation between released of ATP and release of ACh, noradrenaline, and dopamine from brain and spinal cord synaptosomes (Sawynok *et al.*, 1993). Thus, it is conceivable that, in different central synaptic terminals, the secretory vesicles may contain only principal neurotransmitter, principal neurotransmitter and ATP or contain only ATP. This can confer large variability across central synapses in terms of participation of ATP and purinoreceptors in signal transmission and modulation of synaptic strength.

3.2 Release of ATP from glial cells

Another level of complexity in purinergic modulation of synaptic signalling and plasticity is associated with possibility of release of ATP from the glial cells, in particular astrocytes and microglia. An ability of astrocytes to release ATP was initially suggested by observations of participation of ATP in the propagation of glial Ca^{2+}-waves and the significant contribution of ATP and adenosine to the astroglia-driven modulation of neuronal activity (Bal-Price *et al.*, 2002; Fields and Burnstock, 2006; Gordon et al., 2005). Further research into mechanisms in astrocyte-to-neuron communications suggested that the release of ATP from astrocytes may occur via exocytosis. Exocytosis is a universal and evolutionary conserved mechanism of molecular export by eukaryotic cells and astrocytic release of ATP exhibits main characteristic features of vesicular neurotransmitter release such as a dependence on the proton gradient and vesicular transporters, the SNARE proteins and intracellular Ca^{2+} elevation (Araque et al., 2014; Halassa et al., 2007; Hamilton and Attwell, 2010; Lalo *et al.*, 2014a; Zhang et al., 2007). Interestingly, that seminal work of (Sawada et al. 2008) reporting a discovery of vesicular ATP transporter (which was a final argument in favour of ATP as neurotransmitter), also reported a high level of expression of this transporter (VNUT) in astrocytes.

From the early days of research into the topic, a notion of fast vesicular release of ATP from astrocytes was one of the cornerstones of popular concept of tripartite synapse (Halassa *et al.*, 2007) which had implied the equal importance of astrocytes for synaptic function. Later, the concept of tripartite synapses was scrutinized (Hamilton and Attwell, 2010; Sahlender et al., 2014; Savtchouk and Volterra, 2018) highlighting several factors which could putatively undermine the efficiency of astroglial Ca^{2+}-dependent exocytosis *in vivo* and *in situ*, as compared to astrocytes in culture or synaptic terminals. Such factors include different sub-type composition of SNARE proteins in astrocytes and lack of any kind of "active zones" with high density of vesicles and Ca^{2+}-channels in astrocytes microdomains (Hamilton and Attwell, 2010; Savtchouk and Volterra, 2018). Therefore, in contrast to the synaptic exocytosis, the of ATP and other gliotransmitters from astrocytes might be more widely distributed in time and space, preferentially targeting extra-synaptic neuronal membrane (Araque *et al.*, 2014; Sahlender *et al.*, 2014). Nevertheless, the last decade has brought the substantial body of evidence of important role for vesicular release of ATP in glial physiology.

Firstly, Ca^{2+}-dependent release of ATP from astrocytes, most likely by exocytosis, has been implicated into regulation of LTP in the hippocampus (Pascual et al., 2005), and sleep homeostasis in hypothalamus (Halassa et al., 2009) and control of breathing (Gourine et al., 2010). Later, a definitive evidence that SNARE-dependent exocytosis of ATP from astrocytes in situ can be triggered by the cytosolic Ca^{2+}-elevation attainable in physiological conditions have been provided (Lalo *et al.*, 2014a; Lalo *et al.*, 2016; Lalo and Pankratov, 2022; Rasooli-Nejad et al., 2014). It has also been shown, using targeted astrocyte-specific manipulation of exocytosis and overexpression of ATP-degrading enzymes, that astroglial release of ATP plays important roles in the regulation of brain oxygenation and generation of the BOLD fMRI responses (Angelova et al., 2015; Wells et al., 2015).

Exocytosis of ATP from cortical astrocytes occurred, most likely, from synaptic-like micro-vesicles and lysosomes (Lalo *et al.*, 2014a). Importantly, these works also have directly demonstrated that astrocyte-derived ATP can activates P2X receptors in the neocortical and hippocampal neurons (Lalo *et al.*, 2014a; Lalo and

Pankratov, 2022). The purinergic transmembrane currents elicited in the neurons by astrocyte-derived ATP exhibited smaller amplitude and slower kinetics, so they occurred, most likely, at the extra-synaptic site (Lalo *et al.*, 2014a).. The frequencies and net charge transferred by spontaneous purinergic currents of synaptic and astroglial origin were comparable under baseline conditions. However, the number of purinergic events of astroglial origin exhibited a dramatic increase even at moderate levels of Ca^{2+}-signalling in astrocytes (Lalo *et al.*, 2014a; Lalo and Pankratov, 2022). Such levels of cytosolic Ca^{2+}-elevation could be elicited in astrocytes, for example by short episodes of high-frequency stimulation (10-100 Hs) of neighbouring synapses or by short exposure of astrocytes to physiological levels of noradrenaline or endocannabinoids (Lalo *et al.*, 2014a; Lalo and Pankratov, 2022; Pankratov and Lalo, 2015; Rasooli-Nejad *et al.*, 2014). So, upon certain conditions, the major component of postsynaptic purinergic signalling could be activated by ATP released by astrocytes rather than nerve terminals. Existence of powerful non-synaptic source of ATP, which activates extra-synaptic P2X receptors, could explain the above mentioned inconsistencies between the data on the exclusion of P2X receptors from synapses and data on involvement of ATP and P2X receptors in the plasticity of central synapses (Khakh and North, 2012).

Apart from the exocytosis, a variety of non-vesicular pathways of ATP release from astrocytes were proposed including concentration gradient-driven diffusion through large conductance channels such as gap-junction hemichannels, anion channels and dilated P2X7 receptors (Abbracchio *et al.*, 2009; Fields and Burnstock, 2006; Hamilton and Attwell, 2010). However, it is not very likely for non-vesicular mechanisms to play a dominant role in the astrocytic release of ATP, at least in the neocortex, hippocampus and brainstem where dominant role of vesicular mechanisms has been demonstrated (Gourine *et al.*, 2010; Lalo *et al.*, 2014a). Rather, non-vesicular pathways may bring some (minor) contribution to astrocyte-derived ATP under physiological conditions, but their role can increase during development of brain pathologies. Indeed, a large bulk of data supporting a major role for non-vesicular mechanisms in glial ATP release were obtained in experiments aimed to explore the excitotoxicity of ATP after ischemic tissue damage or during neurological disorders (Burnstock, 2007; Fields and Burnstock, 2006; Franke *et al.*, 2012; Rivera *et al.*, 2016; Rodrigues *et al.*, 2015). The notion of dynamic changes in contributions of vesicular and non-vesicular mechanisms in ATP release has been supported by the results obtained in the spinal cord astrocytes (Garre et al., 2010) which showed that fast initial astroglial exocytosis of ATP triggered a robust, but slow, secondary release of ATP through the connexin hemichannels. Thus, a consensus view on mechanisms of glial ATP release might be a synergy of both exocytosis- and channel-dependent pathways, with the relative contribution of non-vesicular pathways increasing under pathological conditions. Also, the exocytosis of ATP from astrocytes shows significant age-related decline (Lalo *et al.*, 2019; Lalo et al., 2014b) so relative contributions of vesicular and non-vesicular mechanisms might undergo age-related changes. In the context of pathologies, in particular neuroinflammation, significant amount of ATP can be released from microglia, (Beamer et al., 2016; Beggs et al., 2012; Butt, 2011; George et al., 2016). Microglia-derived ATP has been reported to activate P2X receptors in the hippocampal and spinal cord neurons (Beamer *et al.*, 2016; Beggs *et al.*, 2012; George *et al.*, 2016).

Thus, release of ATP from astrocytes provides a powerful pathway of glia-neuron interaction which acts synergically with synaptic release to activate the neuronal P2X and P2Y receptors which, in turn, mediate a variety of physiological and pathological processes in the CNS (Boue-Grabot and Pankratov, 2017; Burnstock, 2018; Rivera *et al.*, 2016; Rodrigues *et al.*, 2015).

4. Role for ATP-receptors in the function of central synapse

Discovery of ATP-mediated synaptic transmission in the peripheral nervous system, in particular in sympathetic neurons, neuromuscular junction, and myenteric nervous system, and accumulating evidence of expression of P2X receptors and release of ATP in the CNS inspired a quest for fast purinergic transmission in central synapses (Abbracchio *et al.*, 2009; Burnstock, 2007). Although direct experimental evidence of P2X receptor-mediated synaptic transmission in the brain, obtained during last three decades, is not that abundant and might even seem underwhelming in comparison to the glutamatergic signalling, purinergic signalling can bring important contribution to function of central synapses.

4.1. Fast synaptic signalling vs slow neuromodulation

While the modulatory action of pre-synaptic P2X, P2Y and adenosine receptors on release of various neurotransmitters in the CNS have been studied for last three decades (Abbracchio *et al.*, 2009; Burnstock,

2007; Cunha and Ribeiro, 2000; Rodrigues *et al.*, 2005; Rodrigues *et al.*, 2015), the physiological importance of P2X receptor-mediated postsynaptic signals is yet to be established. Despite the compelling evidence of contribution of postsynaptic P2X receptors to the fast synaptic transmission in several brain regions (Table 2), no example of action potential firing induced in central neurons by the activation of P2X receptors has been reported so far. With exception of medial habenula, the P2X receptor-mediated component of excitatory synaptic input exhibits rather weak amplitude and often requires rather strong electrical stimulation of afferent fibres (Lalo *et al.*, 2016; Pankratov *et al.*, 2007) or synchronous discharge from multiple presynaptic inputs (Mori et al., 2001) to be observed. At the same time, central neurons of the several brain areas exhibit rather high frequency of spontaneous purinergic excitatory postsynaptic currents, originating both from the synaptic and glial exocytosis of ATP, as discussed above. Thus, the central neurons can be exposed to the diffused but sustained purinergic ionotropic signalling which physiological function remains elusive.

Most likely, the main function of the P2X receptors in the CNS is the slow neuromodulation rather than fast excitatory neurotransmission (Khakh and North, 2012). There are several factors which render the P2X receptors very efficient for this role. Activated by ATP of both synaptic and glial origin, the presynaptic P2XRs contribute to the Ca^{2+} -influx and can enhance release of other neurotransmitters, such as glutamate, GABA and glycine (Donato *et al.*, 2008; Khakh et al., 2003; Khakh and North, 2006; Shigetomi and Kato, 2004; Zhang et al., 2003). At postsynaptic cites, ATP-evoked modulatory effects on synaptic strength exhibit high complexity due to the ability of P2X receptors to regulate Ca^{2+}-dependent phosphorylation of synaptic proteins and physically interact with various neurotransmitters receptors (Boue-Grabot and Pankratov, 2017; Illes *et al.*, 2019).

The P2X receptors can exert the bidirectional effects on the efficacy of excitatory synapses by regulating the trafficking and internalisation of AMPA receptors (Gordon *et al.*, 2005; Pougnet et al., 2014). The P2X receptors have been reported to increase the quantal size of glutamatergic EPSCs in the glutamatergic synapses on neocortical layer 2/3 neurons (Lalo and Pankratov, 2017a) and magnocellular neurosecretory cells (MNCs) in the hypothalamus (Gordon *et al.*, 2005; Gordon et al., 2009). In the MNCs, the P2X-triggered postsynaptic facilitation was mediated by the Ca^{2+}- and PIK3 kinase-dependent insertion of AMPA receptors (Gordon *et al.*, 2005). Interestingly, PIK3 kinase also mediates the NMDA receptor-dependent AMPAR insertion and LTP (Reiner and Levitz, 2018) suggesting that P2X receptors may contribute to (or even substitute) the postsynaptic Ca^{2+}-signalling mediated by the NMDARs (Gordon *et al.*, 2005). Another molecular mechanism of synaptic modulation where P2XR can intervene with glutamate receptors, is synaptic depression via endocytosis of AMPA receptors, which is traditionally associated with NMDARs and mGluRs (Collingridge et al., 2010; Pougnet *et al.*, 2014). Recent data show that, acting via mechanisms distinct from those ones activated by NMDARs and mGluRs, postsynaptic P2X receptors can facilitate removal of synaptic GluA1/GluA2 receptors (Compans et al., 2021; Pougnet et al., 2016). In particular, the P2X2-mediated depression of CA1 synapses has been associated with CaMKII-dependent phosphorylation of S567 and S831 located in the cytoplasmic Loop1 and C-terminal tail of GluA1 subunit (Pougnet *et al.*, 2016) whereas mGluR-dependent internalisation of AMPARs has been linked to the pathway mediated by p38 and MAPK-activated protein kinases 2 and 3 (Eales et al., 2014) and NMDAR-mediated LTD has been associated with synaptic autophagy machinery (Compans *et al.*, 2021). So, similarly to NMDARs, the Ca^{2+}-influx via the postsynaptic P2X receptors can lead to both insertion and endocytosis of AMPA receptors. Whether these opposite effects on the strength of excitatory synapses caused by recruiting different subtypes of P2X receptors or some other factors, like source of ATP or interaction with different synaptic scaffolding proteins, is yet to be explored.

Purinoreceptors can modulate the NMDA receptor-mediated signalling in central synapses. P2X receptors have been reported to down-regulate the NMDARs in the hippocampal and neocortical neurons (Lalo *et al.*, 2016; Pankratov *et al.*, 2002b). Their action occurs via the Ca^{2+}-dependent de-phosphorylation and interactions between NMDARs and calcineurine within PSD-95 protein complex (Lalo *et al.*, 2016; Migaud et al., 1998; Pankratov *et al.*, 2002b) as verified by the deficit of purinergic down-regulation of NMDA receptors in the mutant mice, lacking PSD-95/Dlg4 domain (PSD-95 mutants) and the P2X4 knock-out mice (Lalo *et al.*, 2016). In addition to Ca^{2+}- and phosphorylation-dependent mechanism, the P2X2 and P2X4 receptors can directly inhibit the NMDARs in Ca^{2+}-independent manner via physical interaction of intracellular C-termini, as suggested by recent data of (Rodriguez et al., 2020) obtained in the heterologous expression system; implications of this mechanism for synaptic signalling is yet to be investigated. The cross-talk between

P2Y and NMDA receptors has also been observed in the cerebellar and cortical neurons (Cavaliere *et al.*, 2004; Luthardt *et al.*, 2003; Maiolino *et al.*, 2019). Although the mechanism underlying the P2Y-mediated down-regulation of NMDARs remains unclear, it might occur, similar to the P2X receptors, via Ca^{2+}-dependent de-phosphorylation. Interestingly, the P2 receptor-mediated down-regulation of NMDARs in central neurons can be triggered by purinergic transmitters released both synaptically (Lalo *et al.*, 2016; Pankratov *et al.*, 2002b) and from astrocytes (Lalo *et al.*, 2016; Maiolino *et al.*, 2019) which can confer a high importance of this mechanism not only for synaptic plasticity but also for the protection of neurons against glutamate-mediated excitotoxicity (Maiolino *et al.*, 2019).

The interaction between the postsynaptic P2X and GABAA receptors is rather widely documented, and its molecular mechanisms and physiological implications are relatively well-explored (Boue-Grabot et al., 2004; Jo *et al.*, 2011; Lalo *et al.*, 2014a; Toulme et al., 2007). An ability of several P2X subtypes to modulate the GABAA-mediated inhibitory currents has been observed in the different central neurons and recombinant cells (Boue-Grabot *et al.*, 2004; Jo *et al.*, 2011; Toulme *et al.*, 2007). In particular, the cross-talk between P2X4 and GABAA in the neurons of the ventromedial nucleus of the hypothalamus has been reported to rely on direct physical contact rather than on calcium influx (Jo *et al.*, 2011). For this interaction, two aminoacid residues (Tyr374 and Val375) located at the C-terminus of P2X4 subunit appeared to be crucial; on a side of GABA receptors, this interaction could occur at various α and β subunits but did not depend on γ subunit (Jo *et al.*, 2011). The down-regulation of the GABAA receptors by the P2X2 and P2X3 receptors followed the similar pattern of molecular interactions but relied on the different intracellular motifs in the P2X subunits (Boue-Grabot *et al.*, 2004; Toulme *et al.*, 2007). At the same time, purinergic down-regulation of the postsynaptic GABAA receptors in the neocortical neurons was shown to require the Ca^{2+}-entry via P2X receptors and phosphorylation by the protein kinase C (Lalo *et al.*, 2014a). This type of P2X-GABA interactions underlined the down-regulation of both tonic and phasic GABAergic transmission in neocortical pyramidal neurons and was significantly reduced in the P2X4 KO mice (Lalo *et al.*, 2014a).

Apart from the glutamate and GABA receptors, P2X receptors are known to interact with nicotinic ACh receptors, in particular of α3β4 and α4β2 subtypes (Khakh et al., 2000) and 5-HT3A receptors (Boue-Grabot et al., 2003; Emerit et al., 2016). These interactions manifest in the cross-inhibition when activation of one channel type affects the conductance of the other, and nonadditive responses when receptors are co-activated and occur most likely via conformational coupling (Boue-Grabot *et al.*, 2003; Emerit *et al.*, 2016; Toulme *et al.*, 2007). Also, cross-talk between P2X5 receptors and acid sensing ion channels (Birdsong et al., 2010) as well as between P2X3 receptors and TRPV1 channels (Stanchev et al., 2009) have been reported. The physiological implications of these interactions for the function of central synapses remain mostly unexplored.

Thus, even though P2X receptors do not bring a main contrition into direct depolarisation of postsynaptic membrane in the central neurons (except medial habenula), the P2X-mediated signalling can exert a variety of regulatory effects on synaptic transmission via diverse mechanisms including physical protein-protein or Ca^{2+}-dependent interactions with other ligan-gated channels leading to modulation of their activity and density on the synaptic membrane. In turn, these interactions can produce a variety of modulatory effects on membrane potential and firing of neuronal networks.

4.2 Purinergic signalling and synaptic plasticity

A variety of molecular interactions of different P2X and P2Y purinoreceptors with other synaptic receptors and intracellular proteins underlined the diversity and complexity of purinergic modulation of acute ("conventional" LTP / LTD) and homeostatic synaptic plasticity (Gordon *et al.*, 2009; Illes *et al.*, 2019; Lalo *et al.*, 2018; Lalo *et al.*, 2016; Pankratov *et al.*, 2009; Pankratov *et al.*, 2002b). As one might expect, down-regulation of NMDAR-mediated synaptic signalling by the P2X receptors can have a significant impact on long-term synaptic plasticity. This pathway has been shown to affect the tetanus-induced LTP in the CA1 area of hippocampus and layer 2/3 of somatosensory cortex (Lalo *et al.*, 2016; Pankratov *et al.*, 2002b). Pharmacological blockade of non-P2X4 receptors in CA1 pyramidal neurones (most likely, P2X1-3 subtypes) enable the induction of LTP by the weak sub-threshold stimuli (Pankratov et al 2002b). Impairment of purinergic modulation of NMDAR in the P2X4 KO and PSD-95 mutant mice facilitated the induction of the LTP in the neocortical synapse (Lalo *et al.*, 2016) by shifting the threshold of LTP induction towards much weaker stimuli but also led to the moderate reduction in the net LTP amplitude. These results go in line with

observations of (Sim *et al.*, 2006) that enhancement of P2X4 receptor-mediated signalling by specific modulator ivermectin facilitates the induction of LTP in CA1 region of wild-type but not the P2X4 KO mice.

Combined, the above results suggest an importance of the interaction between P2X and NMDA receptors for the regulation of synaptic plasticity at postsynaptic *locus*. Coupling of NMDA receptors to the PSD-95 multiprotein complex, which underlies this interaction (Lalo *et al.*, 2016), has been implicated into bidirectional modulation of synaptic strength underlying several different types of learning (Migaud *et al.*, 1998; Nithianantharajah et al., 2013). Thus, postsynaptic P2X receptors can be an integral part of the important pathway controlling the activity of NMDA receptors. This pathway can be activated by ATP co-released with glutamate from nerve terminals and ATP released astrocytes and, by down-regulating the NMDA receptors, perform the pre-moderation of synaptic plasticity to avoid unwanted or excessive LTP. This mechanism can also increase a dynamic range of potentiation by pre-setting the lower baseline level of synaptic activity which can also be assisted by P2X-mediated endocytosis of AMPA receptors (Pougnet *et al.*, 2016).

The interactions between P2X and AMPA receptors have been implicated into several specific forms of synaptic plasticity, different from the "conventional" LTP induced by high-frequency tetanic stimulation. The P2X-trigerred endocytosis of postsynaptic AMPA receptors (Pougnet *et al.*, 2016) was shown to participate in the long-term depression induced by paired-pulse low- frequency stimulation or application of agonists of Group I metabotropic glutamate receptors (Lalo and Pankratov, 2022). This prominent form of synaptic depression is traditionally considered to be mediated via postsynaptic mGluRs and usually referred as "mGluR-LTD". Still, the data of (Lalo and Pankratov, 2022) show that postsynaptic P2X-receptors, activated by the ATP-releasing astrocytes (activated via mGluRs), can bring substantial contribution to the down-regulation of the excitatory synapses in the pyramidal neurons of neocortical layer 2/3 and CA1 hippocampal area.

An opposite effect – Ca^{2+}-dependent upregulation of AMPAR-mediated signalling by P2X receptors – has been shown to underlie a distributed up-scaling of hypothalamic synapses (Gordon *et al.*, 2005; Gordon *et al.*, 2009) and homeostatic scaling of the neocortical synapses (Lalo *et al.*, 2018; 2019; Pankratov and Lalo, 2015). These forms of synaptic strength regulation can be viewed as manifestation of homeostatic synaptic plasticity – an ability of neurons to autonomously scale their synaptic strength in response to hyper- or hypo-excitability of neighbouring network (Baroncelli et al., 2010; Nithianantharajah and Hannan, 2006). Such form of responsiveness underlies a capability of brain neural networks to adapt to biochemical challenges during development and ageing (Baroncelli *et al.*, 2010) and can underlie beneficial effects of active lifestyle on ageing brain (Hulme et al., 2013; Lalo *et al.*, 2018; Mercken et al., 2012). Both in the hypothalamus (Gordon *et al.*, 2005) and neocortex (Lalo and Pankratov, 2017a; Pankratov and Lalo, 2015), P2X receptors were activated in response to exocytosis of ATP from astrocytes, which could be activated by astroglial noradrenaline receptors. Since astroglial adrenergic Ca^{2+}-signalling is responsive to the state of wakefulness and physical activity (Ding et al., 2013), such way of activation of postsynaptic P2X receptors can make them instrumental for the beneficial effects of physical exercise and environmental enrichment on synaptic plasticity, in particular in aging brain (Lalo *et al.*, 2018).

Since phasic and tonic GABA-conductance can affect membrane depolarization and thereby influence the induction of NMDAR-dependent synaptic plasticity, one might expect some role for the cross-talk between P2X and GABA receptors in the modulation of the LTP. Indeed, activation of P2X by astrocyte-derived ATP was shown to facilitate the induction of LTP in neocortical neurons (Pankratov and Lalo, 2015; Rasooli-Nejad *et al.*, 2014). Most likely, P2X-mediated attenuation of GABAergic inhibition facilitates LTP induction via increasing the depolarization of postsynaptic neurons and therefore, alleviating the Mg^{2+}-block of NMDA receptors. Also, the interaction between P2X and GABA receptors was implicated into age- and experience-dependent homeostatic regulation of inhibitory synaptic transmission in the neocortex (Lalo *et al.*, 2018; 2019).

Apart from P2X, the P2Y receptors also can take part in modulation of synaptic plasticity in CNS. In the CA1 neurons, the presynaptic P2Y receptors were found to induce the heterosynaptic LTD upon activation with ATP released from neighbouring astrocytes; this mechanism relied on P2Y-mediated attenuation of glutamate release (Chen *et al.*, 2013). The postsynaptic P2Y receptors were found to negatively regulate the synaptic plasticity in the layer 5 pyramidal neurons, where inhibition of P2Y1 receptors increased the number of cells developing LTD (Guzman et al., 2005; Guzman *et al.*, 2010). The most likely mechanism for this effect is downregulation of voltage-gated calcium channels by P2YRs (Guzman *et al.*, 2010). In the cerebellar

Purkinje neurons, the P2Y receptor-mediated elevation of cytosolic Ca^{2+} was reported to induce potentiation of GABAergic input from the cerebellar interneurons, most likely via upregulation of GABAA receptor agonist sensitivity (Saitow *et al.*, 2005).

Many brain pathologies, such as ischemia, stroke, chronic pain, Amyotrophic Lateral Sclerosis or Alzheimer disease are associated with excessive release of ATP from various sources, including astroglia, (Burnstock, 2007; Burnstock *et al.*, 2011; Rivera *et al.*, 2016; Rodrigues *et al.*, 2015) and upregulation of surface density of P2X and P2Y receptors in various pathophysiological contexts was observed as well (Alves *et al.*, 2018; Burnstock, 2015; Cieslak and Wojtczak, 2018; Montilla et al., 2020; Tsuda et al., 2013; Zarrinmayeh and Territo, 2020). So, one might assume that effects of P2 purinoreceptors on synaptic transmission and plasticity might be augmented under pathological circumstances. Indeed, there is growing evidence linking pathologic plasticity of purinergic signalling with various neurodegenerative and psychiatric diseases (Burnstock, 2018; Burnstock *et al.*, 2011; Illes *et al.*, 2019; Montilla *et al.*, 2020; Pankratov *et al.*, 2009). One should note, however, that P2X and P2Y receptors can exert opposing effects on the AMPAR and NMDAR-mediated signalling and therefore the net effect of excessive purinergic signalling on synaptic plasticity could hardly be predicted at current state of our knowledge. Yet, one should note that many pathways discussed above provide several feedback loops which could maintain the overall balance between glutamatergic excitation and GABA inhibition. Thus, putative enhancement of ATP-driven modulation of synaptic signalling under pathological conditions might have a homeostatic, "stabilizing" effect on synaptic plasticity. One should also note another powerful feed-back loop of purinergic regulation of synaptic strength which is mediated by the adenosine rapidly formed from ATP released either from synaptic terminals or glia (Halassa *et al.*, 2007; Pascual *et al.*, 2005; Rivera *et al.*, 2016; Rodrigues *et al.*, 2015). The adenosine receptors can exert a variety of effects on neuronal signalling, the most prominent of which is pre-synaptic inhibition (Burnstock *et al.*, 2011; Cieslak and Wojtczak, 2018; Rodrigues *et al.*, 2015). There is a plethora of evidence that disruption of adenosine-mediated feedback cascades contributes into various brain pathologies; for review, one can refer to (Abbracchio *et al.*, 2009; Burnstock *et al.*, 2011; Cieslak and Wojtczak, 2018; Zarrinmayeh and Territo, 2020).

Other confounding factors in the pathological alteration of purinergic regulation of synaptic plasticity are neuroinflammation and significant molecular and morphological changes in astrocytes and microglia (Franke *et al.*, 2012; Morita et al., 2019; Singh and Abraham, 2017; Tsuda *et al.*, 2013) which can in turn affect important neuroprotective and metabolic functions of glial cells and release of gliotransmitters (including ATP) as well (Illes *et al.*, 2019; Lalo *et al.*, 2019; Rivera *et al.*, 2016). Thus, to disentangle the mechanisms of purinergic modulation of pathological synaptic plasticity further studies are needed.

5. Conclusions

To conclude, there is compelling evidence that ATP released from the synaptic terminals, astrocytes and microglia can activate neuronal P2 receptors, both at the post- and pre-synaptic loci, at a variety of locations across CNS including spinal cord, hypothalamus, hippocampus and neocortex. The ATP-mediated signalling brings only minor contribution into the net postsynaptic depolarisation but it is instrumental for slow and diffuse modulation of synaptic dynamics and neuronal firing in many CNS areas, so it may play some specific roles in brain computation. There is growing evidence of participation of purinoreceptors in the regulation of synaptic homeostasis and plasticity in different physiological and pathological context. Although there are many gaps in our current knowledge of ATP-mediated signalling in brain synapses, this topic is definitely worthwhile of further exploration.

Table 1. Contribution of P2 purinoreceptors in the synaptic transmission and physiological functions of central neurons

Receptor[1]	Location/cell type	Species & Age	Experimental evidence and main physiological effects	Reference:
P2Y	Cerebellar cortex	Adult rats	P2Y receptors inhibit release of serotonin and noradrenaline from neocortical neurons	(von Kugelgen et al., 1997)
P2Y1,13	Hippocampal CA1 pyramidal neurons	Young adult rats	P2Y receptors inhibit release of glutamate and noradrenaline from hippocampal neurons	(Csolle *et al.*, 2008; Mendoza-Fernandez et al., 2000)
P2Y1	Hippocampal CA1 interneurons	Juvenile mice	P2Y1 receptors, activated either by exogenous ATP or endogenous release of ATP, caused Ca^{2+}-elevation and depolarisation in interneurons, resulting in the increased GABAergic inhibition	(Bowser and Khakh, 2004)
P2Y	Cerebellar Purkinje neurones	Juvenile rats	P2Y agonists induced long-term postsynaptic increase of GABAA receptor efficacy in Ca^{2+}-dependent manner; P2Y1 specific agonists induced short-term increase in the frequency of GABAergic mIPSCs.	(Saitow *et al.*, 2005)
P2Y4	Cerebellar Purkinje neurones	Juvenile rats	Presynaptic P2Y4 receptors inhibited release of GABA from basket cells onto Purkinje neurons	(Donato *et al.*, 2008)
P2Y1,12 P2Y13	Spinal cord dorsal horn neurons	Juvenile rats	P2Y receptors inhibit release of glutamate and noradrenaline from spinal cord neurons	(Heinrich *et al.*, 2008)
P2Y1	Pyramidal neurons of prefrontal cortex	Adult rats	P2Y receptors inhibit the induction of mGluR-dependent LTD via inhibition of voltage-gated Ca^{2+}-channels in the dendrites and spines of pyramidal neurons	(Guzman *et al.*, 2010)
P2Y1	Olfactory bulb neuron	Juvenile mice	Activation of P2Y receptors by un-caging of ATP or ADP enhanced firing in mitral cells (output neurons of olfactory bulb), most likely, via activation of glutamatergic presynaptic interneurons	(Fischer *et al.*, 2012)
P2Y	Hippocampal CA1 neurons	Juvenile mice	Neuronal P2Y receptors mediated heterosynaptic depression triggered by astrocyte-derived ATP	(Chen *et al.*, 2013)
P2X1-3	Medial habenula neurons	Young adult rats	P2X receptors mediate the main component of excitatory synaptic input in medial habenula neurons; ATP and glutamate are released from functionally different synapses.	(Edwards *et al.*, 1992; Robertson and Edwards, 1998)
P2X2, P2X4	Spinal cord dorsal horn neurons	Juvenile rats	P2X receptors participate in excitatory synaptic input and transmission of nociceptive information in the subpopulation of spinal cord lamina II neurons	(Bardoni *et al.*, 1997)
P2X1-3	Locus coeruleus neurons	Adult rats	P2X receptors participate in the fast excitatory synaptic transmission	(Nieber *et al.*, 1997)
P2X1,3,4	Hippocampal CA1 neurons	Young adult rats	P2X receptors mediate minor component of excitatory synaptic input in the CA3-CA1 synapses, participate in the Ca^{2+}-signalling, and modulate long-term synaptic plasticity	(Pankratov *et al.*, 1998; Pankratov *et al.*, 2002b)
P2X	Hypothalamic neurons *in vitro*	Chick and mice	Co-release of ATP with GABA and concurrent activation of P2X and GABAA receptors	(Jo and Role, 2002)
P2X1,3,4	Somatosensory cortex layer 2/3 pyramidal neurons	Adult rats and mice	ATP co-released with glutamate from neocortical synapses activate P2X receptors which mediate minor component of fast excitatory synaptic and modulate activity of AMPA and NMDA receptors, and modulate long-term synaptic plasticity	(Pankratov *et al.*, 2002a; 2003; Pankratov *et al.*, 2007); (Lalo *et al.*, 2016)
P2X1, P2X3	MNTB neurones	Juvenile mice and rats	Application of specific P2X agonists enhanced the frequency of spontaneous EPSCs and IPSCs (most likely at presynaptic *loci*); modulation of IPSCs involved both P2X1 and P2X3 receptors	(Watano *et al.*, 2004)

P2X4	Hippocampal CA1 neurons	Adult mice	P2X4 receptors, located at the periphery of glutamatergic synapses, facilitate the NMDAR-dependent LTP; the magnitude of tetanus-induced LTP was decreased in the P2X4 knockout mice	(Sim *et al.*, 2006)
P2X	Brainstem NTS neurons	Adult rats	P2X receptors mediate minor component of excitatory synaptic input elicited by stimulation of the tractus solitarius and minor fraction of spontaneous EPSCs	(Li and Yang, 2007)
P2X1/3	Cerebellar Purkinje neurones	Juvenile rats	Presynaptic P2X receptors enhanced release of GABA from basket cells onto Purkinje neurons	(Donato *et al.*, 2008)
P2X2, P2Y1	spherical bushy cells of cochlear nucleus	Juvenile gerbils	Application of ATPγS induced postsynaptic inward current, membrane depolarisation and somatic Ca^{2+}-entry; Ca^{2+}-elevation mediate by P2X2 and P2Y1 caused changes in the SBC firing pattern	(Milenkovic et al., 2009)
P2X	Cerebellar Purkinje neurones	Juvenile mice	Application of ATP induced the P2X-dependent postsynaptic depolarisation and Ca^{2+}-entry which, in turn, activated CB1 receptor-dependent retrograde synaptic suppression	(Kovacs et al., 2011)
P2X2,3,4	Somatosensory cortex pyramidal neurons	Adult and aged mice	Extrasynaptic P2X activated by ATP released from astrocytes down-regulate phasic and tonic GABAergic inhibitory transmission, and facilitate the induction of LTP in the layer 2/3 neurons	(Lalo *et al.*, 2014a) (Pankratov and Lalo, 2015)
P2X2	Hippocampal pyramidal neurons	Young adult mice	Postsynaptic P2X receptors, activated by glia-derived ATP, down-regulate the strength of glutamatergic synapses by triggering the dynamin-dependent internalization of AMPA receptors	(Pougnet *et al.*, 2014)
P2X2,4	Somatosensory cortex pyramidal neurons	Adult and aged mice	Postsynaptic P2X activated by ATP released from astrocytes up-regulate the strength of glutamatergic synapses; expression of ATP receptors undergoes age- and experience-dependent plasticity	(Lalo and Pankratov, 2017b) (Lalo *et al.*, 2019)

[1] Note: receptors subtypes are mentioned if they were verified by any approach including: specific pharmacological agents, mRNA expression, immunolabeling, transgenic modifications or their combination.

Table 2. Contribution of ATP receptors in fast synaptic transmission in central neurons

Receptor[1]	Location Cell type	Species & age	Experimental evidence					Reference:
			Synaptic currents		Agonist-induced responses[2]		Quantal/vesicular release of ATP[4]	
			evoked	mEPSCs	ATP	Other[3]		
P2X1-3	Medial habenula neurons *in situ*	Young adult rats	+	+				(Edwards *et al.*, 1992; Robertson and Edwards, 1998)
P2X2, P2X4	spinal cord dorsal horn neurons *in situ*	Juvenile rats	+		+	+		(Bardoni *et al.*, 1997)
P2X1-3	Locus coeruleus neurons *in situ*	Adult rats	+			+		(Nieber *et al.*, 1997)
P2X1,3,4	Hippocampal CA1 neurons *in situ*	Young adult rats	+		+	+		(Pankratov *et al.*, 1998; Pankratov *et al.*, 2002b)
P2X	CA3 neurons of organotypic culture	Rats	+		+			(Mori *et al.*, 2001)
P2X	Hypothalamic neurons *in vitro*	Chick and mice	+	+			+	(Jo and Role, 2002)
P2X1,3,4	Neocortex layer 2/3 pyramidal neurons	Adult rats and mice	+	+	+	+	+	(Pankratov *et al.*, 2002a; Pankratov *et al.*, 2007) (Lalo *et al.*, 2016)
P2X	Brainstem NTS neurons *in situ*	Juvenile rats	+	+				(Li and Yang, 2007)
P2X2	spherical bushy cells of cochlear nucleus	Juvenile gerbils				+		(Milenkovic *et al.*, 2009)
P2X	Cerebellar Purkinje neurones *in situ*	Juvenile mice			+			(Kovacs *et al.*, 2011)

[1] Receptors subtypes are mentioned if they were verified by any approach including: specific pharmacological agents, mRNA expression, immunolabeling, transgenic modifications or their combination.

[2] Includes direct responses: P2 agonist-induced depolarisation, transmembrane currents or Ca^{2+}-transients; modulation of amplitude or frequency of synaptic currents mediated by other receptors are not included.

[3] Non-hydrolysable and P2X subtype-specific ATP analogs

[4] Verified by biochemical analysis of vesicular fusion or by the quantal behaviour of purinergic currents

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