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6 **P2X7 receptor: an emerging target in CNS diseases**

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26 **Abstract**

27

28 The ATP-sensitive homomeric P2X7 receptor (P2X7R) has received particular

29 attention as a potential drug target because of its widespread involvement in

30 inflammatory diseases as a key regulatory element of the inflammasome

31 complex. However, it has only recently become evident that P2X7Rs also play a

32 pivotal role in central nervous system (CNS) pathology. There is an explosion of

33 data indicating that genetic deletion and pharmacological blockade of P2X7Rs

34 alter responsiveness in animal models of neurological disorders, such as stroke,

35 neurotrauma, epilepsy, neuropathic pain, multiple sclerosis, amyotrophic

36 lateralsclerosis, Alzheimer's disease, Parkinson's disease, and Huntington's

37 disease. Moreover, recent studies suggest that P2X7Rs regulate the

38 pathophysiology of psychiatric disorders, including mood disorders, implicating

39 P2X7Rs as drug targets in a variety of CNS pathology.

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43 It has been known for almost a decade that P2X7Rs convey important

44 physiopathological functions in the CNS [1]. The aim and scope of the present

45 review are to summarize the latest developments in the description of these

46 functions, to redirect interest to those fields, where there are still significant gaps

47 in our present understanding and to promote further development of those

48 therapeutic areas, in which P2X7R is the most promising as a potential drug

49 target.

50

51 **The structure and molecular physiology of P2X7Rs**

52

53 P2X7Rs are ATP-gated, non-selective cation channels belonging to the family of

54 ionotropic P2X receptors. P2X7Rs function in homo-trimeric form and most

55 mammalian P2X7R subunits comprise 595 amino acids [2]. The common

56 structural motifs of P2X7Rs are the two transmembrane domains (TM1, TM2), a

57 large, glycosylated, cysteine-rich extracellular loop, a short intracellular N-

58 terminal domain, and an intracellular C-terminal domain, which is longer than that

59 of other P2X receptor subunits. Within the family of P2X receptors, so far only the

60 crystal structure of zebrafish (zf)P2X4.1R has been solved in the closed [3] and

61 ATP-binding, open state [4]; nevertheless, its considerable homology with

62 mammalian P2X7Rs allowed for the structural modelling of the latter [2]. The

63 molecular architecture of an individual P2X7R subunit is akin to a leaping

64 dolphin, with the extracellular loop forming the body, and the TM domains

65 forming the tail. When co-assembled as a trimeric unit, P2X7R has a chalice-like

66 structure, overarching the channel pore (Figure 1A). There are three ATP binding

67 sites localized at the interface of the three subunits; occupancy of at least two of

68 the three sites is necessary for the activation of the receptors [5]. The adenine

69 base and the β- and γ-phosphate groups of ATP form hydrogen bonds with the

70 respective amino acid residues of the ATP binding pocket, as suggested for the

71 zfP2X4.1R. However, because a residue corresponding to Leu217, which

72 interacts with the ribose moiety, is missing in the mammalian P2X7R, the affinity

73 of ATP to P2X7Rs is more than a hundredfold lower than to other P2XR-

74 subtypes [2]. On the other hand, non-conserved residues surrounding the ATP

75 binding site might confer differences in agonist sensitivity between mammalian

76 P2XR species, (i.e. rat P2X7Rs display substantially higher sensitivity to ATP and

77 BzATP than their human and mouse counterparts [6]). A distinctive feature of the

78 mouse P2X7R is that it can be activated by extracellular nicotinamide adenine

79 dinucleotide (NAD$^+$) by ADP-ribosylation with the ADP-ribosyltransferase 2

80 ectoenzyme [7]. In contrast, less is known about the binding site of antagonists,

81 although potent and selective antagonists of P2X7Rs are now widely available.

82 Earlier data indicated that P2X7R subunits are able to form heterotrimers with

83 P2X4Rs [8], but more recent studies did not confirm this (e.g. [9]).

84 There are several splice variants of mammalian P2X7Rs, all of which are

85 widely expressed in the nervous system. Hence, a naturally occurring truncated

86 isoform of the human P2X7R (P2X7B) has been found in the CNS [10]; a C-

87 terminally truncated variant of mouse P2X7R has also been identified, which

88 partly retains its functionality, when expressed in tissues of the *P2rx7* gene

89 deficient mice [11]. Another mouse isoform is the P2X7(k) variant, which in

90 contrast to P2X7(a), is sensitive to ADP-ribosylation [12, 13].

91 The gene encoding the human P2X7R (*P2RX7*) is also well known to exhibit a

92 number of non-synonymous single nucleotide polymorphisms (NS-SNPs), which

93 results in a change in amino acid sequence and the expression of different

94 human P2X7 variants, further increasing the structural diversity of P2X7Rs. The

95 functional consequence of several individual NS-SNPs has been determined in

96 native and recombinant systems and their association with various human CNS

97 disease states has been extensively investigated in genetic linkage studies [14].

98 The activation of P2X7Rs results in the opening of the channel pore, allowing

99 the passage of small cations (Na^+, Ca^{2+}, and K^+). In addition, a hallmark feature

100 of the P2X7R is the opening of a non-selective pore in response to repeated or

101 prolonged activation, allowing the permeation of large molecular weight organic

102 cations up to 600-800 Da. The pore forming property of P2X7Rs can be studied

103 by the uptake of high molecular weight cations, such as $NMDG^+$, or dyes, such

104 as Yo-Pro-1 or ethidium bromide; nevertheless, its molecular mechanism has

105 remained a highly debated issue, with two alternative, but non excluding

106 possibilities, both having substantial experimental support (Figure 1 B, C). The

107 first potential mechanism is the progressive dilation of the P2X7R-gated channel

108 itself. A conformational change of the receptor-protein could be the structural

109 basis for channel dilation, as previously confirmed for other P2XRs (P2X2, P2X4)

110 by electrophysiological methods [15]. In agreement with the pore dilation theory,

111 the carboxyl terminal domain [16] and the TM2 region of the P2X7R protein are

112   essential for pore formation [17]. Moreover, recent studies revealed that the open

113   channel conformation of the P2X7R can allow the passage of negatively charged

114   fluorescent dyes with molecular diameters of up to 1.4 nm [18], and occupation

115   of one or two agonist binding sites favors transition to the desensitized state,

116   whereas occupation of the third binding site favors the transition to the

117   sensitized/dilated state [19].

118   The alternative mechanism involves the recruitment of an additional pore-

119   forming protein, most likely the pannexin-1 hemichannel (Panx1). Evidence

120   derived from studies using genetic knockdown of Panx1 indicate that this protein

121   is indispensable for the pore formation (e.g. [20]) and can be selectively affected

122   pharmacologically by colhicine [21]. However, other data conflict with the

123   involvement of Panx1 in the formation of the membrane pore (e.g. [22]).

124   Therefore, it appears that although recruitment of pannexin hemichannels is a

125   downstream signaling event closely linked to P2X7R activation, it is not an

126   absolute requirement [23]. A potential dissolution of conflicting results is that

127   different P2X7R splice variants display distinct pore forming properties [12, 23].

128   The opening of the large pore might eventually result in membrane blebbing

129   and cell death; however, this is not an obligatory consequence of P2X7R

130   activation. Pore formation might gain significance in the pathological sensitization

131   underlying chronic pain as highlighted by a recent study [24]. This paper reported

132   that mutations of the gene encoding the P2X7R, which result in hypofunctional

133   pore formation, affect chronic pain sensitivity in both mice and humans. Moreover

134   treatment with a peptide corresponding to the P2X7R C-terminal domain, which

135 blocks pore formation, but not cation channel activity, selectively reduced

136 allodynia only in mice with the pore-forming P2rx7 allele. These findings illustrate

137 that the pore formation associated with P2X7R, by itself could be a potential

138 target of personalized therapy to combat chronic pain disorders.

139

140 **Tissue and cell type specific distribution of P2X7Rs**

141

142 P2X7Rs are expressed by many cell types, including cells of hematopoietic origin

143 (lymphocytes, monocyte-macrophages, microglia) and intrinsic cells of the

144 nervous system (neurons, astrocytes, oligodendrocytes, Schwann cells). P2X7R

145 binding sites have been explored in autoradiographic studies using the

146 radioligand [^{3}H]-A-804598, and a dense P2X7R binding was found throughout the

147 brain and spinal cord [25], including hypothalamic nuclei, thalamic nuclei,

148 hippocampus, spinal trigeminal nucleus and tract, cortical regions, cerebellum

149 and caudate putamen [25]. Nevertheless, the cell-type specific localization of the

150 P2X7Rs in the CNS has been the subject of a long-standing debate, which has

151 not reached general consensus even after a decade: immunohistochemical

152 findings are inhomogeneous and contradict findings obtained by physiological

153 and neurochemical methods. Whereas early studies found a prominent

154 expression of P2X7R immunoreactivity (IR) on excitatory nerve terminals [26],

155 and later studies confirmed these findings throughout the CNS [27, 28]; other

156 groups questioned these findings, revealing P2X7R-immunoreactivity in brain

157 sections obtained from P2X7R deficient animals [29]. Subsequently however,

158 functional splice variants of rodent P2X7R [11, 12] were identified which are likely

159 to be responsible for P2X7-pseudo-immunoreactivities, found in the brain of

160 P2X7R$^{-/-}$ mice. These variants represent either gain- or loss-of function P2X7Rs,

161 and may explain the high variability of responses induced by P2X7R stimulation.

162 Other studies reported an activity-dependent expression pattern of P2X7Rs,

163 induced or upregulated following an insult such as a seizure [30], ischemia [31],

164 sleep deprivation [32], undernourishment [33], or morphine tolerance [34]. A

165 recent study utilizing single particle tracking photoactivated localization

166 microscopy (sptPALM) revealed that Dendra2 tagged P2X7Rs transfected to

167 hippocampal neurons formed two dynamic populations within the extrasynaptic

168 membrane of proximal dendrites: one was composed of rapidly diffusing

169 receptors and another stabilized within nanoclusters, both being rarely

170 appositioned to synaptic sites [35].

171 In contrast to immunohistochemistry, the available evidence on functional

172 P2X7Rs on different cell types of the CNS is convincing. Functional studies,

173 verifying P2X7Rs on neurons, astrocytes and microglia are presented in Table 1.

174 The most parsimonious explanation for the contradictory findings is that the

175 expression of P2X7Rs dynamically changes in response to experimental

176 variables such as age or different levels of stressful stimuli prior to sample

177 collection (freshly prepared *vs.* fixed sections). Moreover, under *in vivo* conditions

178 even mild stimuli, such as saline injection, may cause a dramatic change in the

179 expression level of P2X7Rs.

180

Physiopathology of P2X7 receptors

P2X7R function can be studied with a selection of pharmacological and genetic tools (Box 1). The activation of P2X7Rs is followed by Ca^{2+} influx and a variety of cellular responses depending on the cell type investigated (Figure 2). Outside the nervous system, the most prominent role of P2X7R is in the regulation of cytokine response to inflammatory challenge. In fact, P2X7R is a key regulatory element of the inflammasome molecular complex, providing the external stimulus necessary for the posttranslational modification and subsequent release of the pro-inflammatory cytokine IL-1β. The role of P2X7Rs has been confirmed in the regulation of central cytokine response after LPS priming [36]. This effect could be involved in physiological and pathological actions controlled by P2X7Rs, such as memory formation [37]; sleep [32], fever [38], hyperalgesia [39] and depression [40, 41].

However, a major caveat in our understanding of the physiopathology of P2X7R function is how the endogenous activation of P2X7Rs is achieved, given the low affinity of the endogenous agonist ATP. ATP is present in the synaptic vesicles and is co-released as a co-transmitter with various other transmitters in the autonomic nervous system under physiological conditions [42]. This holds also true to a certain extent for central synapses and the increase in extracellular ATP in response to normal neuronal activity might transiently reach the high micromolar concentration required for the activation of P2X7R, at least in the synaptic cleft. However, a more widespread activation of P2X7Rs is expected

204 under pathological conditions, when tissue damage, trauma or other pathological

205 signals provide an ATP-rich extracellular milieu, which might lead to the

206 activation of extrasynaptic and extraneuronal P2X7Rs. In addition, the possibility

207 of constitutive activity without the presence of the endogenous agonist cannot be

208 excluded either and should be further investigated. In the CNS, the best

209 characterized consequence of P2X7R activation is the release of

210 neurotransmitters, in particular of glutamate to the extracellular space [43]. This

211 effect could be evoked both from synaptosomes [44] and from astrocytes [45]. In

212 nerve terminals and cell lines expressing recombinant P2X7Rs, the P2X7R

213 mediated glutamate release appears to be both exocytotic and non-exocytotic,

214 [46, 47]. P2X7R mediated excitatory amino acid efflux can be detected in acutely

215 prepared brain slices by neurochemical (e.g. [48, 49]) and electrophysiological

216 techniques [50]. In rat hippocampal (hilar neurons; [51] CA1 neurons [52]), and

217 midbrain slices (locus coeruleus; [50]), stimulation of P2X7Rs by BzATP elicited

218 an increase of the frequency but not amplitude of spontaneous excitatory

219 postsynaptic currents (sEPSCs) and miniature (m)EPSCs. Occasionally [49, 50]

220 the P2X7R-mediated glutamate release was sensitive to blockade by fluorocitric

221 acid, a glia-selective metabolic poison, and to antagonists of glutamate receptors.

222 These findings imply that glutamate release induced by P2X7R stimulation from

223 neurons could also be indirect, mediated by glutamate release from astrocytes,

224 acting subsequently on glutamatergic nerve terminals.

225 To add further complexity to neuron-glia and glia-neuron P2X7R signaling,

226 P2X7R stimulation elicits or reinforces the release of ATP, thereby providing an

227 auto-stimulatory loop. This effect was observed in retinal ganglion cells [53]

228 hippocampal brain slices [49] and cultured spinal cord astrocytes [54]. The

229 mechanism of P2X7R-driven ATP release could be exocytotic, as observed by

230 total internal reflection microscopy in neuroblastoma cells [55], whereas in other

231 studies it appears to involve connexin and/or pannexin hemichannels [49, 54].

232   A further interesting function of P2X7Rs is to regulate differentiation and cell-

233 fate during development. P2X7Rs are expressed by both embryonic [56] and

234 adult neural progenitor cells (NPCs) in the subventricular zone of the lateral

235 ventricle [57]. Whereas stimulation of P2X7Rs induces neuronal differentiation in

236 embryonic NPCs [56], other studies indicated that P2X7Rs stimulate gliogenesis

237 [58]. In contrast, the activation of P2X7Rs on adult, cultured NPCs decrease cell

238 proliferation and induce necrotic/apoptotic cell death [57].

239   Of note, a very recent study showed that P2X7Rs regulate ion channel density

240 and protein composition/function of the axon initial segment, a key structural

241 element of neuronal excitability and in consequence action potential initiation in

242 cultured hippocampal neurons and brain slices [59].

243 It has been known for a long time that P2X7R activation might lead to cell death

244 through pore formation as it has been described for peripheral immune cells.

245 However, a more recently emerging view is that P2X7Rs also convey trophic

246 function against cell-death promoting physiological or pathological stimuli: for

247 example the microglial "suicide" P2X7R promotes cell cycle progression and

248 proliferation [60, 61], and this receptor might act as a scavenger for the removal

249 of apoptotic cells in the absence of its ATP ligand [62, 63].

P2X7R as a potential target in neurological diseases

ATP is released in large quantities following any kind of cell injury, and the ensuing stimulation of the low affinity P2X7R results in necrosis/apoptosis or proliferation as the two opposing end-points of neuroinflammation. P2X7R antagonists are potential therapeutics of traumatic brain injury, stroke, epilepsy, neuropathic pain, and neurodegenerative illnesses, because in these cases secondary cell damaging conditions accompany the primary pathological condition.

Middle cerebral artery occlusion, the most widely used animal model of cerebral ischemia, results in cell death in the core of the affected neuronal tissue, while around it, in the so called penumbra, the cellular damage is reversible. Both infarct size and neurological deficits were reduced by P2X7R antagonists [64, 65]. In combination with the sequential up-regulation of P2X7R-IR in microglia and then in astrocytes and neurons, this receptor-type was considered to be a primary target of the considerable amounts of ATP released. Similar results were reported for subarachnoid hemorrhage [66], traumatic brain [67, 68] or spinal cord injury [69] and ischemic retina degeneration [70]. However, a later study failed to reconfirm the protective action of P2X7R in spinal cord injury [71]. Reperfusion after transient global cerebral ischemia exacerbates the consequences of oxygen/glucose deprivation (OGD) due to microglial and astroglial activation [72]. The ensuing neuroinflammatory reaction is also

273   alleviated by P2X7R antagonists [73, 74].   BBG partially reversed the OGD-

274   induced anoxic depolarization and cell damage in cultured oligodendrocyte cells

275   [75]. Accordingly, left common carotid artery occlusion decreased P2X7R-

276   immunoreactivity at oligodendrocyte precursor cells in cerebral cortex, subcortical

277   white matter and hippocampus [76].

278   Status epilepticus (SE)-like seizures, modelled in rodents by pilocarpine or

279   kainate, up-regulate P2X7R-immunoreactivity in microglial cells [77] astrocytes

280   and neurons [78]; quantification by western-blotting confirmed these results [79,

281   80]. Utilizing the intra-amygdala application of kainate as an epileptic stimulus

282   [79, 80], it was shown that (1) Bz-ATP facilitated and prolonged the EEG activity

283   caused by seizures, and (2) P2X7R antagonists had a neuroprotective effect

284   after epilepsy due to suppression of IL-β production and microglial response.

285   More recent findings suggest that the effect of P2X7Rs during SE depends on

286   the nature of the chemical stimulus used. A-438079 decreased pilocarpin-

287   induced seizure susceptibility in mice by interrupting a direct facilitatory

288   interaction between P2X7- and muscarinic receptors [81] or blockade of the

289   release of the protective TNF-α [82]. P2X7R activation also influenced leukocyte

290   infiltration [83] and reactive astrogliosis following SE [84].

291   The involvement of P2X7Rs in different models of inflammatory and

292   neuropathic pain and the potential therapeutic effect of P2X7R antagonists are

293   well documented [85]. Down regulation of P2X7Rs with siRNA or BBG prevented

294   the induction of spinal long-term potentiation *in vitro* and at the same time

295   alleviated mechanical allodynia in naive rats *in vivo* [39]. Central sensitization of

296 nociceptive neurons could be produced by intrathecal superfusion of Bz-ATP and

297 was depressed by P2X7R antagonists [86]. Additional studies extended these

298 findings to mechanisms participating in the development of neuropathic or

299 orofacial pain [87-89], bone cancer pain [90] and migraine [91]. Recent studies

300 highlighted the association between human P2X7R variants with chronic pain

301 sensitivity [24].

302 Multiple sclerosis (MS) is a chronic degenerative disease of the CNS that is

303 characterized by focal lesions with inflammation, infiltration of immune cells,

304 demyelination, oligodendroglial death and axonal damage [92]. A putative

305 association of the *P2X7R* gene with this illness was indicated by the most

306 frequent expression of the gain-of-function T allele of rs17525809 polymorphism

307 of the receptor, which yields an Ala-76 to Val change in its extracellular domain

308 [93]. The overexpression of P2X7Rs was detected in experimental autoimmune

309 encephalomyelitis (EAE), an animal model of SM [94], whereas the amelioration

310 of EAE was found in P2X7R deficient animals [95, 96], but see [97]. Further,

311 pannexin-1 knockout mice with restricted ability to mediate pore development/dye

312 uptake after P2X7R stimulation, also displayed a delayed onset of clinical signs

313 of EAE and decreased mortality when compared with wild-type mice [98].

314 Amyotrophic lateral sclerosis (ALS) is characterized by the progressive

315 degeneration of motor neurons in the spinal cord, brainstem and motor cortex,

316 leading to respiratory failure and death of the affected patients within a few years

317 of diagnosis [99]. Microglia and astrocytes are major contributors to motor neuron

318 dysfunction in ALS through the maintenance of a chronic inflammatory response.

14

319 Transgenic mice expressing a mutant protein Cu^+/Zn^+ superoxide dismutase

320 SOD1-G93A, which directly enhances the activity of the main reactive oxygen

321 species producing enzyme in microglia (NADPH oxidase 2; NOX2) is used widely

322 as a model of ALS [100]. P2X7R activation by BzATP induced the death of motor

323 neurons in mixed astrocytic/neuronal cultures prepared from wild-type mice [101].

324 Further, apyrase, an enzyme degrading ATP or BzATP, decreased neuronal

325 death observed in cultures prepared from SOD-G93A spinal cord. Bz-ATP also

326 increased the activity of NOX2, leading to motor neuron damage, an effect which

327 did not occur in primary microglia cultures of SOD-G93A mice lacking P2X7Rs

328 [102].

329 A neuropathological hallmark of Alzheimer's disease (AD) is the appearance of

330 plaques consisting of extracellular β-amyloid peptide (Aβ) surrounded by reactive

331 microglial cells [103]. Aβ triggered increases in intracellular Ca^{2+}, ATP release,

332 IL-1β secretion and plasma membrane permeabilization in microglia from wild-

333 type but not P2X7R$^{-/-}$ mice [104]. These findings and the neuroprotective effects

334 of BBG against intrahippocampally injected Aβ suggest that Aβ activates a

335 purinergic autocrine/paracrine stimulatory loop of which the P2X7R is an

336 obligatory component. In fact, *in vivo* inhibition of the P2X7R in mice transgenic

337 for mutant human APP indicated a significant decrease of the number of

338 hippocampal amyloid plaques [105].

339 Parkinson's disease (PD) is a motor disease affecting the striatal

340 dopaminergic system, by damaging dopaminergic neurons in the substantia

341 nigra. In the disease model induced by unilateral intrastriatal injection of 6-

342 hydroxydopamine, BBG and A-438079 prevented the ensuing synaptotoxicity,

343 gliosis and neurotoxicity [106]. In another study, A-438079 prevented the

344 depletion of striatal dopamine stores by 6-hydroxydopamine treatment, but this

345 was not associated with a reduction of dopaminergic cell loss [107]. Similarly, the

346 effects of P2X7R antagonists appeared to depend on the neurotoxin used,

347 because in MPTP- or rotenone-induced Parkinson models, the genetic deletion of

348 the P2X7R did not increase survival rates of mice compared to wild-type

349 counterparts [108].

350   Huntington's disease (HD) is an autosomal dominant neurodegenerative

351 disorder caused by a triplet repeat expansion coding for a polyglutamine

352 sequence in the N-terminal region of the huntingtin protein. A higher P2X7R level

353 was documented by western-blot analysis in the striatum of transgenic mice

354 models of this disease [109]. In addition, P2X7R antagonists prevented neuronal

355 apoptosis and attenuated body weight loss and motor-coordination deficits.

356

357 **P2X7R as a potential target in psychiatric disorders**

358

359 Mood disorders arise from complex interactions between genetic, developmental

360 and environmental factors [110, 111]. Linkage studies suggested that variations

361 of the chromosome 12q24.31 containing candidate genes for P2X7R, P2X4R and

362 calmodulin-dependent protein kinase b (CaMKKb) may be associated with major

363 depressive, bipolar and anxiety disorders. It has repeatedly been reported that

364 the NS-SNP rs2230912 coding for the P2X7R-Glu460Arg is associated with

365 major depressive disorder [112, 113]. Further, relevant SNP mutations identified

366 by linkage studies were introduced into the human recombinant P2X7R and were

367 expressed in human embryonic kidney cells [114]. The measurement of their

368 functional properties by the patch-clamp technique indicated that some of them,

369 including Glu460Arg, exhibited a strong impairment of the current response to

370 ATP, while other mutants demonstrated significant increases in sensitivity. In

371 contrast, other studies failed to confirm the allelic or genotypic association of

372 rs2230912 or other SNPs of P2X7R with mood disorders [115, 116]. The reasons

373 for this discrepancy are presently unknown. Eventually, variations in the *P2X7R*

374 gene were described to be associated with cognitive manic symptoms in bipolar

375 disorders [117], but not in schizophrenia [118].

376 Production of TNF-α and IL-6 is initiated by the activation of Toll-like receptors

377 (TLRs) by e.g. bacterial lipopolysaccharide. The formation of IL-1β also requires

378 TLR4 induction of gene transcription but requires an additional step, the

379 processing of pro-IL-1β to the mature form of IL-1β, which is then released via

380 NLRP3 referred to as the "inflammasome" [110, 119]. P2X7Rs are indispensable

381 activators of NLRP3. Inflammatory cytokines have been suggested to play key

382 roles in the development of depressive behavior. Their levels are elevated in

383 depressed patients [110, 120] and rodents exposed to stressful stimuli [111].

384 These cytokines are potent activators of the hypothalamic-pituitary-adrenal axis

385 through which the secretion of hypothalamic corticotropin releasing hormone

386 (CRH), pituitary adrenocorticotropic hormone (ACTH) and corticosterone are

387 stimulated. In this respect it is interesting to note that P2X7R stimulation also

388   directly leads to increased ACTH secretion from the terminals of hypothalamic

389   magnocellular neurons [121].

390   The interrelationship between inflammatory cytokines, P2X7Rs and mood

391   related behavior has been intensively studied in animal models. The genetic

392   deletion of P2X7Rs resulted in antidepressive-like behavior in the forced swim

393   and tail suspension tests and alleviated amphetamine induced hyperactivity [40,

394   41]. Although P2X7Rs are present at peripheral/central immunocytes, glial cells

395   and neurons, it was shown that macrophages and microglia are not responsible

396   for the detected changes in mood measured by tail suspension test and

397   amphetamine-induced hyperlocomotion in P2X7R$^{-/-}$ mice [41]. On a larger scale,

398   several potential mechanisms were identified for the antidepressant phenotype of

399   P2X7R$^{-/-}$ mice, such as the absence of P2X7R-mediated glutamate release,

400   elevated basal brain-derived neurotrophic factor (BDNF) production, enhanced

401   neurogenesis and increased serotonin bioavailability in the hippocampus [48]. It

402   has also been observed that P2X7Rs are downregulated in the hippocampus in

403   response to chronic stress [122] and P2X7R$^{-/-}$ mice exhibited impaired adaptive

404   coping responses to repeated stress [123], which enlighten the potential role of

405   P2X7Rs as a protective adaptive mechanism in the process leading to mood

406   disorders.

407   The above data illustrate that P2X7R seems to be activated in a number of

408   different pathological conditions raising the possibility that the receptor is one

409   common avenue of cellular stress signaling pathways (Figure 2). However, one

410   should keep in mind that the pathophysiology of CNS diseases is very complex

411 involving a multiplicity of mediators and signaling pathways and the P2X7R is

412 only one among the multiple signaling pathways activated. Moreover, the

413 significance of this avenue is probably not uniform in all CNS pathologies and

414 could be more prominent in certain disease conditions (e.g. chronic pain, status

415 epilepticus) than in other ones (e.g. Parkinson's disease), depending on the

416 expression of P2X7Rs in the brain area afflicted. Finally, important physiological

417 functions mediated by P2X7Rs should not be neglected. For instance, taking into

418 account that the purportedly necrotic/apoptotic P2X7Rs also convey trophic and

419 adaptive changes, their role might vary or even reverse during the course of the

420 same disease, because neuroinflammation, regulated by P2X7Rs has also a

421 double-faced role. In fact, inflammation initially is a protective reaction and

422 becomes detrimental only, when it progresses to an excessive or chronic phase.

423 These aspects serve as explanations to conflicting results with P2X7R inhibition

424 on the disease outcome (e.g. [95-97]) and should also be addressed when

425 P2X7R is considered as a potential human drug-target.

426

427

428 **Current development of P2X7R ligands**

429

430 Although end-products of the pioneering developments of P2X7R antagonists,

431 such as CE-224,535 [124] and AZD 9056 [125] have not proved efficacious in

432 Phase II trials in rheumatoid arthritis patients, clinical studies revealed an

433 acceptable safety and tolerability profile of such antagonists as a whole [124-

434 126], opening up the possibility of developing P2X7R-targeting compounds in

435 new areas, such as CNS disorders.

436 In recent years, a number of different classes of small molecular weight, drug-

437 like P2X7R ligands have been developed (Table 2), and P2X7Rs have been

438 qualified as the most "druggable" target within the P2X receptor family [85, 127].

439 More recently, the development of centrally penetrating potent P2X7R

440 antagonists has also been reported (Table 2). In addition, systematic search

441 through compound libraries resulted in the further discovery of novel P2X7R

442 antagonists and allosteric modulators utilizable either for basic research or drug

443 development. Analyses of natural compounds have also resulted in several

444 valuable P2X7R ligands (Table 2).

445

446 **Concluding remarks**

447

448 In conclusion, P2X7R mediated pathways appears to be a common avenue of

449 many CNS disorders of different aetiology and P2X7R antagonists are potential

450 drugs to treat them. Their immense advantage may lie in the absence or low

451 density of P2X7Rs in healthy tissue and therefore in the limited systemic side

452 effects of these compounds. However, major caveats in our understanding of the

453 physiopathological functions of central P2X7Rs should be further elucidated (Box

454 2). Though the majority of known antagonists fail to pass the blood-brain barrier,

455 BBG and some new and high affinity P2X7R antagonists readily enter the CNS

456 [128]. Further, recently identified negative allosteric modulators of P2X7Rs (e.g.

457 certain phenothiazine-type antipsychotic drugs), already registered for human

458 use [129], may become important therapeutic tools.

459   The future development of new P2X7R antagonists has to take into

460 consideration that P2X7R isoforms may exhibit large variability between different

461 species in their agonist/antagonist sensitivities. Therefore, the classic search for

462 new pharmacologically active compounds based on the use of laboratory

463 animals, may lead to spurious negative or positive results. A further complicating

464 factor is the presence of numerous splice variants and SNPs widely distributed in

465 the animal and human organism; their sensitivities to pharmacological blockade

466 is often different from that of the wild-type receptor. Hence, the development of

467 new and therapeutically valuable P2X7R antagonists is a tedious task but the

468 reward may be enormous.

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References

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480 1 Sperlagh, B. *et al.* (2006) P2X7 receptors in the nervous system. *Prog Neurobiol*

481   78, 327-346

482 2 Jiang, L.H. *et al.* (2013) Insights into the Molecular Mechanisms Underlying

483   Mammalian P2X7 Receptor Functions and Contributions in Diseases, Revealed

484   by Structural Modeling and Single Nucleotide Polymorphisms. *Front Pharmacol*

485   DOI: 10.3389/fphar.2013.00055

486 3 Kawate, T. *et al.* (2009) Crystal structure of the ATP-gated P2X(4) ion channel in

487   the closed state. *Nature* 460, 592-598

488 4 Hattori, M. and Gouaux, E. (2012) Molecular mechanism of ATP binding and ion

489   channel activation in P2X receptors. *Nature* 485, 207-212

490 5 Stelmashenko, O. *et al.* (2012) Activation of trimeric P2X2 receptors by fewer than

491   three ATP molecules. *Mol Pharmacol* 82, 760-766

492 6 Bradley, H.J. *et al.* (2011) Residues 155 and 348 contribute to the determination

493   of P2X7 receptor function via distinct mechanisms revealed by single-nucleotide

494   polymorphisms. *J Biol Chem* 286, 8176-8187

495 7 Adriouch, S. *et al.* (2008) ADP-ribosylation at R125 gates the P2X7 ion channel

496   by presenting a covalent ligand to its nucleotide binding site. *FASEB J* 22, 861-

497   869

498 8 Guo, C. *et al.* (2007) Evidence for functional P2X4/P2X7 heteromeric receptors.

499   *Mol Pharmacol* 72, 1447-1456

500 9 Antonio, L.S. *et al.* (2011) P2X4 receptors interact with both P2X2 and P2X7

501   receptors in the form of homotrimers. *Br J Pharmacol* 163, 1069-1077

502 10 Adinolfi, E. *et al.* (2010) Trophic activity of a naturally occurring truncated isoform

503   of the P2X7 receptor. *FASEB J* 24, 3393-3404

504 11 Masin, M. *et al.* (2012) Expression, assembly and function of novel C-terminal

505   truncated variants of the mouse P2X7 receptor: re-evaluation of P2X7 knockouts.

506   *Br J Pharmacol* 165, 978-993

507 12 Nicke, A. *et al.* (2009) A functional P2X7 splice variant with an alternative

508   transmembrane domain 1 escapes gene inactivation in P2X7 knock-out mice. *J*

509   *Biol Chem* 284, 25813-25822

510 13 Schwarz, N. *et al.* (2012) Alternative splicing of the N-terminal cytosolic and

511   transmembrane domains of P2X7 controls gating of the ion channel by ADP-

512   ribosylation. *PLoS One* DOI: 10.1371/journal.pone.0041269

513 14 Fuller, S.J. *et al.* (2009) Genetics of the P2X7 receptor and human disease.

514   *Purinergic Signal* 5, 257-262

515 15 Chaumont, S. and Khakh, B.S. (2008) Patch-clamp coordinated spectroscopy

516   shows P2X2 receptor permeability dynamics require cytosolic domain

517   rearrangements but not Panx-1 channels. *Proc Natl Acad Sci U S A* 105, 12063-

518   12068

519 16 Alloisio, S. *et al.* (2010) Evidence for two conductive pathways in P2X receptor:

520   differences in modulation and selectivity. *J Neurochem* 113, 796-806

521 17 Sun, C. *et al.* (2013) The second transmembrane domain of P2X7 contributes to

522   dilated pore formation. *PLoS One* DOI: 10.1371/journal.pone.0061886

523 18 Browne, L.E. *et al.* (2013) P2X7 receptor channels allow direct permeation of

524   nanometer-sized dyes. *J Neurosci* 33, 3557-3566

525 19 Khadra, A. *et al.* (2013) Dual gating mechanism and function of P2X7 receptor

526   channels. *Biophys J* 104, 2612-2621

527 20 Suadicani, S.O. *et al.* (2012) ATP signaling is deficient in cultured Pannexin1-null

528   mouse astrocytes. *Glia* 60, 1106-1116

529 21 Marques-da-Silva, C. *et al.* (2011) Colchicine inhibits cationic dye uptake

530   induced by ATP in P2X2 and P2X7 receptor-expressing cells: implications for its

531   therapeutic action. *Br J Pharmacol* 163, 912-926

532 22 Alberto, A.V. *et al.* (2013) Is pannexin the pore associated with the P2X7

533   receptor? *Naunyn Schmiedebergs Arch Pharmacol* 386, 775-787

534 23 Xu, X.J. *et al.* (2012) Splice variants of the P2X7 receptor reveal differential

535   agonist dependence and functional coupling with pannexin-1. *J Cell Sci* 125,

536   3776-3789

537 24 Sorge, R.E. *et al.* (2012) Genetically determined P2X7 receptor pore formation

538   regulates variability in chronic pain sensitivity. *Nat Med* 18, 595-599

539 25 Able, S.L. *et al.* (2011) Receptor localization, native tissue binding and ex vivo

540   occupancy for centrally penetrant P2X7 antagonists in the rat. *Br J Pharmacol*

541   162, 405-414

542 26 Deuchars, S.A. *et al.* (2001) Neuronal P2X7 receptors are targeted to

543   presynaptic terminals in the central and peripheral nervous systems. *J Neurosci*

544   21, 7143-7152

545 27 Atkinson, L. *et al.* (2004) Differential co-localisation of the P2X7 receptor subunit

546   with vesicular glutamate transporters VGLUT1 and VGLUT2 in rat CNS.

547   *Neuroscience* 123, 761-768

548 28 Puthussery, T. and Fletcher, E.L. (2004) Synaptic localization of P2X7 receptors

549   in the rat retina. *J Comp Neurol* 472, 13-23

550 29 Sim, J.A. *et al.* (2004) Reanalysis of P2X7 receptor expression in rodent brain. *J*

551   *Neurosci* 24, 6307-6314

552 30 Henshall, D.C. *et al.* (2013) P2X receptors as targets for the treatment of status

553   epilepticus. *Front Cell Neurosci* DOI: 10.3389/fncel.2013.00237

554 31 Milius, D. *et al.* (2008) Up-regulation of P2X7 receptor-immunoreactivity by in

555   vitro ischemia on the plasma membrane of cultured rat cortical neurons. *Neurosci*

556   *Lett* 446, 45-50

557 32 Krueger, J.M. *et al.* (2010) ATP and the purine type 2 X7 receptor affect sleep. *J*

558   *Appl Physiol (1985)* 109, 1318-1327

559 33 Girotti, P.A. *et al.* (2013) Differential effects of undernourishment on the

560   differentiation and maturation of rat enteric neurons. *Cell Tissue Res* 353, 367-

561   380

562 34 Zhou, D. *et al.* (2010) Involvement of spinal microglial P2X7 receptor in

563   generation of tolerance to morphine analgesia in rats. *J Neurosci* 30, 8042-8047

564 35 Shrivastava, A.N. *et al.* (2013) Dynamic micro-organization of P2X7 receptors

565   revealed by PALM based single particle tracking. *Front Cell Neurosci* DOI:

566   10.3389/fncel.2013.00232

567 36 Csolle, C. and Sperlagh, B. (2010) Peripheral origin of IL-1beta production in the

568   rodent hippocampus under in vivo systemic bacterial lipopolysaccharide (LPS)

569   challenge and its regulation by P2X(7) receptors. *J Neuroimmunol* 219, 38-46

570 37 Labrousse, V.F. *et al.* (2009) Impaired interleukin-1beta and c-Fos expression in

571 the hippocampus is associated with a spatial memory deficit in P2X(7) receptor-

572 deficient mice. *PLoS One* DOI: 10.1371/journal.pone.0006006

573 38 Barbera-Cremades, M. *et al.* (2012) P2X7 receptor-stimulation causes fever via

574 PGE2 and IL-1beta release. *FASEB J* 26, 2951-2962

575 39 Chu, Y.X. *et al.* (2010) Involvement of microglial P2X7 receptors and

576 downstream signaling pathways in long-term potentiation of spinal nociceptive

577 responses. *Brain Behav Immun* 24, 1176-1189

578 40 Basso, A.M. *et al.* (2009) Behavioral profile of P2X7 receptor knockout mice in

579 animal models of depression and anxiety: relevance for neuropsychiatric

580 disorders. *Behav Brain Res* 198, 83-90

581 41 Csolle, C. *et al.* (2013) The absence of P2X7 receptors (P2rx7) on non-

582 haematopoietic cells leads to selective alteration in mood-related behaviour with

583 dysregulated gene expression and stress reactivity in mice. *Int J*

584 *Neuropsychopharmacol* 16, 213-233

585 42 Burnstock, G. (2004) Cotransmission. *Current opinion in pharmacology* 4, 47-52

586 43 Sperlagh, B. *et al.* (2002) Involvement of P2X7 receptors in the regulation of

587 neurotransmitter release in the rat hippocampus. *J Neurochem* 81, 1196-1211

588 44 Marcoli, M. *et al.* (2008) P2X7 pre-synaptic receptors in adult rat cerebrocortical

589 nerve terminals: a role in ATP-induced glutamate release. *J Neurochem* 105,

590 2330-2342

591 45 Fu, W. *et al.* (2013) Activity and metabolism-related Ca2+ and mitochondrial

592 dynamics in co-cultured human fetal cortical neurons and astrocytes.

593 *Neuroscience* 250, 520-535

594 46 Cervetto, C. *et al.* (2013) The P2X7 receptor as a route for non-exocytotic

595 glutamate release: dependence on the carboxyl tail. *J Neurochem* 124, 821-831

596 47 Cervetto, C. *et al.* (2012) Calmidazolium selectively inhibits exocytotic glutamate

597 release evoked by P2X7 receptor activation. *Neurochem Int* 60, 768-772

598 48 Csolle, C. *et al.* (2013) Neurochemical Changes in the Mouse Hippocampus

599 Underlying the Antidepressant Effect of Genetic Deletion of P2X7 Receptors.

600 *PLoS One* DOI: 10.1371/journal.pone.0066547

601 49 Heinrich, A. *et al.* (2012) K+ depolarization evokes ATP, adenosine and

602 glutamate release from glia in rat hippocampus: a microelectrode biosensor

603 study. *Br J Pharmacol* 167, 1003-1020

604 50 Khakpay, R. *et al.* (2010) Potentiation of the glutamatergic synaptic input to rat

605 locus coeruleus neurons by P2X7 receptors. *Purinergic Signal* 6, 349-359

606 51 Cho, J.H. *et al.* (2010) P2X7 receptors enhance glutamate release in

607 hippocampal hilar neurons. *Neuroreport* 21, 865-870

608 52 Ficker, C. *et al.* (2014) Astrocyte-neuron interaction in the substantia gelatinosa

609 of the spinal cord dorsal horn via P2X7 receptor-mediated release of glutamate

610 and reactive oxygen species. *Glia,* DOI: 10.1002/glia.22707.

611 53 Xia, J. *et al.* (2012) Neurons respond directly to mechanical deformation with

612 pannexin-mediated ATP release and autostimulation of P2X7 receptors. *J*

613 *Physiol* 590, 2285-2304

614 54 Bennett, M.V. *et al.* (2012) Connexin and pannexin hemichannels in

615   inflammatory responses of glia and neurons. *Brain Res* 1487, 3-15

616 55 Gutierrez-Martin, Y. *et al.* (2011) P2X7 receptors trigger ATP exocytosis and

617   modify secretory vesicle dynamics in neuroblastoma cells. *J Biol Chem* 286,

618   11370-11381

619 56 Tsao, H.K. *et al.* (2013) PKC-dependent ERK phosphorylation is essential for

620   P2X7 receptor-mediated neuronal differentiation of neural progenitor cells. *Cell*

621   *Death Dis* DOI: cddis2013274 [pii]

622 57 Messemer, N. *et al.* (2013) P2X7 receptors at adult neural progenitor cells of the

623   mouse subventricular zone. *Neuropharmacology* 73, 122-137

624 58 Zou, J. *et al.* (2012) ATP-P2X7 receptor signaling controls basal and TNFalpha-

625   stimulated glial cell proliferation. *Glia* 60, 661-673

626 59 del Puerto, A. *et al.* (2012) Adenylate cyclase 5 coordinates the action of ADP,

627   P2Y1, P2Y13 and ATP-gated P2X7 receptors on axonal elongation. *J Cell Sci*

628   125, 176-188

629 60 Bianco, F. *et al.* (2006) A role for P2X7 in microglial proliferation. *J Neurochem*

630   99, 745-758

631 61 Monif, M. *et al.* (2009) The P2X7 receptor drives microglial activation and

632   proliferation: a trophic role for P2X7R pore. *J Neurosci* 29, 3781-3791

633 62 Gu, B.J. *et al.* (2011) P2X(7) is a scavenger receptor for apoptotic cells in the

634   absence of its ligand, extracellular ATP. *J Immunol* 187, 2365-2375

635 63 Yamamoto, M. *et al.* (2013) P2X7 receptors regulate engulfing activity of non-

636   stimulated resting astrocytes. *Biochemical and biophysical research*

637   *communications* 439, 90-95

638 64 Arbeloa, J. *et al.* (2012) P2X7 receptor blockade prevents ATP excitotoxicity in

639   neurons and reduces brain damage after ischemia. *Neurobiol Dis* 45, 954-961

640 65 Lammer, A.B. *et al.* (2011) The P2 receptor antagonist PPADS supports

641   recovery from experimental stroke in vivo. *PLoS One* DOI:

642   10.1371/journal.pone.0019983

643 66 Chen, S. *et al.* (2013) P2X7 receptor antagonism inhibits p38 mitogen-activated

644   protein kinase activation and ameliorates neuronal apoptosis after subarachnoid

645   hemorrhage in rats. *Crit Care Med* 41, e466-474

646 67 Kimbler, D.E. *et al.* (2012) Activation of P2X7 promotes cerebral edema and

647   neurological injury after traumatic brain injury in mice. *PLoS One* DOI:

648   10.1371/journal.pone.0041229

649 68 Roth, T.L. *et al.* (2014) Transcranial amelioration of inflammation and cell death

650   after brain injury. *Nature* 505, 223-228

651 69 Peng, W. *et al.* (2009) Systemic administration of an antagonist of the ATP-

652   sensitive receptor P2X7 improves recovery after spinal cord injury. *Proc Natl*

653   *Acad Sci U S A* 106, 12489-12493

654 70 Niyadurupola, N. *et al. (2013)* P2X7 receptor activation mediates retinal ganglion

655   cell death in a human retina model of ischemic neurodegeneration. *Invest*

656   *Ophthalmol Vis Sci* 54, 2163-2170

657 71 Marcillo, A. *et al.* (2012) A reassessment of P2X7 receptor inhibition as a

658 neuroprotective strategy in rat models of contusion injury. *Experimental*

659 *neurology* 233, 687-692

660 72 Stoll, G. *et al.* (2010) Combating innate inflammation: a new paradigm for acute

661 treatment of stroke? *Annals of the New York Academy of Sciences* 1207, 149-

662 154

663 73 Chu, K. *et al.* (2012) Inhibition of P2X7 receptor ameliorates transient global

664 cerebral ischemia/reperfusion injury via modulating inflammatory responses in

665 the rat hippocampus. *J Neuroinflammation* DOI: 1742-2094-9-69 [pii]

666 74 Yu, Q. *et al.* (2013) Block of P2X7 receptors could partly reverse the delayed

667 neuronal death in area CA1 of the hippocampus after transient global cerebral

668 ischemia. *Purinergic Signal* 9, 663-675

669 75 Domercq, M. *et al.* (2010) P2X7 receptors mediate ischemic damage to

670 oligodendrocytes. *Glia* 58, 730-740

671 76 Wang, L.Y. *et al.* (2009) Downregulation of P2X7 receptor expression in rat

672 oligodendrocyte precursor cells after hypoxia ischemia. *Glia* 57, 307-319

673 77 Kim, J.E. *et al.* (2009) Blockade of P2X receptor prevents astroglial death in the

674 dentate gyrus following pilocarpine-induced status epilepticus. *Neurol Res* 31,

675 982-988

676 78 Dona, F. *et al.* (2009) Alteration of purinergic P2X4 and P2X7 receptor

677 expression in rats with temporal-lobe epilepsy induced by pilocarpine. *Epilepsy*

678 *Res* 83, 157-167

679 79 Engel, T. *et al.* (2012) Seizure suppression and neuroprotection by targeting the

680   purinergic P2X7 receptor during status epilepticus in mice. *FASEB J* 26, 1616-

681   1628

682 80 Jimenez-Pacheco, A. *et al.* (2013) Increased neocortical expression of the P2X7

683   receptor after status epilepticus and anticonvulsant effect of P2X7 receptor

684   antagonist A-438079. *Epilepsia* 54, 1551-1561

685 81 Kim, J.E. and Kang, T.C. (2011) The P2X7 receptor-pannexin-1 complex

686   decreases muscarinic acetylcholine receptor-mediated seizure susceptibility in

687   mice. *J Clin Invest* 121, 2037-2047

688 82 Kim, J.E. *et al.* (2011) P2X7 receptor activation ameliorates CA3 neuronal

689   damage via a tumor necrosis factor-alpha-mediated pathway in the rat

690   hippocampus following status epilepticus. *J Neuroinflammation* DOI: 1742-2094-

691   8-62 [pii]

692 83 Kim, J.E. *et al.* (2010) P2X7 receptor regulates leukocyte infiltrations in rat

693   frontoparietal cortex following status epilepticus. *J Neuroinflammation* DOI: 1742-

694   2094-7-65 [pii]

695 84 Kim, J.E. *et al.* (2013) The effect of P2X7 receptor activation on nuclear factor-

696   kappaB phosphorylation induced by status epilepticus in the rat hippocampus.

697   *Hippocampus* 23, 500-514

698 85 North, R.A. and Jarvis, M.F. (2013) P2X receptors as drug targets. *Mol*

699   *Pharmacol* 83, 759-769

700 86 Itoh, K. *et al.* (2011) Central sensitization of nociceptive neurons in rat medullary

701   dorsal horn involves purinergic P2X7 receptors. *Neuroscience* 192, 721-731

702 87 Ando, R.D. *et al.* (2010) A comparative analysis of the activity of ligands acting at

703   P2X and P2Y receptor subtypes in models of neuropathic, acute and

704   inflammatory pain. *Br J Pharmacol* 159, 1106-1117

705 88 He, W.J. *et al.* (2012) Spinal P2X(7) receptor mediates microglia activation-

706   induced neuropathic pain in the sciatic nerve injury rat model. *Behav Brain Res*

707   226, 163-170

708 89 Ito, G. *et al.* (2013) P2X7 receptor in the trigeminal sensory nuclear complex

709   contributes to tactile allodynia/hyperalgesia following trigeminal nerve injury. *Eur*

710   *J Pain* 17, 185-199

711 90 Huang, Z.X. *et al.* (2014) Involvement of RVM-expressed P2X7 receptor in bone

712   cancer pain: Mechanism of descending facilitation. *Pain* 155, 783-791

713 91 Gölöncsér, F. and Sperlágh, B. (2014) Effect of genetic deletion and

714   pharmacological antagonism of P2X7 receptors in a mouse animal model of

715   migraine. *The Journal of Headache and Pain* DOI: 10.1186/1129-2377-15-24

716 92 Amadio, S. *et al.* (2011) Purinergic signalling at the plasma membrane: a

717   multipurpose and multidirectional mode to deal with amyotrophic lateral sclerosis

718   and multiple sclerosis. *J Neurochem* 116, 796-805

719 93 Oyanguren-Desez, O. *et al.* (2011) Gain-of-function of P2X7 receptor gene

720   variants in multiple sclerosis. *Cell Calcium* 50, 468-472

721 94 Grygorowicz, T. *et al.* (2010) Temporal expression of P2X7 purinergic receptor

722   during the course of experimental autoimmune encephalomyelitis. *Neurochem Int*

723   57, 823-829

724 95 Matute, C. *et al.* (2007) P2X(7) receptor blockade prevents ATP excitotoxicity in

725 oligodendrocytes and ameliorates experimental autoimmune encephalomyelitis. *J*

726 *Neurosci* 27, 9525-9533

727 96 Sharp, A.J. *et al.* (2008) P2x7 deficiency suppresses development of

728 experimental autoimmune encephalomyelitis. *J Neuroinflammation* DOI: 1742-

729 2094-5-33 [pii]

730 97 Chen, L. and Brosnan, C.F. (2006) Exacerbation of experimental autoimmune

731 encephalomyelitis in P2X7R-/- mice: evidence for loss of apoptotic activity in

732 lymphocytes. *J Immunol* 176, 3115-3126

733 98 Lutz, S.E. *et al.* (2013) Contribution of pannexin1 to experimental autoimmune

734 encephalomyelitis. *PLoS One* DOI: 10.1371/journal.pone.0066657

735 99 Volonte, C. *et al.* (2011) ALS: focus on purinergic signalling. *Pharmacology &*

736 *therapeutics* 132, 111-122

737 100 Apolloni, S. *et al.* (2013) Ablation of P2X7 receptor exacerbates gliosis and

738 motoneuron death in the SOD1-G93A mouse model of amyotrophic lateral

739 sclerosis. *Hum Mol Genet* 22, 4102-4116

740 101 Gandelman, M. *et al.* (2010) Extracellular ATP and the P2X7 receptor in

741 astrocyte-mediated motor neuron death: implications for amyotrophic lateral

742 sclerosis. *J Neuroinflammation* DOI: 1742-2094-7-33 [pii]

743 102 Apolloni, S. *et al.* (2013) The NADPH oxidase pathway is dysregulated by the

744 P2X7 receptor in the SOD1-G93A microglia model of amyotrophic lateral

745 sclerosis. *J Immunol* 190, 5187-5195

746 103 Delarasse, C. *et al.* (2011) The purinergic receptor P2X7 triggers alpha-

747 secretase-dependent processing of the amyloid precursor protein. *J Biol Chem*

748 286, 2596-2606

749 104 Sanz, J.M. *et al.* (2009) Activation of microglia by amyloid {beta} requires P2X7

750 receptor expression. *J Immunol* 182, 4378-4385

751 105 Diaz-Hernandez, J.I. *et al.* (2012) In vivo P2X7 inhibition reduces amyloid

752 plaques in Alzheimer's disease through GSK3beta and secretases. *Neurobiol*

753 *Aging* 33, 1816-1828

754 106 Carmo, M.R. *et al.* (2014) The P2X7 receptor antagonist Brilliant Blue G

755 attenuates contralateral rotations in a rat model of Parkinsonism through a

756 combined control of synaptotoxicity, neurotoxicity and gliosis.

757 *Neuropharmacology* 81, 142-152

758 107 Marcellino, D. *et al.* (2010) On the role of P2X(7) receptors in dopamine nerve

759 cell degeneration in a rat model of Parkinson's disease: studies with the P2X(7)

760 receptor antagonist A-438079. *J Neural Transm* 117, 681-687

761 108 Hracsko, Z. *et al.* (2011) Lack of neuroprotection in the absence of P2X7

762 receptors in toxin-induced animal models of Parkinson's disease. *Mol*

763 *Neurodegener* DOI: 1750-1326-6-28 [pii]

764 109 Diaz-Hernandez, M. *et al.* (2009) Altered P2X7-receptor level and function in

765 mouse models of Huntington's disease and therapeutic efficacy of antagonist

766 administration. *FASEB J* 23, 1893-1906

767 110 Iwata, M. *et al.* (2013) The inflammasome: pathways linking psychological

768 stress, depression, and systemic illnesses. *Brain Behav Immun* 31, 105-114

769 111 Sperlagh, B. *et al.* (2012) The role of purinergic signaling in depressive

770   disorders. *Neuropsychopharmacol Hung* 14, 231-238

771 112 McQuillin, A. *et al.* (2009) Case-control studies show that a non-conservative

772   amino-acid change from a glutamine to arginine in the P2RX7 purinergic receptor

773   protein is associated with both bipolar- and unipolar-affective disorders. *Mol*

774   *Psychiatry* 14, 614-620

775 113 Soronen, P. *et al.* (2011) P2RX7 gene is associated consistently with mood

776   disorders and predicts clinical outcome in three clinical cohorts. *Am J Med Genet*

777   *B Neuropsychiatr Genet* 156B, 435-447

778 114 Roger, S. *et al.* (2010) Single nucleotide polymorphisms that were identified in

779   affective mood disorders affect ATP-activated P2X7 receptor functions. *J*

780   *Psychiatr Res* 44, 347-355

781 115 Grigoroiu-Serbanescu, M. *et al.* (2009) Variation in P2RX7 candidate gene

782   (rs2230912) is not associated with bipolar I disorder and unipolar major

783   depression in four European samples. *Am J Med Genet B Neuropsychiatr Genet*

784   150B, 1017-1021

785 116 Viikki, M. *et al.* (2011) P2RX7 polymorphisms Gln460Arg and His155Tyr are

786   not associated with major depressive disorder or remission after SSRI or ECT.

787   *Neurosci Lett* 493, 127-130

788 117 Backlund, L. *et al.* (2011) Cognitive manic symptoms associated with the

789   P2RX7 gene in bipolar disorder. *Bipolar Disord* 13, 500-508

790 118 Hansen, T. *et al.* (2008) Variation in the purinergic P2RX(7) receptor gene and

791   schizophrenia. *Schizophr Res* 104, 146-152

792 119 Di Virgilio, F. (2013) The therapeutic potential of modifying inflammasomes and

793   NOD-like receptors. *Pharmacol Rev* 65, 872-905

794 120 Dowlati, Y. *et al.* (2010) A meta-analysis of cytokines in major depression. *Biol*

795   *Psychiatry* 67, 446-457

796 121 Lemos, J.R. *et al.* (2012) Modulation/physiology of calcium channel sub-types

797   in neurosecretory terminals. *Cell Calcium* 51, 284-292

798 122 Kongsui, R. *et al.* (2014) Chronic stress induces prolonged suppression of the

799   P2X7 receptor within multiple regions of the hippocampus: A cumulative

800   threshold spectra analysis. *Brain Behav Immun* DOI: 10.1016/j.bbi.2014.05.017

801 123 Boucher, A.A. *et al.* (2011) Resilience and reduced c-Fos expression in P2X7

802   receptor knockout mice exposed to repeated forced swim test. *Neuroscience*

803   189, 170-177

804 124 Stock, T.C. *et al.* (2012) Efficacy and safety of CE-224,535, an antagonist of

805   P2X7 receptor, in treatment of patients with rheumatoid arthritis inadequately

806   controlled by methotrexate. *J Rheumatol* 39, 720-727

807 125 Keystone, E.C. *et al.* (2012) Clinical evaluation of the efficacy of the P2X7

808   purinergic receptor antagonist AZD9056 on the signs and symptoms of

809   rheumatoid arthritis in patients with active disease despite treatment with

810   methotrexate or sulphasalazine. *Ann Rheum Dis* 71, 1630-1635

811 126 Ali, Z. *et al.* (2013) Pharmacokinetic and pharmacodynamic profiling of a P2X7

812   receptor allosteric modulator GSK1482160 in healthy human subjects. *Br J Clin*

813   *Pharmacol* 75, 197-207

814 127 Gum, R.J. *et al.* (2012) P2X receptor antagonists for pain management:

815   examination of binding and physicochemical properties. *Purinergic Signal* 8, 41-

816   56

817 128 Bhattacharya, A. *et al.* (2013) Pharmacological characterization of a novel

818   centrally permeable P2X7 receptor antagonist: JNJ-47965567. *Br J Pharmacol*

819   170, 624-640

820 129 Hempel, C. *et al.* (2013) The phenothiazine-class antipsychotic drugs

821   prochlorperazine and trifluoperazine are potent allosteric modulators of the

822   human P2X7 receptor. *Neuropharmacology* 75, 365-379

823 130 Patel, D. *et al.* (2014) Connexin hemichannel and pannexin channel

824   electrophysiology: How do they differ? *FEBS Lett* 588, 1372-1378

825 131 Wang, J. *et al.* (2013) The food dye FD&C Blue No. 1 is a selective inhibitor of

826   the ATP release channel Panx1. *J Gen Physiol* 141, 649-656

827 132 Sikora, A. *et al.* (1999) Cutting edge: purinergic signaling regulates radical-

828   mediated bacterial killing mechanisms in macrophages through a P2X7-

829   independent mechanism. *J Immunol* 163, 558-561

830 133 Solle, M. *et al.* (2001) Altered cytokine production in mice lacking P2X(7)

831   receptors. *J Biol Chem* 276, 125-132

832 134 Garcia-Huerta, P. *et al.* (2012) The specificity protein factor Sp1 mediates

833   transcriptional regulation of P2X7 receptors in the nervous system. *J Biol Chem*

834   287, 44628-44644

835 135 Costa-Junior, H.M. *et al.* (2011) C terminus of the P2X7 receptor: treasure

836   hunting. *Purinergic Signal* 7, 7-19

837 136 Torres, G.E. *et al.* (1999) Hetero-oligomeric assembly of P2X receptor subunits.

838 Specificities exist with regard to possible partners. *J Biol Chem* 274, 6653-6659

839 137 Marin-Garcia, P. *et al.* (2008) Synaptic terminals from mice midbrain exhibit

840 functional P2X7 receptor. *Neuroscience* 151, 361-373

841 138 Cuadra, A.E. *et al.* (2014) P2X7 receptors in neurohypophysial terminals:

842 evidence for their role in arginine-vasopressin secretion. *J Cell Physiol* 229, 333-

843 342

844 139 D'Amico, M. *et al.* (2010) AMPA- and P2X7-receptor-mediated facilitation of

845 [^{3}H]D-aspartate release from nerve terminals isolated from the rat caudal

846 brainstem. *Neurochem Int* 57, 623-628

847 140 Bhattacharya, A. *et al.* (2013) Potentiation of inhibitory synaptic transmission by

848 extracellular ATP in rat suprachiasmatic nuclei. *J Neurosci* 33, 8035-8044

849 141 Gandelman, M. *et al.* (2013) P2X7 receptor-induced death of motor neurons by

850 a peroxynitrite/FAS-dependent pathway. *J Neurochem* 126, 382-388

851 142 Nishida, K. *et al.* (2012) Mitochondrial dysfunction is involved in P2X7 receptor-

852 mediated neuronal cell death. *J Neurochem* 122, 1118-1128

853 143 Oliveira, J.F. *et al.* (2011) Rodent cortical astroglia express in situ functional

854 P2X7 receptors sensing pathologically high ATP concentrations. *Cereb Cortex*

855 21, 806-820

856 144 Norenberg, W. *et al.* (2010) Electrophysiological classification of P2X7

857 receptors in rat cultured neocortical astroglia. *Br J Pharmacol* 160, 1941-1952

858 145 Carrasquero, L.M. *et al.* (2010) Mechanisms of protein kinase D activation in

859 response to P2Y(2) and P2X7 receptors in primary astrocytes. *Glia* 58, 984-995

860 146 Hashioka, S. *et al.* (2014) Purinergic responses of calcium-dependent signaling

861   pathways in cultured adult human astrocytes. *BMC Neurosci* DOI: 1471-2202-15-

862   18 [pii]

863 147 Habbas, S. *et al.* (2011) Purinergic signaling in the cerebellum: Bergmann glial

864   cells express functional ionotropic P2X7 receptors. *Glia* 59, 1800-1812

865 148 Chen, Y. *et al.* (2012) P2X7 receptors in satellite glial cells mediate high

866   functional expression of P2X3 receptors in immature dorsal root ganglion

867   neurons. *Mol Pain* DOI: 1744-8069-8-9 [pii]

868 149 Arnoux, I. *et al.* (2013) Adaptive phenotype of microglial cells during the normal

869   postnatal development of the somatosensory "Barrel" cortex. *Glia* 61, 1582-1594

870 150 Friedle, S.A. *et al.* (2011) The P2X7-Egr pathway regulates nucleotide-

871   dependent inflammatory gene expression in microglia. *Glia* 59, 1-13

872 151 Chrovian, C.C. *et al.* (2014) P2X7 Antagonists as Potential Therapeutic Agents

873   for the Treatment of CNS Disorders. *Progress in medicinal chemistry* 53, 65-100

874 152 Beswick, P.J. *et al.* (2010) Structure-activity relationships and in vivo activity of

875   (1H-pyrazol-4-yl)acetamide antagonists of the P2X(7) receptor. *Bioorg Med*

876   *Chem Lett* 20, 4653-4656

877 153 Chambers, L.J. *et al.* (2010) Synthesis and structure-activity relationships of a

878   series of (1H-pyrazol-4-yl)acetamide antagonists of the P2X7 receptor. *Bioorg*

879   *Med Chem Lett* 20, 3161-3164

880 154 Chen, X. *et al.* (2010) Discovery of 2-chloro-N-((4,4-difluoro-1-

881   hydroxycyclohexyl)methyl)-5-(5-fluoropyrimidin-2-yl)b enzamide as a potent and

882   CNS penetrable P2X7 receptor antagonist. *Bioorg Med Chem Lett* 20, 3107-3111

883 155 Subramanyam, C. *et al.* (2011) Discovery, synthesis and SAR of azinyl- and

884   azolylbenzamides antagonists of the P2X(7) receptor. *Bioorg Med Chem Lett* 21,

885   5475-5479

886 156 Gleave, R.J. *et al.* (2010) Synthesis and biological activity of a series of

887   tetrasubstituted-imidazoles as P2X(7) antagonists. *Bioorg Med Chem Lett* 20,

888   4951-4954

889 157 Abberley, L. *et al.* (2010) Identification of 2-oxo-N-(phenylmethyl)-4-

890   imidazolidinecarboxamide antagonists of the P2X(7) receptor. *Bioorg Med Chem*

891   *Lett* 20, 6370-6374

892 158 Wilkinson, S.M. *et al.* (2014) The First CNS-Active Carborane: A Novel P2X

893   Receptor Antagonist with Antidepressant Activity. *ACS Chem Neurosci* 55, 335-

894   339

895 159 Norenberg, W. *et al.* (2011) Clemastine potentiates the human P2X7 receptor

896   by sensitizing it to lower ATP concentrations. *J Biol Chem* 286, 11067-11081

897 160 Norenberg, W. *et al.* (2012) Positive allosteric modulation by ivermectin of

898   human but not murine P2X7 receptors. *Br J Pharmacol* 167, 48-66

899 161 Fischer, W. *et al.* (2013) Natural compounds with P2X7 receptor-modulating

900   properties. *Purinergic Signal* DOI: 10.1007/s11302-013-9392-1

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904   **Boxes**

905

906   **Box 1.** Tools to study P2X7 receptors

907

908   The continuously evolving interest in this receptor resulted in the generation

909   of various tools to study its function. P2X7Rs could be identified based on

910   the following distinctive pharmacological features:

911   • The affinity of the endogenous agonist ATP is low, in the high micromolar-

912   millimolar range.

913   • BzATP is a more potent agonist than ATP itself. It has been frequently

914   used mistakenly as a selective agonist of P2X7R. This is, however, not

915   valid, because BzATP also binds to other P2X receptors with high affinity.

916   • The effect of ATP and BzATP are potentiated by a low Ca^{2+}/no Mg^{2+}-

917   containing external medium.

918   • There are several potent antagonists available, such as A-438079, A-

919   740003, the negative allosteric modulator AZ-10606120 and Brilliant blue

920   G (BBG); among them BBG is selective in concentrations below 1 µM.

921   This antagonist is also a useful tool in *in vivo* experiments. The

922   penetration of BBG through the blood-brain barrier has already been

923   determined and using doses not higher than 50 mg/kg, the resultant brain

924   concentration remains below 1 µM [105]. It should be noted, however,

925   that many P2X7R antagonists, including BBG also inhibit Panx1

926   channels. Therefore, BBG alone is inadequate to prove the involvement

927   of P2X7Rs [130]. In this respect, a valuable compound could be Brilliant

928   blue FCF, which inhibits Panx1, but not P2X7R [131].

929   • Novel radioligands, i.e. [³H]A-804598 are also available to characterize

930   the affinity of newly developed compounds to rodent P2X7Rs [25].

931 In addition to pharmacological approaches,

932   • genetic knock-down by siRNA has been increasingly used to silence

933   P2X7Rs in the past years in both *in vitro* and *in vivo* studies (e.g. [34, 39]).

934   • Mouse lines, genetically deficient in P2X7Rs, initially generated by the

935   companies Glaxo (LacZ gene and neomycin cassette insertion into exon

936   1; [132]) and Pfizer (Neo insertion in exon 13, close to the carboxyl

937   terminal; [133]), have also been widely used. However, none of these

938   mouse lines could be regarded as fully deficient in P2X7Rs, as individual

939   splice variants evaded inactivation [11, 12].

940   • For studies of P2X7R function in morphologically identified neurons,

941   astrocytes or microglia, the GFP-P2X7 reporter mouse seems to be a

942   crucial tool [134].

943

944

Box 2. Outstanding Questions

Despite the large interest in P2X7Rs and the correspondingly high number of publications dealing with this receptor, many questions still remain unresolved.

- The C-terminus of the P2X7R has been implicated in regulating receptor function including signaling pathway activation, cellular localization, protein-protein interactions, and post-translational modification [135]. It would be important to learn the three-dimensional structure of the P2X7R C-terminal tail, which is yet to be determined [4].

- Although repetitive or long-lasting stimulation of P2X7Rs by ATP allows the passage of 600-800 Da organic molecules through the cell membrane, the mechanism of pore opening is still a matter of debate. There are good arguments favouring an accessory protein, with Panx1-hemichannels probably involved in this effect, but the cationic channel-dilation theory is also an attractive alternative.

- Original work based on co-immunoprecipitation with epitope tagged subunits demonstrated that overexpressed recombinant P2X1-6 subunits could form hetero-oligomeric complexes, while P2X7 was able to form only homomeric receptor channels [136]. However, it remains to be established whether true functional P2X4/7 heteromers are formed in native systems, which might have great significance for CNS immune functions e.g. in microglia.

- A lot of controversy has arisen on the issue of whether P2X7Rs are located exclusively at microglia and astroglia in the CNS or also at neurons (see the

968   discussion on "Tissue and cell type specific distribution of P2X7Rs"). The

969   solution of this enigma might be that under normal conditions P2X7Rs are

970   dormant but after various types of damaging conditions (mechanical trauma,

971   ischemia, inflammation, etc.) they become unmasked, mostly at central

972   immunocytes but probably also at neurons. Already the tissue damage

973   afflicted to cells during the culturing procedure or the preparation of brain

974   slices may be sufficient to induce the expression of previously absent

975   P2X7Rs.

976   • Although endogenous activation of P2X7Rs under disease conditions has

977   repeatedly been proven, its exact mechanism is not fully understood, given

978   the low affinity of ATP. The possibility of constitutive activity of this receptor as

979   well as its potential endogenous ligands other than ATP should be explored.

980   • Whereas available gene deficient mouse models are not fully deficient in

981   P2X7Rs, more advanced mouse models, such as cell-type specific and/or

982   inducible knockouts, optogenetic constructs, as well as humanized mouse

983   models reproducing human gene polymorphisms in rodents are yet to be

984   generated for probing P2X7R function.

985

986

987 **Tables**

988 Table 1. Examples from recent studies verifying functional P2X7Rs on different

989 cell types of the rodent central nervous system.

Cell type/Brain area, preparation	Technique	Refs
Neurons		
Cerebral cortex, purified synaptosomes	neurochemistry, Ca^{2+} fluorimetry	[44]
Midbrain, synaptic terminals	Ca^{2+} microfluorimetry	[137]
Neurohypophysis, nerve terminals	patch clamp electrophysiology	[138]
Caudal brainstem, nerve terminals	neurochemistry	[139]
Hippocampus, isolated hilar neurons	patch clamp electrophysiology	[51]
Retina, isolated ganglion cells	patch clamp electrophysiology	[53]
Suprachiasmatic nucleus, isolated neurons	Ca^{2+} imaging	[140]
Embryonic spinal cord, cultured neurons	neurochemistry	[141]
Cortex, cultured neurons	neurochemistry	[142]
Astrocytes		

Cortex, *in situ*	patch clamp electrophysiology	[143]
Cortex, cultured	patch clamp electrophysiology	[144]
Cerebellum, cultured	neurochemistry	[145]
Human, cultured	Ca^{2+} fluorimetry	[146]
Bergmann glia		
Cerebellum, *in situ*	patch clamp electrophysiology, Ca^{2+} imaging	[147]
Satellite glia		
Immature dorsal root ganglion, isolated	electrophysiology	[148]
Microglia		
Cortex, *in situ*	patch clamp electrophysiology	[149]
N9 microglia, cultured	neurochemistry	[150]

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991

992   Table 2. Non-comprehensive list of different classes of P2X7 receptor

993   antagonists and allosteric modulators. For more information see [151]

994

Class/Compound	Function	Refs
Novel, small molecule		
(1H-pyrazol-4-yl) acetamides	antagonist	[152, 153]
benzamides	antagonist	[154, 155]
tetrasubstituted-imidazoles	antagonist	[156]
2-oxo-N-(phenymethyl)-4-imidazolinecarboxamides	antagonist	[157]
Novel, small molecule, CNS active		
JNJ-47965567	antagonist	[128]
polycyclic carboranes	antagonist	[158]
Identified by screening compound libraries		
clemastine	Positive allosteric modulator	[159]
perazine-type antipsychotic drugs	Negative allosteric modulator	[129]
ivermectine	Negative allosteric modulator	[160]

Natural compounds		
teniposide	antagonist	[161]

995

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Figure Legends

Figure 1. The simplified schematic structure of the P2X7R in open state (A) and

1000 during pore formation (B and C). The P2X7R functions as a homo-trimer, forming

1001 a chalice-like structure, while the individual P2X7R subunit is akin to a leaping

1002 dolphin. The agonist binding sites are located at the subunit interfaces and the

1003 occupation of two out of three binding sites is necessary for opening of the

1004 channel. In addition to ATP, which is the presumed endogenous agonist, the

1005 mouse P2X7R receptor could also be activated by NAD^+ through ADP-

1006 ribosylation. The activation of the receptor-ion channel leads to the inward flux of

1007 cationic current. Prolonged and /or repeated activation of P2X7R and occupation

1008 of the third agonist binding site renders the membrane permeable for high

1009 molecular weight organic cations and dyes such as $NMDG^+$ and Yo-Pro-1^+ (B

1010 and C). B. One potential mechanism of the pore formation is the dilation of the

1011 P2X7R-mediated channel pore itself. C. Alternatively, but not exclusively,

1012 additional pore forming proteins, such as pannexin (Panx1) might be recruited,

1013 which seem to be indispensable for pore formation under certain circumstances.

1014

1015

1016 **Figure 2.** Common disease mechanism by P2X7R mediated pathways in CNS

1017 disorders of different etiology. P2X7 receptors are expressed on nerve terminals,

1018 astrocytes and microglia and they are upregulated upon various disease

1019 conditions. Stress signals, such as hypoxia/ischemia (metabolic limitations),

mechanical injury, and bacterial or chemical toxins elicit the endogenous activation of P2X7R and leads to a self-amplifying ATP release and to further activation of P2X7 receptors on neighbouring cells. Following the influx of Ca^{2+} through the receptor ion channel complex, P2X7 receptor activation (a) releases glutamate from nerve terminals and astrocytes by both exocytotic and non-exocytotic mechanisms, which may give rise excitotoxicity; (b) leads to the posttranslational processing of pro-IL-1β to the leaderless, mature IL-β and to its further release by the NLRP3 inflammasome and that of other cytokines, which contribute to neuroinflammation; (c) enhance ROS production and thereby aggravate protein misfolding and neuronal damage; (d) leads directly or indirectly to cell death and the following reactive astrogliosis (e) directly or indirectly downregulates the production of BDNF and the following neuroplasticity. These key mechanisms could be manifested and contribute to disease pathology in Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), status epilepticus (SE), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), stroke, pain and mood disorders in different forms and proportion, depending on the etiology. GLU, glutamate, ROS, reactive oxygen species.

A

K⁺

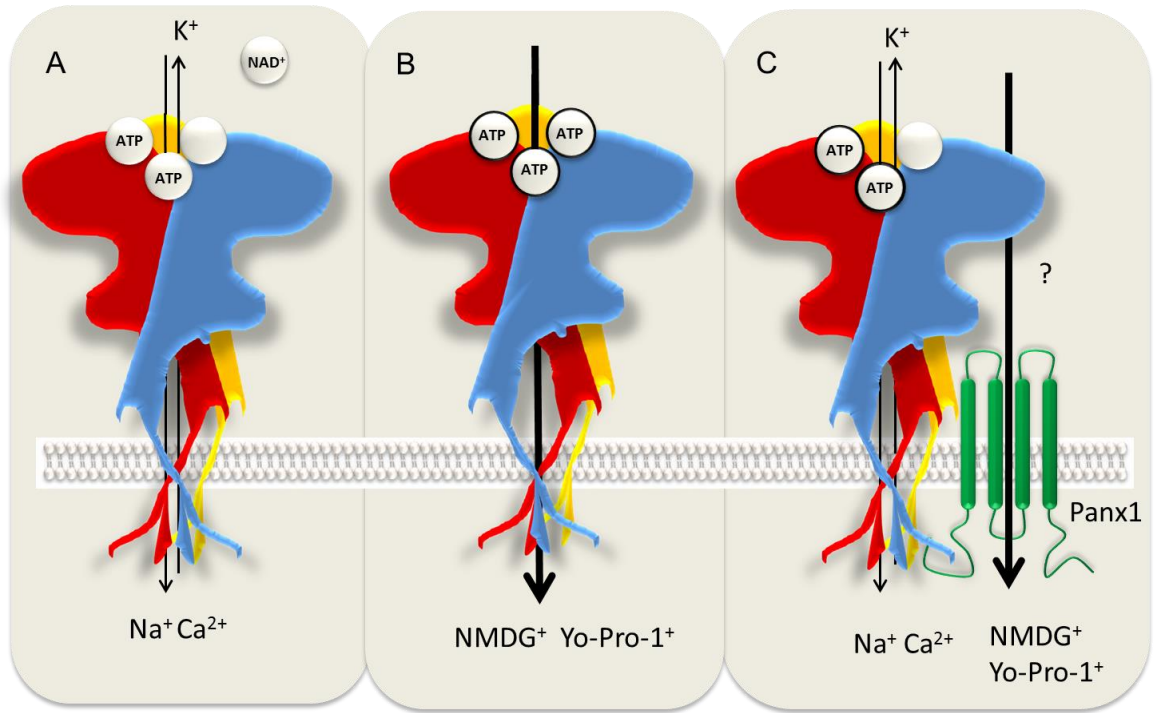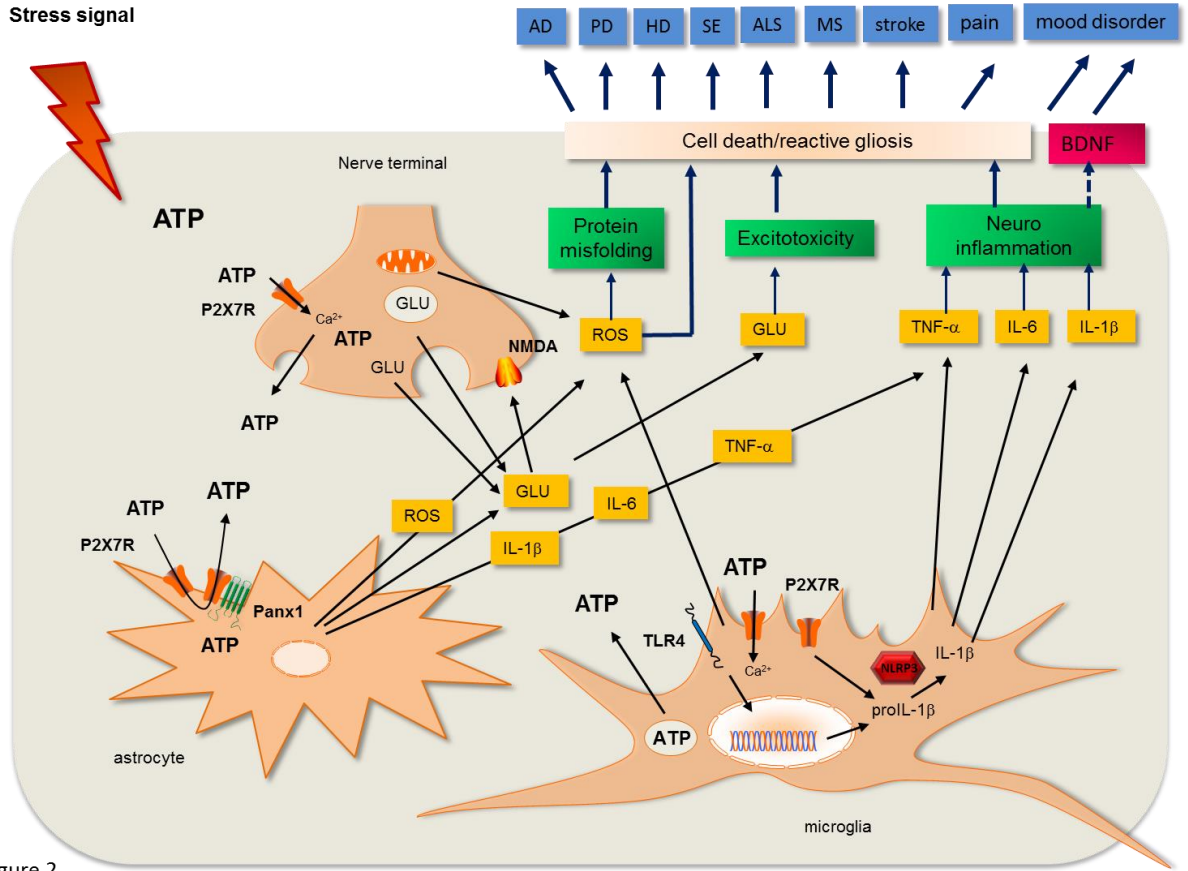NAD⁺

ATP

ATP

Na⁺ Ca²⁺

B

ATP ATP

ATP

NMDG⁺ Yo-Pro-1⁺

C

K⁺

ATP

ATP

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Panx1

Na⁺ Ca²⁺

NMDG⁺
Yo-Pro-1⁺

1043   Figure 1

Figure 2

1044