PERSPECTIVES

P2X receptors in maintenance and differentiation of neural progenitor cells

Purinergic receptors are among the first cell surface receptors expressed during embryonic development (Burnstock and Ulrich, 2011). These are characterized based on their pharmacological properties of being activated by adenosine or purine/pyrimidine nucleotides as P1 and P2 receptors. P2 receptors are further classified by their structure as P2Y metabotropic and P2X ionotropic receptors. P2Y receptors in mammals consist of P2Y1, 2, 4, 6, 11, 12 and 14 subtypes, which couple to Gq or Gi proteins and induce the release of Ca\(^{2+}\) from intracellular stores or affect accumulation of intracellular cAMP. P2X receptors are ATP-activated ion channels, assembled from three homo- or heteromeric P2X1–7 subunits.

In the past decades, it has become increasingly clear that purinergic signaling is not limited to neuronal transmission, but participates in various vital functions in almost every cell type and tissue. Recently, expression of purinergic receptor subtypes has been reported in stem cells, and functions of purinergic signaling have been observed for maintenance of stemness, proliferation, differentiation and programmed cell death (reviewed by Burnstock and Ulrich, 2011). It has been proposed that a distinct expression and activity pattern of purinergic receptors promotes proliferation or differentiation in a defined cellular context, while different purinergic expression features could contribute to senescence and cell death. Dysfunction of purinergic signaling has been proposed for numerous brain diseases, and the purinergic system provides targets for therapeutic intervention in tissue regeneration (Burnstock and Ulrich, 2011).

We will here focus on P2X receptors expressed by neural progenitor cells (NPCs), which can be obtained by primary cultivation of embryonic brain or the subventricular zone (SVZ)/subgranular zone (SGZ) of the postnatal or adult rodent (mouse/rat) brain, or alternatively by in vitro differentiation of embryonic or induced pluripotent stem (iPS) cells.

Ionotrophic P2X receptors participate in regulation of differentiation and final phenotype determination at all developmental stages. P2X7 receptors appear to inhibit differentiation into neurons, as their expression becomes down-regulated with the onset of neurogenesis (Glaser et al., 2014). In agreement with such observation, neuroblast differentiation from mouse embryonic stem cells was enhanced in conditions of pharmacological inhibition of P2X receptor activity. On the other hand, P2X7 receptor expression in NPCs favors proliferation and gliogenic differentiation. Stable down-regulation of P2X7 receptor expression negatively affected proliferation and gliogenesis of NPCs pre-differentiated from a pluripotent mouse cell line (Yuahasi et al., 2012).

Different from the developing CNS, where stem and progenitor cells continuously proliferate and differentiate for increasing the neuronal and glial population, in the adult brain the balance between neurogenic proliferation and cell death of excess proliferating NPCs is a continuous process. Such a regulatory mechanism is of particular importance during brain injury, including ischemia and epilepsy. During these pathophysiological events, unregulated proliferation of NPCs may occur, resulting in excessive formation of new neurons or glial cells. The P2X7 receptors expressed by adult SVZ NPCs have been suggested to participate in cell death mechanisms to limit disproportionate proliferation (Messemer et al., 2013a). At high extracellular ATP concentrations, which occur as consequence of tissue damage and dying of disintegrating cells, this receptor is able to introduce pore formation in the cell membrane leading to an overload of cells with inflowing ions, including Ca\(^{2+}\), resulting in the activation of the apoptotic caspase enzyme cascade and of other mechanisms leading to cell death (Sperlagh and Illés, 2014). The functionality of P2X7 receptors in SVZ NPC neurospheres and acute brain slices was confirmed by patch-clamp recording (Messemer et al., 2013a).

Expression levels of P2X7 receptors were increased in the hippocampus of a rat model of temporal-lobe epilepsy, being in line with the upregulation of hippocampal P2X7 receptors following prolonged seizures (Engel et al., 2012). Thus, it may be hypothesized that P2X7 receptors keep the balance between neurogenic proliferation and cell death in disproportionately proliferating NPCs of the adult brain. However, different from the cell-death activating mechanism proposed for pathophysiological conditions in order to control aberrant proliferation, P2X7 receptor activation in the SVZ of E15.5 rats resulted in differentiation of NPCs, observed by reduction of progenitor marker expression, such as Wnt7a, ms1 and glial fibrillary acidic protein (GFAP). Accelerated neurogenesis was revealed by enhanced expression of doublecortin and neuron-specific enolase (Tsao et al., 2013).

P2X2 receptor expression has been related to the progress of neurogenesis. First, P2X2 as well as P2X6 receptor subunit expression was enhanced in embryonic rat telencephalon neurospheres induced to neuronal differentiation in mitogen-free culture medium (Schwindt et al., 2011). Second, using a pluripotent mouse stem cell line model (P19 cells), pre-differentiated to NPCs, P2X2 receptor expression and activity, studied by expression and receptor inhibition assays, was crucial for the progress of differentiation into neurons and phenotype determination, such as expression of cholinergic and glutamatergic receptors (reviewed by Burnstock and Ulrich 2011).

P2X2 and P2X7 receptors may exert functions in embryonic neurogenesis and neural phenotype determination, based on in vitro studies involving RNA interference and pharmacological assays, respectively (Yuahasi et al., 2012), however, other P2X receptor subtypes are also present in these cells. P2X6 subunits are believed of not being capable to form functional homomeric receptors, and, therefore, they are found in heteromeric receptors consisting of additional P2X2 or P2X4 receptor subunits. Such heteromeric receptors reveal pharmacological properties different from those of homomeric receptors. In view of that, we believe that the inclusion of P2X6 subunits into P2X receptors may contribute to regulatory functions during neural differentiation. For instance, Schwindt and co-workers (2011) demonstrated significant expression of P2X6 subunits in NPCs undergoing neurogenesis, supposedly forming functional P2X2/6 heteromeric receptors. A full length and alternatively spliced variant of the P2X6
subunit is present in P19 cells throughout their differentiation into NPCs and neurons. Full-length and truncated P2X6 variants were also present during postnatal development of the mouse brain. During postnatal development, however, expression levels of the full-length transcript predominated when compared to the spliced truncated form. Just as the truncated P2X6 isoform may attribute reduced activity to the assembled heteromeric receptors, alternative splicing is suggested to regulate P2X6 subunit function during neuronal differentiation (da Silva et al., 2007).

The expression of P2X3 and P2X4 receptors was reduced when NPC neurosphere cultures from the embryonic rat telencephalon were induced to differentiate into neurons (Schwindt et al., 2011). These results agree with those obtained with the P19 embryonic cell line, used as in vitro model of neuroectodermal differentiation, in which down-regulation of P2X4 receptor expression and activity occurred along the progress of neurogenesis (reviewed by Burnstock and Ulrich 2011). Furthermore, upregulated P2X4 expression in human embryonic stem cell-derived NPCs significantly diminished when the cells differentiated into neurons (Young et al., 2011). Functional co-expression of P2X4 and P2X7 receptors was observed at adult NPCs of the mouse SVZ; ivermectin, a positive allosteric modulator of P2X4 receptors; potentiated the current responses induced by the ATP structural analogue dibenzoyl-ATP and this effect was abolished by 5-(3-bromophenyl)-1,3-dihydro-2H-benzofuro[3,2-e]-1,4-diazepin-2-one (5-BDBD), a negative allosteric modulator of this receptor type (Messemer et al., 2013b).

P2X3 receptor expression has also been detected in human embryonic stem cell-derived neural progenitors, supposed to contribute to the plasticity of calcium signaling. Such as reviewed in detail by Glaser et al. (2013), during neuronal development P2X receptors trigger intracellular calcium transients with higher amplitudes when compared to calcium transients promoted by P2Y receptors. The process of neurogenesis and neuronal phenotype determination, i.e. neurotransmitter specification, is coded by calcium wave and spike signaling (reviewed by Glaser et al., 2013).

In summary, the here demonstrated examples point to the importance of P2X receptor-mediated signaling in promoting differentiation of neural stem and progenitor cells. Among the discussed subtypes, the P2X7 receptor appears to possess crucial functions in the decision, whether a NPC is going to proliferate, differentiate or die because of necrosis/apoptosis. Further studies should focus on the mechanisms underlying such divergent actions, which could be the expression of different P2X7 isoforms (Sperlágh and Illes, 2014), such as observed during differentiation of mouse embryonic stem cells (Glaser et al., 2014) and/or extra- and intracellular cues affecting P2X7 receptor downstream-signaling. The evidence concerning the involvement of other P2X receptor subtypes than the P2X7 one is only circumstantial, because usually correlations between their expression in progenitor cells - as measured by quantitative PCR or immunohistochemistry - and neuronal differentiation, rather than the direct effects of subtype selective agonists or antagonists were determined.

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References
Burnstock G, Ulrich H (2011) Purinergic signaling in embryonic and stem cell development.Cell Mol Life Sci 68:1369-1394.
da Silva RL, Resende RR, Ulrich H (2007) Alternative splicing of P2X6 receptors in developing mouse brain and during in vitro neuronal differentiation. Exp Physiol 92:139-145.
Engel T, Jimenez-Pacheco A, Miras-Portugal MT, Diaz-Hernandez M, Henshall DC (2012) P2X7 receptor in epilepsy: role in pathophysiology and potential targeting for seizure control. Int J Physiol Pathophysiol Pharmacol 4:174-187.
Glaser T, de Oliveira SL, Cheffer A, Beco R, Martins P, Fornazari M, Lameu C, Junior HM, Coutinho-Silva R, Ulrich H (2014) Modulation of mouse embryonic stem cell proliferation and neural differentiation by the P2X7 receptor. PLoS One 9:e96281.
Glaser T, Resende RR, Ulrich H (2013) Implications of purinergic receptor-mediated intracellular calcium transients in neural differentiation. Cell Commun Signal 11:12.
Messemer N, Kunert C, Grohmann M, Sobottka H, Nieber K, Zimmermann H, Franke H, Nörenberg W, Straub I, Schafer M, Riedel T, Illes P, Rubini P (2013a) P2X7 receptors at adult neural progenitor cells of the mouse subventricular zone. Neuropharmacology 73:122-137.
Messemer N, Kunert C, Illes P, Rubini P (2013b) Co-expression of functional P2X4 and P2X7 receptors at adult neural progenitor cells of the mouse subventricular zone. Open Neurosci J 17:1-4.
Schwindt TT, Trujillo CA, Negroes PD, Lameu C, Ulrich H (2011) Directed differentiation of neural progenitors into neurons is accompanied by altered expression of P2X purinergic receptors. J Mol Neurosci 44:141-146.
Sperlágh B, Illes P (2014) The P2X7 receptor: an emerging target in CNS diseases. Trends Pharmacol Sci 35:537-547.
Tsao HK, Chiu PH, Sun SH (2013) PKC-dependent ERK phosphorylation is essential for P2X7 receptor-mediated neuronal differentiation of neural progenitor cells. Cell Death Dis 4:e751.
Young A, Machacek DW, Dhara SK, Macleish PR, Benveniste M, Dodda MC, Sturkie CD, Stice SL (2011) Ion channels and ionotropic receptors in human embryonic stem cell derived neuronal progenitors. Neuroscience 192:793-805.
Yuaashi KK, Demasi MA, Tamajusuki AS, Lenz G, Sogayar MC, Fornazari M, Lameu C, Nascimento IC, Glaser T, Schwindt TT, Negroes PD, Ulrich H (2012) Regulation of neurogenesis and gliogenesis of retinoic acid-induced P19 embryonal carcinoma cells by P2X2 and P2X7 receptors studied by RNA interference. Int J Dev Neurosci 30:91-97.