Advanced Review

P2X receptor-mediated synaptic transmission

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Activation of postsynaptic P2X receptors, which occur in many synapses in the central nervous system, following vesicular adenosine triphosphate (ATP) released from presynaptic terminals contributes to the fast excitatory neurotransmission. Postsynaptic P2X receptors mediate fast excitatory postsynaptic currents in synapses located in various brain regions, including medial habenula, hippocampus, and cortex. Synaptic stimulation of P2X receptors can trigger substantial Ca^{2+} entry and cytosolic Ca^{2+} signaling at the resting membrane potential due to a high Ca^{2+} permeability of P2X receptors. In addition, P2X receptors dynamically interact with *N*-methyl *D*-aspartate (NMDA) receptors, γ-aminobutyric acid (GABA) receptors, and nicotinic acetylcholine (ACh) receptors. Activation of P2X receptors in the course of synaptic transmission has multiple modulatory effects on synaptic plasticity, either inhibiting or facilitating the long-term changes of synaptic strength depending on physiological context.

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INTRODUCTION

The purinergic transmission that employs adenosine triphosphate (ATP), adenosine, and related nucleotides as universal extracellular signaling molecules is widely present in the nervous system.[1–4] ATP is physiologically released from both neurones and neuroglia by Ca^{2+}-dependent vesicular exocytosis[5] and by diffusion through several types of plasmalemmal channels.[6–9] Neural cells, as a rule, express several types of purinoceptors[10] represented by P1 metabotropic adenosine receptors,[11,12] P2Y metabotropic nucleotide receptors,[13] and P2X ionotropic ATP receptors.[14–16]

The ionotropic P2X receptors assembled (in homo- or heteromeric fashion) from seven subunits are expressed in both the peripheral nervous system (PNS) and the central nervous system (CNS) and mediate fast purinergic excitatory neurotransmission in a variety of synapses in the brain, in the spinal cord, and in peripheral nerve terminals.[4,15,17] In addition, ATP is intimately involved in synaptic communication between neurones and neuroglia,[18–20] these effects being also partially mediated by P2X receptors. In this article, we shall overview the main properties of ATP-mediated synaptic signaling through P2X receptors.

IDENTIFICATION OF P2X RECEPTOR-MEDIATED SYNAPTIC TRANSMISSION

The very first demonstration of physiological ATP release from excited neuronal structure was made by Pamela Holton,[21] who found that electric stimulation of the great auricular nerve of rabbit resulted in a transient increase in the concentration of extracellular ATP. Some year afterwards, ATP release was also identified in sympathetic nerve terminals.[22] These early observations prompted the concept of ATP-mediated neurotransmission, which was introduced by Geoffrey Burnstock.[23,24]

In late 1970s, ATP release was identified in the suspension of brain synaptosomes treated with

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veratridine or high extracellular K^+.[25,26] Slightly later, the excitatory action of ATP was shown in isolated dorsal horn neurones, in which ATP triggered $[Na^+]_o$-dependent depolarization indicating the involvement of specific cationic channel.[27] Another decade, however, passed before ATP-mediated synaptic currents were identified in the medial habenula slices,[28] in cultured celiac ganglion cells,[29] and in the myenteric neurones.[30] The role of ATP as fast neurotransmitter in the brain was further corroborated when P2X receptor-mediated excitatory synaptic transmission was found in the spinal cord,[31] in the hippocampus,[32,33] locus coeruleus,[34] and in the somatosensory cortex.[35,36]

P2X-MEDIATED SYNAPTIC CURRENTS

In the CNS, ATP is generally coreleased with glutamate from excitatory synapses[37]; a small population (~10%) of synapses in the medial habenula utilize ATP as the sole transmitter.[38] There are also indications[39,40] that ATP can be coreleased with GABA from GABAergic terminals.[39,40] As a result in the majority of synapses, P2X receptors mediate only a fraction of total postsynaptic current.

In the experiments *in situ*, the ATP-mediated component of synaptic currents can be dissected from other components of synaptic transmission using pharmacological assays. In glutamatergic excitatory synapses, the P2X-mediated component of the excitatory postsynaptic current (EPSC) can be revealed after inhibition of 2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl)propanoic acid (AMPA)/NMDA glutamate receptors with specific agents (mixture of 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX)/2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulfonamide (NBQX) and D-(-)-2-Amino-5-phosphonopentanoic acid (D-AP5), see Figure 1). The purinergic nature of the residual synaptic currents remaining in the presence of glutamate receptor blockers was corroborated by their sensitivity to P2/P2Xantagonists suramin, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS), NF023, and NF279. These purinoceptor agonists usually produce an almost complete block of the residual (i.e., nonglutamatergic) EPSC in cortical and hippocampal synapses; in contrast, antagonists of nicotinic cholinoreceptors, P1 adenosine receptors, and serotonin receptors are ineffective (Figure 1 and Refs 35, 36, and 41). Alternatively, the role for P2X receptors can be confirmed by their desensitization induced by incubation with nonhydrolyzable ATP analogs such as α,β-methylene-ATP or ATP-γ-S.[28,33,35]

It should be noted, however, that the pharmacological approach of dissecting of P2X-mediated synaptic currents in CNS preparations has certain limitations. Although most of P2X receptor antagonists (except suramin) show good selectivity with respect to other ionotropic receptors (being inactive against glutamatergic, GABAergic, and cholinergic receptors), many of them (e.g., PPADS and suramin) readily inhibit metabotropic P2Y purinoceptors. Therefore, the use of these antagonists in brain slices may affect the release of neurotransmitters and special attention should be paid to discrimination between post- and presynaptic effects. Similarly, P2X receptor agonists may have side effects. For example, inhibition of synaptic responses in the hippocampus by γ-phosphorus-substituted ATP analogs resulted from their catabolism to adenosine with subsequent activation of P1 receptors.[42] This inhibition, however, developed slowly and required 15–20-min exposure of tissue to high concentration of agonist (IC_{50} about 20 μM). Conversely, α,β-methyleneATP and 2-methylthioATP had weak inhibitory action and are not converted to adenosine. Taken together, the selective action of α,β-methyleneATP on some P2X subtypes and the absence of the effects on P2Y receptors makes this agent a reliable tool for studying P2X-mediated synaptic currents.

QUANTAL ATP RELEASE IN CENTRAL SYNAPSES: SPONTANEOUS P2X-MEDIATED EPSCs

To all likelihood, Ca^{2+}-regulated exocytosis represents the preferred physiological mechanism of ATP release from presynaptic terminals in the CNS. ATP can be accumulated and stored in synaptic vesicles either together with other neurotransmitters or in specific ATP-containing vesicles. ATP accumulation into vesicles is mediated by transporters such as, for example, Cl^--dependent vesicular nucleotide transporter (VNUT), which belongs to the family of SLC17 anion transporters that also includes vesicular glutamate transporters.[43] The VNUT is widely expressed in the CNS with preferential localization in astroglia. The costorage and corelease of ATP with other neurotransmitters is abundant in the PNS[4]; in the CNS, ATP was reported to corelease with GABA (in cultured neurones from spinal cord and lateral hypothalamus[39,40]) and with glutamate (hippocampal organotypic slices[32]; see also Ref 5 for review). Alternatively, ATP may be released from specific ATP-containing vesicles, which can be present in specific purinergic terminals (in the medial habenula[38]) or colocalize with glutamate-containing vesicles in the

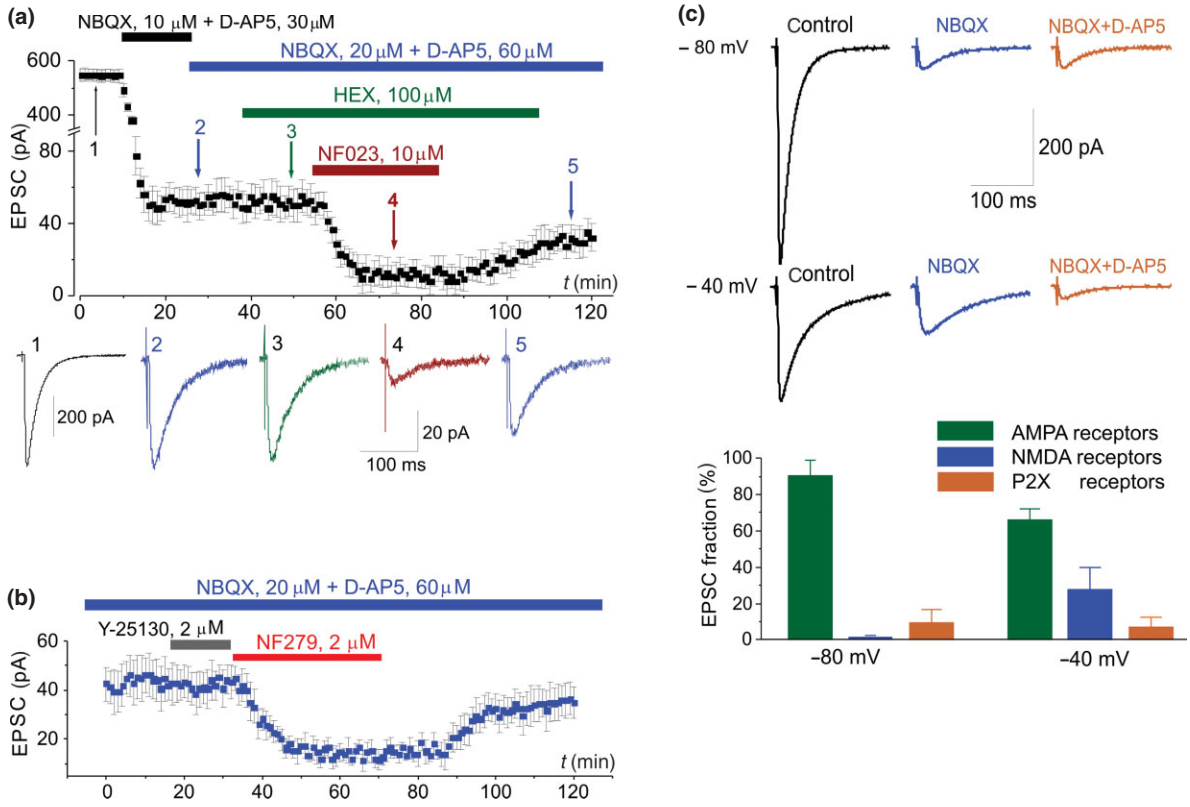

FIGURE 1 | Contribution of P2X receptors to the excitatory synaptic currents in the somatosensory cortex. (a–c) Excitatory postsynaptic currents (EPSCs) were elicited in the pyramidal neuron of somatosensory cortex layer II by field stimulation of thalamocortical afferents in the presence of bicuculline, 20 μM. (a) Changes in the amplitude of EPSC following bath application of glutamatergic antagonists NBQX and D-AP5, cholinergic antagonist hexamethonium (HEX), and P2X receptor antagonist NF023 as indicated in the graph. Each point represents the mean ± SD for six sequential trials, holding potential of −80 mV. Bottom, the examples of EPSC (average of six traces) recorded as indicated in the upper graph. Note the presence of nonglutamatergic EPSCs sensitive to P2X receptor antagonist. (b) Changes in the amplitude of nonglutamatergic EPSC following bath application of 5-HT3 receptor antagonist Y-25130 and P2X receptor antagonist NF279. Recordings were made at a holding potential of −80 mV in the presence of 10 μM NBQX, μM D-AP5, and μM bicuculline. Each point represents the mean ± SD for six sequential trials. (c) *Upper panel*, representative EPSCs recorded in the cortical pyramidal neuron at different membrane potentials after consecutive application of 20 μM NBQX and 60 μM D-AP5. Each trace represents the average of six consecutive sweeps. *Bottom panel*, the average relative contribution of the AMPA, NMDA, and adenosine triphosphate (ATP) receptors to the total EPSC measured at different membrane potentials. Each column represents the mean ± SD for 16 cells. All data were obtained in the presence of 20 μM bicuculline. (Reprinted with permission from Ref 36. Copyright 2003 Elsevier)

excitatory glutamatergic terminals (e.g., in the cortex and hippocampus[5,37]). The nonvesicular (diffusional) ATP release may occur through unpaired hemichannels or through volume-sensitive chloride channels, or even through $P2X_7$ receptors, known to form a large transmembrane pore following stimulation.[7,8,44–47] These latter mechanisms are predominantly associated with neuroglial cells and may also be operational in pathological conditions.

Electrophysiological correlate for vesicular neurotransmitter release is represented by spontaneous (or 'miniature') excitatory postsynaptic currents (mEPSCs) that represent stochastic release of a single quantum of neurotransmitter in conditions of inhibited neuronal electrical excitability.[48] The quantal release of ATP from synaptic terminals in the CNS was revealed by the analysis of P2X receptor-mediated mEPSCs (Figure 2) in the neocortical and hippocampal pyramidal neurones.[5,37] In these synapses, the ATP- and glutamate-mediated transmission occurs at the very same nerve terminals, which host ATP and glutamate-containing vesicles. Both ATP- and glutamate-mediated miniature excitatory postsynaptic potentials (mEPSPs) were sensitive to toxins known to modify vesicular release (Figure 2b, see also Refs 5 and 37). For example, α-latrotoxin significantly increased the frequency of P2X-mediated and glutamatergic mEPSCs. The P2X-mediated mEPSCs were distinguished from mEPSPs mediated by glutamate receptors by their amplitude distribution, kinetics, and pharmacology (Figure 2, Refs 5 and 37).

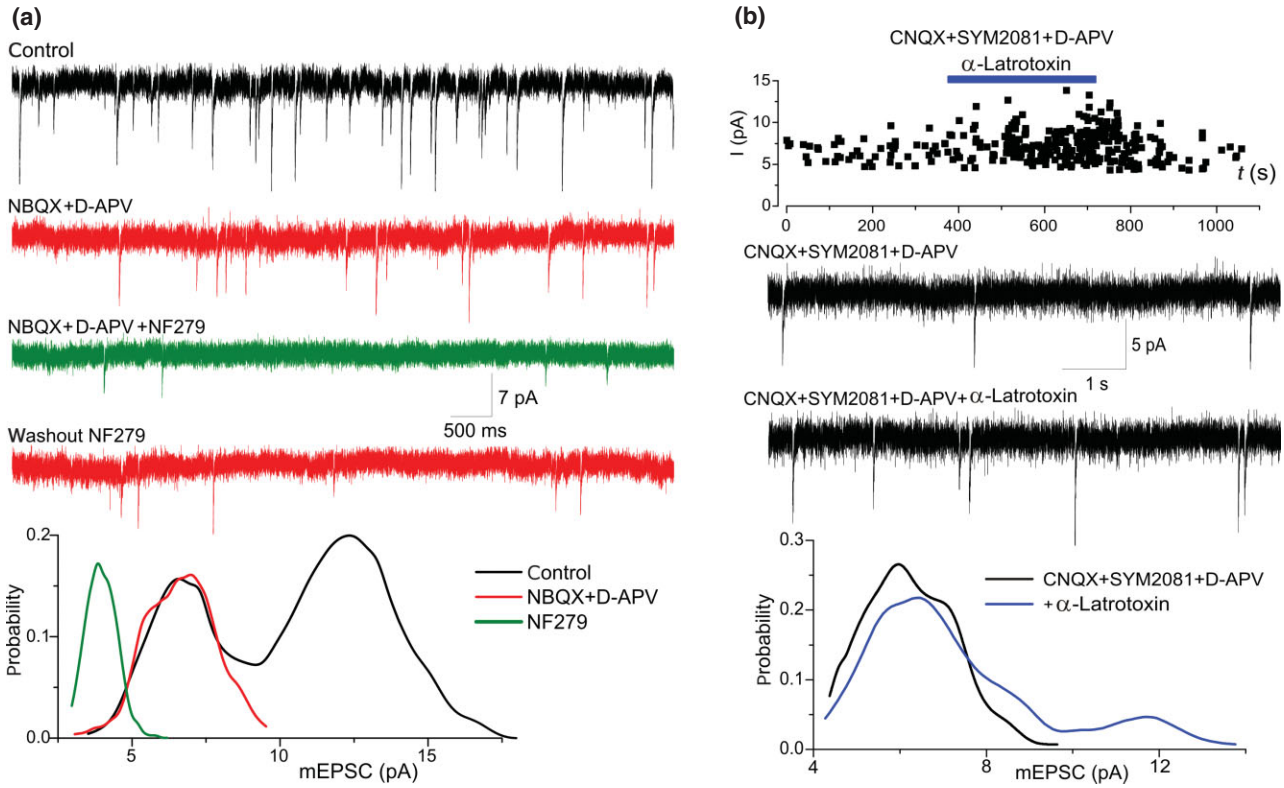

FIGURE 2 | Two populations of spontaneous miniature excitatory postsynaptic currents (mEPSCs) in the neocortical pyramidal neurones. (a) Representative mEPSCs recorded in the somatosensory cortex layer II/III pyramidal neurone at membrane potential −80 mV in the presence of 1 μM tetrodotoxin (TTX) and 100 μM picrotoxin (control, black lines) after application of the glutamate receptor blockers (100 μM NBQX and 30 μM D-AP5; red lines) and after subsequent addition of P2X receptor antagonist NF279 (1 μM). Note the disappearance of high-amplitude mEPSCs after the inhibition of glutamate receptors. *Lower panel* shows the amplitude distribution (probability density functions) of mEPSCs constructed from the recordings illustrated in the upper panel. The amplitude distribution in the control conditions (black line) exhibits two clear peaks at 7.2 pA (smaller peak) and 12.3 pA (larger peak). Note the disappearance of the larger peak after the inhibition of glutamate receptors (red line) and shift of smaller peak toward lower amplitude values under action of P2X receptor antagonist (green line). (b) Purinergic spontaneous currents are potentiated by α-latrotoxin, a positive modulator of exocytosis. The representatives are time course of amplitude and examples of mEPSCs recorded in the neocortical pyramidal neuron at membrane potential −80 mV in the presence of 1 μM TTX and 100 μM picrotoxin and the mixture of glutamate receptor blockers (50 μM CNQX, 10 μM SYM2081, and 30 μM D-AP5). Each dot on the upper graph represents single mEPSC. Application of α-latrotoxin causes a burst of purinergic mEPSCs. The amplitude distribution in the lower graph does not show considerable changes in the amplitude of main peak, but shows an appearance of secondary peak of double amplitude. These data indicate that purinergic mEPSCs originate from quantal vesicular release of adenosine triphosphate (ATP).

In cortical and hippocampal excitatory synapses, P2X receptors mediate the distinct population of synaptic currents (Figure 3). In these synapses, ATP is most likely released from a separate pool of vesicles localized in a subset of excitatory glutamatergic synaptic terminals.[5,37] In the medial habenula, ATP is exocytotically released from a relatively minor subset of specific ATPergic terminals.[38] The segregation of neurotransmitter vesicles and the coexistence of separate pools of vesicles containing ATP and other neurotransmitters in the same presynaptic compartment have also been reported for the PNS. For example, the stimulation of postganglionic sympathetic nerves in the vas deference induced corelease of noradrenaline, ATP, and neuropeptide Y, which all are stored in separate vesicles.[49]

FUNCTIONAL ROLE OF P2X EPSCs

P2X-Mediated Ca^{2+} Signaling

As a rule, the contribution of P2X receptors to the total synaptic current in the central synapses is rather small; the amplitude of P2X-mediated component of EPSCs rarely exceeds 50–100 pA, which represent 5–15% of the amplitude of the synaptic current mediated by glutamate receptors. Nonetheless, the ATP-mediated synaptic transmission is functionally important, especially considering that activation of

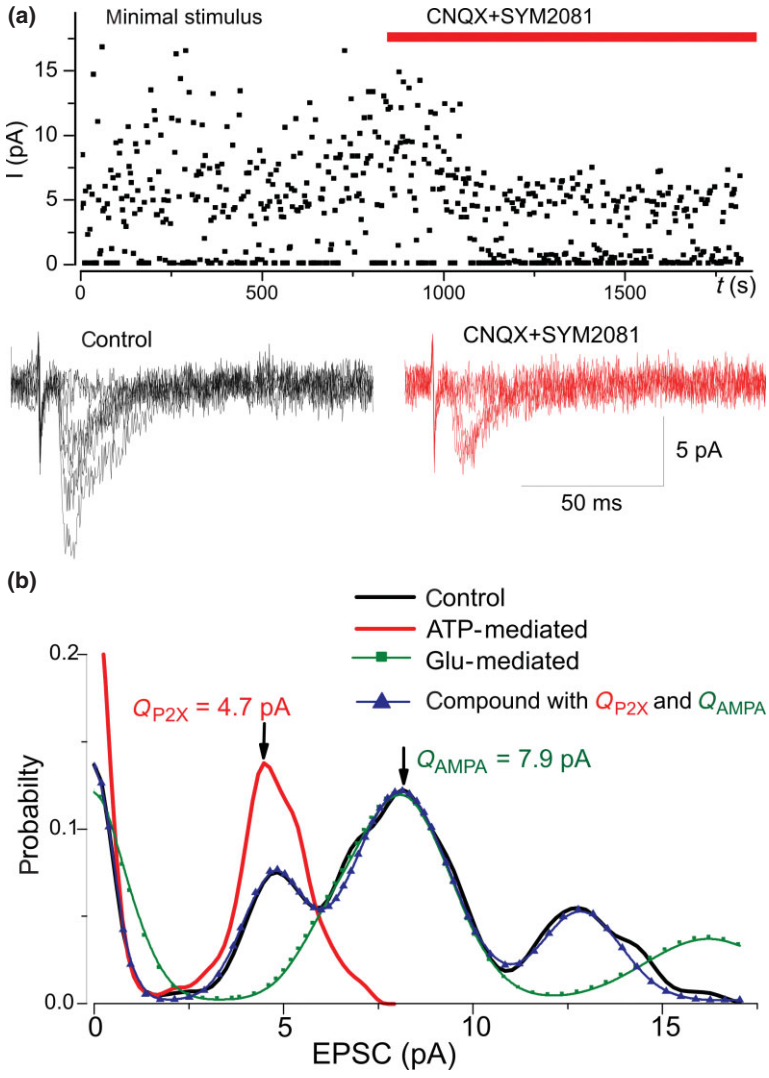

FIGURE 3 | Quantal adenosine triphosphate (ATP)-mediated current evoked in neocortical pyramidal neurons by stimulation of single axon. (a) Time course of amplitude of excitatory postsynaptic currents (EPSCs) evoked in the layer II/III pyramidal neuron by minimal field stimulation of thalamocortical afferents, at which only a single axon can be activated (see Ref 37 for details). EPSCs were recorded at −80 mV in the presence of 100 μM picrotoxin (control). Each dot on the graph represents a response to single stimulus; note the significant number of failures (zero responses) and clear quantization of EPSCs. Application of glutamatergic antagonists CNQX (50 μM) and SYM2081 (10 μM) did not eliminate quantal miniature excitatory postsynaptic current (mEPSC) completely. Bottom graphs show representative quantal mEPSCs recorded before (black) and after (red) application of glutamatergic antagonists. The residual mEPSCs were sensitive to P2X receptor antagonists (data not shown). (b) Amplitude distribution of EPSCs in control conditions (black line, peaks at 4.7 and 7.9 pA) and in the presence of CNQX and SYM2081 (red line, peak at 4.7 pA). Dotted lines represent two quantal models: binomial model (green line and dots) with single quantal size of 7.9 pA (AMPA component) and compound fit (blue line and dots) with two quantal sizes (4.7 pA, P2X component; 7.9 pA, AMPA component). Best fit is provided by compound model, indicating the evoked EPSCs in the control originate from quantal release of either glutamate or ATP or glutamate and ATP together. Thus, the quantal release of ATP can be elicited in the same synaptic terminals as glutamate. (Reprinted with permission from Ref 37. Copyright 2007 The Rockefeller University Press)

postsynaptic P2X receptors can provide a route for Ca^{2+} entry at resting membrane potentials. This contrasts to NMDA receptor-mediated Ca^{2+} entry into postsynaptic compartment, which requires cell predepolarization to remove Mg^{2+} block.[50,51]

Permeability of P2X receptors to Ca^{2+} is determined by a subunit composition and the $P_{Ca}/P_{monovalent}$ ratio may vary between 1 and >10 (Table 1, Refs 52 and 53). Similarly, the ratio of Ca^{2+} to monovalent cation permeability of P2X-mediated synaptic currents can be quite high reaching the values of 10–12.[36,53,54] On average, relative calcium/monovalent permeability of P2X receptors is much higher than for AMPA and kainate receptors and is generally similar to that for neuronal ACh receptors ($P_{Ca}/P_{monovalent}$ = 4–6[55,56]) and NMDA receptors ($P_{Ca}/P_{monovalent}$ = 4–10[57]).

Fractional calcium currents through P2X receptors were determined at 8% for recombinant human $P2X_4$ receptors.[69] In superior cervical ganglia neurones, which mostly express homomeric $P2X_2$ receptors, fractional calcium current was ~6.5%.[70] The fractional currents for various homo- and heteromeric P2X receptors expressed in HEK293 vary between 4 and 14%.[71] In summary, fractional Ca^{2+} currents through P2X receptors are similar to or higher than that estimated for neuronal nicotinic ACh (nACh) receptors; these latter vary between 3 and 6.7%.[56,70,72] Likewise, fractional Ca^{2+} currents through some P2X receptors (e.g., $P2X_1$, $P2X_4$, $P2X_{2/6}$, for which fractional Ca^{2+} currents are 8–14%) are equal to or even greater than fractional Ca^{2+} currents for NMDA receptors, which were determined at 10.7%.[73] In general, according to their calcium permeability major neurotransmitter-gated channels could be ranked as:

AMPA/kainate receptors ≪ nACh receptors < NMDA receptors ≤ P2X receptors.

TABLE 1 | Relative Ca^{2+} Permeability of the P2X, NMDA, AMPA, Kainate, and ACh Receptors. (Reprinted with Permission from Ref 53. Copyright 2009 Elsevier)

Receptor/Experimental Preparation	P_{Ca}/P_{mono}	Value	References
ATP (P2X) receptors			
Recombinant $P2X_1$ receptors	P_{Ca}/P_{Na}[1,2,3]	3.9	58
Recombinant $P2X_2$ receptors	P_{Ca}/P_{Na}[1,2,3]	2.2	58
Recombinant $P2X_3$ receptors	P_{Ca}/P_{Na}[1]	1.2	59
Recombinant $P2X_4$receptors	P_{Ca}/P_{mono}[1,3]	4.2	60
$P2X_1$ receptors from vas deferens	P_{Ca}/P_{Na}	4.8	61
$P2X_{2/3}$ receptors from nodose neurones	P_{Ca}/P_{Na}[1]	1.5	59
$P2X_{2/3}$ receptors from dorsal root ganglia (DRG) neurones	P_{Ca}/P_{Na}	4	62
P2X receptors in the medial habenula	P_{Ca}/P_{Cs}[1,3]	>10	54
P2X receptors from pyramidal neocortical neurones	P_{Ca}/P_{Cs}[1,3]	12.3	35
$P2X_{1/5}$ receptors from cortical astrocytes	P_{Ca}/P_{K}[1,3]	2.2	63
NMDA receptors			
Recombinant NMDA receptors	P_{Ca}/P_{Cs}[1,3]	10.4	60
Dendritic CA1–CA3 NMDA receptors	P_{Ca}/P_{Cs}[1,3]	4.2	57
NMDA receptors from cortical astrocytes	P_{Ca}/P_{K}[1,3]	3.1	63
Ca^{2+}-permeable AMPA receptors			
Cultured Bergmann glial cells	P_{Ca}/P_{Cs}[1,2,4]	1.44	64
Nonpyramidal neocortical neurones	P_{Ca}/P_{Cs}[1,2,4]	0.1–1.3	65
Nonpyramidal hippocampal neurones	P_{Ca}/P_{Cs}[1,2,4]	0.5–1.7	66
Kainate receptors			
Recombinant KA receptors	P_{Ca}/P_{Cs}[1,3]	0.74	67
GluR6 kainate receptors	P_{Ca}/P_{mono}	0.4–1.2	68
Neuronal ACh receptors			
Recombinant $\alpha 7$ receptors	P_{Ca}/P_{Na}[1,3]	4.0–6.6	56
Hippocampal $\alpha 7$ receptors	P_{Ca}/P_{Cs}[1,3]	6.1	55

ATP, adenosine triphosphate.
[1]Corrected for activity.
[2]Data obtained at very high (>50 mM) $[Ca^{2+}]_{out}$.
[3]Extended Goldman–Hodgkin–Katz equation (GHK)
[4]GHK constant field theory.

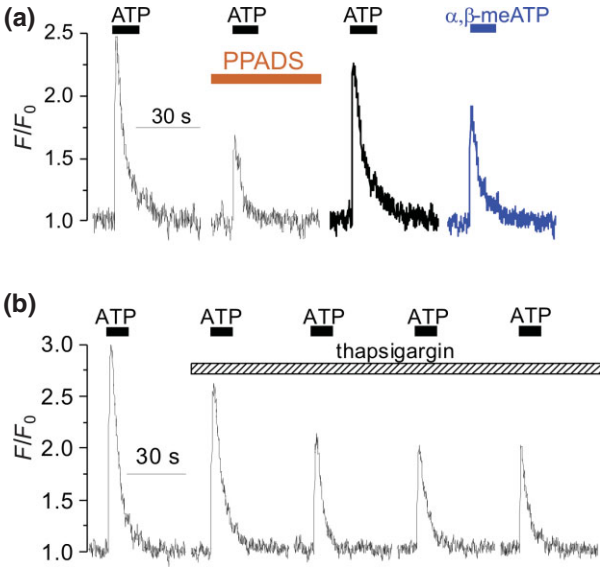

FIGURE 4 | Contribution of P2X receptors to the Ca^{2+} signaling in CA1 pyramidal neurones. (a) Examples of $[Ca^{2+}]_i$ transients elicited in the pyramidal neuron of hippocampal slices by fast application of adenosine triphosphate (ATP) (100 μM) and α,β-methyleneATP (100 μM, blue line) in control and after bath application of 20 μM PPADS. All traces were recorded in the same cell at 5-min intervals. Note the substantial amplitude of the response to α,β-methyleneATP, the selective agonist of ionotropic P2X receptors. (b) Examples of $[Ca^{2+}]_i$ transients elicited by repetitive fast application of ATP (100 μM) in the presence of 1 μM thapsigargin at 5-min time intervals. The first response represents the combined activity of P2X and P2Y purinoceptors, whereas the third and the following transients, recorded after depletion of intracellular Ca^{2+} store, are attributable to entry of the extracellular calcium via ionotropic P2X receptors. Note that the amplitude of P2X-mediated responses reaches 40–50% of total purinergic Ca^{2+} transients. (Reprinted with permission from Ref 77. Copyright 2002 Society for Neuroscience)

Contrary to NMDA receptors or to voltage-gated calcium channels, Ca^{2+} entry through P2X receptors does not require membrane depolarization and therefore can significantly contribute to intracellular calcium signals evoked at resting membrane potentials.[36] Indeed, the application of exogenous ATP triggers high-amplitude transient Ca^{2+} signals in central neurones, which have a mixed origin, being mediated by Ca^{2+} entry through P2X receptors/voltage-gated Ca^{2+} channels and by P2Y-mediated Ca^{2+} release from intracellular stores[74,75] (Figure 4). Calcium influx through P2X receptors is higher at negative membrane potentials and decreases with cell depolarization (Figure 1c). The P2X-mediated Ca^{2+} signaling can also be regulated by the ongoing synaptic transmission because the receptors can be desensitized at higher stimulation frequencies.[76] As a result, P2X-mediated Ca^{2+} signaling has a substantial plastic potential and can be involved in activity-dependent modulation of synaptic strength.

Functional Interaction Between P2X Receptors and Other Neurotransmitter Receptors

Another important property of P2X receptors is their ability to interact with other neurotransmitter receptors. This may involve either direct coupling between receptor molecules, or the inter-receptor cross-talk can be mediated through intracellular Ca^{2+} signals. Functional cross-talk between P2X and nACh receptors has been found in rat sympathetic neurones,[78] which, most likely, originates from direct interaction between receptor molecules.[79]

The negative P2X/GABA receptors interactions in peripheral and central neurones were reported to be mediated through intracellular Ca^{2+} signals.[80,81] Similar mechanisms underlie functional interaction between P2X and NMDA receptors in central neurones. The NMDA receptors, which are intimately involved in synaptic plasticity, are subjected to the calcium-dependent inactivation.[82] The mechanism of modulation of NMDA receptor by intracellular calcium is complex and involves calmodulin and phosphatase B (calcineurin).[83–85] Calcium influx through P2X receptors might therefore modulate an activity of NMDA receptors in hippocampus and neocortex where ATP is coreleased with glutamate.

Activation of P2X receptors by exogenous and endogenous ATP caused the Ca^{2+}-dependent inactivation of NMDA receptors in the hippocampal pyramidal neurones.[77] Brief application of ATP in micromolar concentrations significantly decreased the amplitude of NMDA-evoked current in acutely isolated cells (Figure 5a). The short train of P2X receptor-mediated synaptic current inhibited similarly the NMDA-mediated EPSCs in CA1 neurones in brain slices (Figure 5b). The inhibition of NMDA receptors

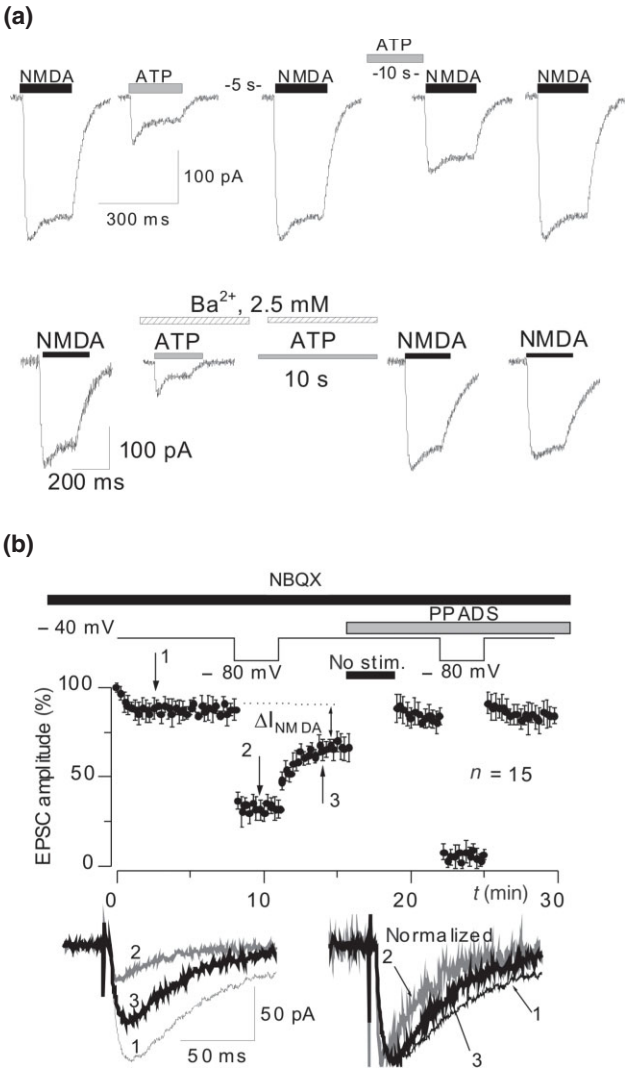

FIGURE 5 | Interaction between P2X and NMDA receptors in CA1 pyramidal neurones. (a) Adenosine triphosphate (ATP)-induced inhibition of NMDA receptors in acutely isolated CA1 pyramidal neurones. *Upper panel*, the traces represent inward currents evoked by fast application of agonists (from *left* to *right*): the control response to NMDA (10 μM), the control response to ATP (30 μM), the response to NMDA evoked 5 s after a 200-ms long application of ATP, the response to NMDA evoked after a 10-s preapplication of ATP, and the control response to NMDA. Note the significant decrease in the amplitude of NMDA response after preapplication of ATP. *Lower panel*, substitution of calcium in the extracellular solution for barium eliminates the ATP-induced inactivation of NMDA receptors. The traces represent (from *left* to *right*) the control response to NMDA, the response to ATP after substitution of extracellular Ca^{2+} for Ba^{2+}, the response to NMDA evoked after a 10-s preapplication of ATP in the Ba^{2+}-containing medium, and the control response to NMDA. NMDA (30 μM) was applied on the background of 10 μM glycine at a holding potential of −40 mV; ATP (20 μM) was applied at −80 mV. Intracellular solution contained 0.1 mM ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA). (b) The NMDA component of synaptic current in the CA1 pyramidal neurone of hippocampal slice is inactivated by low-frequency stimulation at a strongly negative voltage. This effect is inhibited by the P2X receptor antagonist PPADS. *Upper panel* shows the protocol of experiment and time course of EPSC amplitude. EPSCs comprising NMDA and P2X receptor-mediated components were continuously recorded at two membrane potentials, first at −40 mV (practically pure NMDA component) and then at −80 mV (only the P2X-mediated component is responsible for the measured inward current). The EPSCs measured at the moments indicated by the numbers in the *upper panel* are shown in the *lower panel* (each trace is the average of five EPSCs). Note a substantial inhibition of the NMDA component after return of the membrane voltage to −40 mV. The kinetics of the EPSC recorded again at −40 mV became faster because of the decrease in the NMDA receptor-mediated fraction. Application of PPADS (20 μM) to the same cell eliminated this effect. (Reprinted with permission from Ref 77. Copyright 2002 Society for Neuroscience)

was eliminated after chelating of extracellular calcium (Figure 5b) or after blocking of P2X receptors by PPADS (Figure 5b), showing that the effect was dependent on Ca^{2+} influx via P2X receptors. Incidentally, extracellular ATP may directly modulate NMDA receptors, although this occurs at rather high (0.1–0.3 mM) concentrations.[86]

P2X RECEPTORS AND SYNAPTIC PLASTICITY

Two most common forms of synaptic plasticity, the long-term potentiation (LTP) and depression (LTD), are regulated by numerous presynaptic (affecting neurotransmitter release) or postsynaptic (regulating the efficacy and density of receptors and ion channels) cascades. There is also growing evidence of important role for astroglial cells in regulation of neuronal plasticity. A unique combination of factors makes neuronal P2X receptors potentially important for modulation of synaptic plasticity at both presynaptic and postsynaptic loci and glia–neurone interface as well. These factors include (1) release of ATP from nerve terminals and glial cells, (2) segregation of P2X receptors to specific subpopulation of synapses, (3) large calcium permeability of P2X receptors at the resting membrane potential, and (4) their capability to interact with other receptors and modulate/trigger release of other neuro- and gliotransmitters.

There are several lines of evidence suggesting the role for P2X receptors in regulation of synaptic plasticity. First group of experiments have shown that P2X agonists may, by their own, trigger some changes in synaptic strength, and even induce long-term synaptic plasticity generally similar to LPT/LTD, although the data about the efficacy of stimulation of P2X receptors in triggering long-term plasticity remain controversial.[87,88] These experiments, however, are likely to represent a 'pathological plasticity', which may occur upon brain injury when excessive amount of ATP is released into the extracellular space. Indeed, there are certain indications that inhibition of P2X receptors (by broad antagonist PPADS) can be neuroprotective in forebrain ischemia.[89] In addition, ATP applied to brain slices acts indirectly through stimulating astroglial signaling pathways, and indeed recent data indicate the involvement of glial P2X receptors in synaptic plasticity in the spinal cord.[90]

The second group of experiments investigated the action of P2X selective pharmacological agents on LTP/LTD induced by conventional stimulation protocols. It turned out that pharmacological manipulations with P2X receptors can both stimulate and inhibit long-term synaptic plasticity. For example,

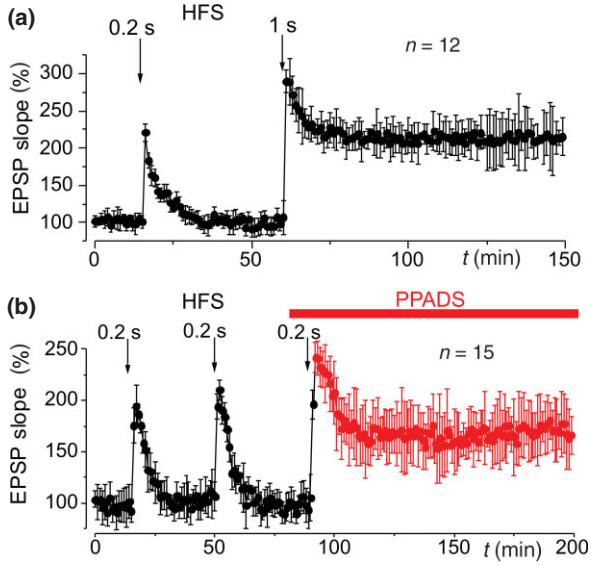

FIGURE 6 | Inhibition of adenosine triphosphate (ATP) receptors facilitates the induction of long-term potentiation in the hippocampus. (a and b) The changes in the CA1 field potentials induced by 100-Hz stimulation delivered to the Schaffer collateral in the control and after inhibition of the ATP receptors. (a) The short (0.2 s) train of high-frequency stimulation (HFS) does not induce long-term changes in the excitatory postsynaptic potential (EPSP) in control conditions, whereas 1-s-long HFS induces robust long-term potentiation (LTP). (b) After inhibition of P2 purinoreceptors by μM PPADS, the subthreshold stimulation (0.2 s HFS) becomes capable to induce LTP. Each point on the graph represents the average slope (mean ± SD) for five consecutive EPSPs; baseline stimulation frequency is 0.08 Hz. (Reprinted with permission from Ref 77. Copyright 2002 Society for Neuroscience)

inhibition of P2X receptors facilitated the induction of LTP in hippocampal excitatory synapses.[77] In these experiments, the LTP was induced in the CA1 hippocampal region by high-frequency stimulation (HFS; 1-s-long train at 100 Hz); this protocol resulted in a long-lasting increase in the amplitude of field EPSPs (Figure 6). Shorter (0.2 s) episodes of HFS triggered only short-term potentiation, even when applied repeatedly (Figure 6a). However, when P2X receptors were inhibited by PPADS or desensitized by nonhydrolyzable ATP analog α,β-methyleneATP, these short, 0.2-s-long episodes of HFS readily induced LTP (Figure 6b).

The depressing effect of P2X receptors on LTP induction is, most likely, associated with Ca^{2+}-dependent inactivation of NMDA receptors. The P2X receptors activated at basal level of synaptic activity may provide for sustained Ca^{2+} entry, which in turn can inhibit NMDA receptors, thereby significantly increasing the threshold for LTP induction. According to this scenario, P2X receptors may function as a dynamic filter or as a coincidence

detector preventing the 'unwanted' spontaneous LTP. When P2X receptors are inhibited, even basal synaptic activity can cause LTP; this was directly demonstrated in hippocampal slices treated with suramin.[91]

The data described above, however, remain controversial because several groups have gathered evidence indicating that activation of P2X receptors enhances LTP in central neurones. For example, ATP was reported to enhance LTP in hippocampal slices, and this effect was antagonized by inhibition of P2X receptors.[92] Similarly, positive modulation of $P2X_4$ receptors by ivermectin facilitated induction of LTP induction in the CA1 area of hippocampus; the genetic deletion of *P2X*$_4$ gene reduced LTP in the same preparations.[93] Interestingly, these effects of P2X receptors stimulation may also be mediated through Ca^{2+} entry and cytosolic Ca^{2+} signaling. One of the Ca^{2+}-sensitive cascades involved in synaptic potentiation is represented by an insertion of new glutamate receptors into the postsynaptic density.[94] It has been shown that activation of postsynaptic P2X receptors by ATP released from astroglia triggered an insertion of AMPA receptors into the postsynaptic membrane of hypothalamic neurones.[95] In this particular study, the authors linked the observed effect to the activation of $P2X_7$ receptors, because of the sensitivity to 2′(3′)-O-(4-Benzoylbenzoyl)adenosine-5′-triphosphate (BzATP) and brilliant blue G (BBG). However, the role for other P2X subtypes (e.g., for

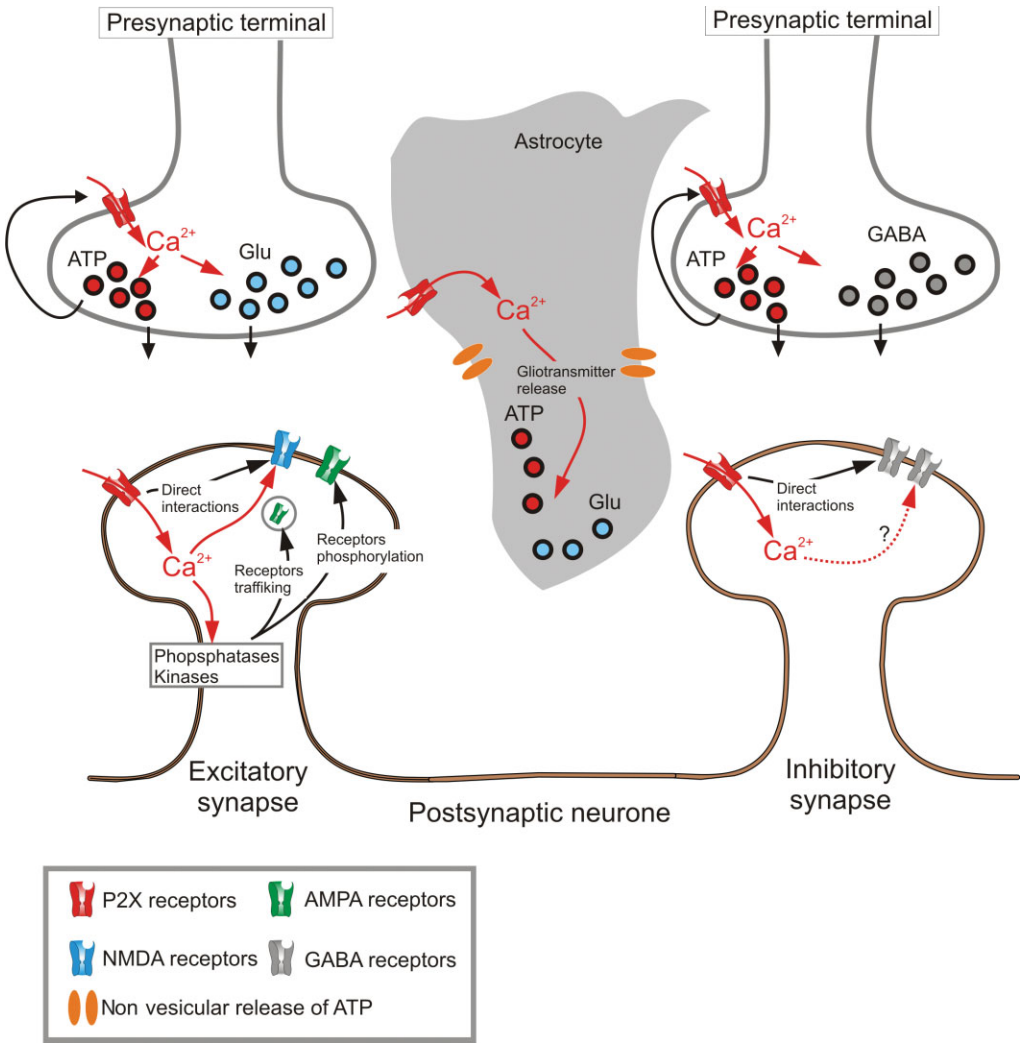

FIGURE 7 | P2X receptors in the context of synaptic transmission and plasticity in the central nervous system (CNS). Adenosine triphosphate (ATP) may be coreleased with glutamate and GABA from nerve terminals and released from glial cells by both vesicular and nonvesicular mechanisms. P2X receptors can trigger postsynaptic excitatory currents and modulate all elements of the tripartite synapse via interaction with postsynaptic NMDA and GABA receptors, by triggering trafficking of AMPA receptors, and by modulating the release of neuro- and gliotransmitters. (Reprinted with permission from Ref 53. Copyright 2009 Elsevier)

$P2X_2$ receptor, which is also sensitive to BzATP and BBG[15]) cannot be excluded. This result nonetheless shows that purinoceptors are capable to trigger the trafficking of neuronal AMPA receptors, thereby directly participating in long-term regulation of synaptic plasticity.

The dual effects of P2X receptors stimulation are in fact very much in line with 'Ca^{2+} hypothesis' of bidirectional synaptic plasticity,[96,97] which suggest that moderate rise in the intracellular calcium level causes depression of synaptic transmission through activation of phosphatases, whereas stronger cytosolic Ca^{2+} signals activate kinases, which initiate LTP. Therefore, due to their diverse functional properties and heterogeneous expression within central synapses, P2X receptors can modulate synaptic plasticity in opposite ways, depending on the physiological context.

CONCLUSION

The P2X receptors are involved in fast excitatory transmission in the CNS. Their activation produces EPSCs and results in entry into postsynaptic compartments. The P2X receptors have a specific role in regulation of the plasticity in central synapses (Figure 7). Effects of P2X receptors on synaptic plasticity result from their high calcium permeability, capability to interact with other receptors, and widespread exocytotic release of ATP from central neurones and glia. Depending on physiological context, P2X receptor can provide for either stimulation or inhibition of long-term synaptic plasticity. In addition, P2X receptors contribute to synaptically activated neuronal–glial interactions, which may form an additional and so far poorly characterized mechanism(s) for dynamic regulation of information processing/plasticity in neuronal–glial networks.

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