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## Nucleotide signaling in nervous system development

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**Abstract** The development of the nervous system requires complex series of cellular programming and intercellular communication events that lead from the early neural induction to the formation of a highly structured central and peripheral nervous system. Neurogenesis continuously takes place also in select regions of the adult mammalian brain. During the past years, a multiplicity of cellular control mechanisms has been identified, ranging from differential transcriptional mediators to inducers or inhibitors of cell specification or neurite outgrowth. While the identification of transcription factors typical for the stage-specific progression has been a topic of key interest for many years, less is known concerning the potential multiplicity of relevant intercellular signaling pathways and the fine tuning of epigenetic gene regulation. Nucleotide receptors can induce a multiplicity of cellular signaling pathways and are involved in multiple molecular interactions, thus opening the possibility of cross talk between several signaling pathways, including growth factors, cytokines, and extracellular matrix components. An increasing number of studies provides evidence for a role of nucleotide signaling in nervous system development. This includes progenitor cell proliferation, cell migration, neuronal and glial cellular interaction and differentiation, and synaptic network formation.

**Keywords** Adenosine · ATP · Development · Ectonucleotidase · Nervous system · Neurogenesis · Nucleotide · P2 receptor

### Introduction

During the development of the mammalian nervous system, neural stem cells and their derivative progenitor cells generate neurons by asymmetric and symmetric divisions [74]. They are also the source of the two types of macroglial cells of the central nervous system (CNS), astrocytes, and oligodendrocytes [97]. The development of the nervous system requires complex series of cellular programming and intercellular communication events that lead from the neural induction of a specified region of the ectoderm to the formation of a highly structured central and peripheral nervous system, with specified cell types and pathways of cellular interconnection [66]. These processes include the control of proliferation and cell fate determination of neurons, astrocytes, and oligodendrocytes, cell migration and maturation, neurite extension and nerve guidance, synapse formation, plasticity and network implementation and, in addition, abandoning of excessive cellular contacts and apoptosis. During the past years, a multiplicity of cellular control mechanisms has been identified, ranging from differential transcriptional mediators to inducers or inhibitors of cell specification or neurite outgrowth. Furthermore, there is now abundant evidence that neurogenesis continuously takes place in select regions of the adult mammalian forebrain [3]. This has initiated a wealth of studies to understand the mechanisms controlling adult neuron formation and raises hopes to reinitiate neurogenesis under conditions of neural trauma or disease [29, 55, 109].

While the identification of transcription factors typical for the stage-specific progression during nervous system development has been a topic of key interest for many years, less is known concerning the potential multiplicity of intercellular signaling substances and the epigenetic gene regulation that define or modulate individual developmental switches [13, 35, 69, 86]. Regarding nervous system development, nucleotides [32] are among the most recently identified and least investigated group of extracellular signaling substances. The present overview highlights recent evidence for a role of nucleotide signaling in

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nervous system development with a focus on the mammalian nervous system. Aspects of the role of nucleotides and nucleosides in nervous system development have previously been reviewed [30, 31, 64].

### Signaling via extracellular nucleotides

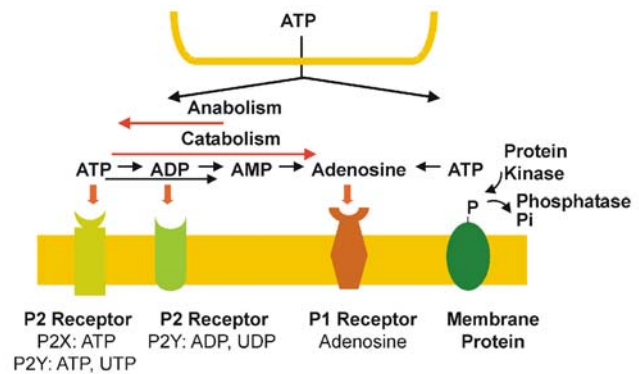
Signaling via extracellular nucleotides is complex (Fig. 1). Nucleotides act via ionotropic or G-protein-coupled receptors (P2 receptors) [146]. The homomeric or heteromeric P2X receptors (seven subtypes, P2X<sub>1-7</sub>) are stimulated by ATP, are permeable to Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup>, and mediate fast signal transmission [139]. Most of these can combine to form heterooligomers, resulting in altered pharmacological properties. The eight subtypes of P2Y receptors (P2Y<sub>1,2,4,6,11,12,13,14</sub>) are activated by ATP, ADP, UTP, UDP, or nucleotide sugars, depending on the subtype, and couple to differential intracellular signaling pathways [104]. In rodents, the most common experimental animals, the ligand preference (in brackets) is as follows: P2Y<sub>1</sub> (ADP, ATP), P2Y<sub>2</sub> (UTP, ATP), P2Y<sub>4</sub> (UTP, ATP), P2Y<sub>6</sub> (UDP), P2Y<sub>11</sub> (ATP), P2Y<sub>12</sub> (ADP), P2Y<sub>13</sub> (ADP), and P2Y<sub>13</sub> (UDP glucose and other nucleotide sugars). The ATP-activated P2Y<sub>11</sub> receptor is not apparent in the rodent genome. The potential role in nervous system development of dinucleoside polyphosphates, which can activate various P2 receptors as well as receptors of their own [116], has not yet been investigated. Extracellular nucleotides can be inactivated or interconverted by a multiplicity of enzymes generally referred to as ectonucleotidases. These belong to several enzyme families that vary regarding catalytic properties and cellular expression pattern [201, 203]. Extracellular ATP is eventually degraded to adenosine that may additionally influence neural development via P1 receptors (A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, A<sub>3</sub>) [146, 163]. ATP (and UTP) can be released from essentially every cell type. Release can be constitutive as well as stimulus-evoked and regulated [164].

### Nucleotide receptor-induced cellular signaling

Nucleotides and nucleosides mediate a considerable number of trophic effects on neurons and glial cells including cell proliferation, cell differentiation, axonal growth, and the synthesis and release of trophic factors such as fibroblast growth factor-2 (FGF-2), nerve growth factor (NGF), or neurotrophin 3 [132, 147]. Recent evidence suggests that cellular communication via nucleotides can involve a considerable variety of molecular interactions and cellular signaling mechanisms.

#### P2Y receptors

Depending on subtype, P2Y receptor activation can induce several intracellular signaling cascades, most notably activation of phospholipase C, activation or inhibition of



**Fig. 1** Potential pathways of extracellular nucleotide metabolism and receptor function. ATP is shown as an example. Released ATP is metabolized by ectonucleotidases to adenosine in a stepwise manner (catabolism). Some of the ectonucleotidases directly degrade ATP to AMP without intermediate formation of ADP. ATP is an agonist at P2X receptors as well as at P2Y<sub>1</sub>, P2Y<sub>2</sub>, P2Y<sub>4</sub>, and P2Y<sub>11</sub> receptors whereas UTP only activates P2Y<sub>2</sub> and P2Y<sub>4</sub> receptors. ADP is an agonist at P2Y<sub>1</sub>, P2Y<sub>12</sub>, and P2Y<sub>13</sub> receptors and UDP at P2Y<sub>6</sub> receptors. Adenosine activates P1 receptors (A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub>). The surface-located enzymes nucleoside diphosphate kinase and adenylate kinase catalyze the extracellular formation of ATP (anabolism). Ectoprotein kinases can phosphorylate surface-located proteins which in turn are dephosphorylated by ectophosphoprotein phosphatase activity (potentially by alkaline phosphatases)

adenylyl cyclase, activation of phospholipase A<sub>2</sub>, coupling to ion channels, activation of the mitogen-activated (MAP) kinase pathways, and induction of immediate early genes. Transactivation of receptor tyrosine kinases opens up additional avenues for cross talk with other signaling pathways [1, 14, 104, 115, 130]. The outcome of individual P2 receptor activation is likely to vary with cell type, with the coexpression of receptors for additional signaling molecules and with the associated intracellular signaling molecules—that can change during development [12, 57]. In addition, P2Y receptors were shown to form homooligomers or heterooligomers with other G-protein-coupled receptors (GPCRs) that could impact on both ligand properties and intracellular signaling. The P2Y<sub>2</sub> receptors can form homooligomers [102], and the P2Y<sub>1</sub> receptor forms heterooligomers with the A<sub>1</sub> adenosine receptor [123].

The multiple interactions of the well-investigated UTP- and ATP-activated P2Y<sub>2</sub> receptor can serve as an example [190]. Through activation of phospholipase C, it induces a rise in intracellular Ca<sup>2+</sup>. This may modulate Ca<sup>2+</sup> signaling pathways including the generation of intercellular Ca<sup>2+</sup> waves that can play a significant role in embryonic development [189]. Furthermore, the human P2Y<sub>2</sub> receptor contains two C-terminal consensus Src-homology-3 (SH3) binding domains that promote recruitment and activation of Src. The Src family of tyrosine kinases was found to be important for embryonic stem-cell renewal [8]. Activation of Src is important for P2Y<sub>2</sub> receptor-mediated transactivation of soluble tyrosine kinases, the epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), and vascular endothelial growth factor receptor (VEGFR)-2 [110, 167], that have all been

implicated in the control of neurogenesis [187]. P2Y<sub>2</sub> receptor activation increases Src kinase-dependent clustering with the EGFR and VEGFR-2 receptors. Similarly, P2Y<sub>2</sub> receptor activation in the presence of NGF leads to the colocalization and association of tyrosine receptor kinase A and P2Y<sub>2</sub> receptors [9]. Furthermore, the P2Y<sub>2</sub> receptor contains an RGD integrin-binding motif in its first extracellular loop. It colocalizes with  $\alpha_v\beta_3/\beta_5$  integrins [56], suggesting that it can form a large signaling complex containing integrins and proteins associated with integrins such as Src, focal adhesion kinase, Pyk2, EGFR, PDGFR, and the actin cytoskeleton [110]. In accordance, the P2Y<sub>2</sub> receptor-mediated astrocyte migration was shown to involve the interaction with  $\alpha_v$  integrin [10, 186].

P2Y receptors activate MAP kinase pathways in a wide variety of cell types [165, 170]. The functional role of MAP kinase protein cascades in P2Y receptor-mediated trophic actions has been particularly intensively investigated in astrocytes [128, 130]. In these cells, extracellular signal-regulated protein kinases (ERKs) can be activated via P2Y<sub>1</sub>, P2Y<sub>2</sub>, or P2Y<sub>4</sub> receptors whereby the intracellular signaling cascades vary between receptor subtypes [100, 107, 127, 129]. Nucleotides can exert a synergistic effect on cell proliferation together with growth factors, chemokines, or cytokines in a variety of cell types, including astrocytes and stem cells [88, 106, 117, 126, 181]. This may be initiated by parallel activation of the MAP kinase pathway and/or by transactivation of growth factor receptors [107, 133] (Fig. 2). Activation of P2 receptors (including P2Y<sub>2</sub> receptors) can also result in CREB phosphorylation [26, 42, 119]. P2Y<sub>2</sub> receptor activation in astrocytes not only induces the expression of bcl-2 and bcl-x1, thus triggering survival signaling cascades, but also stimulates the expression of genes implicated in nervous

system development, neuronal differentiation and survival, and the formation and function of synapses [42].

An additional and very interesting potential concerns the stimulation of  $\alpha$ -secretase activity by the heterologously expressed P2Y<sub>2</sub> receptor, the enhancement of amyloid precursor protein processing, and the concomitant release of the soluble non-amyloidogenic amyloid precursor protein- $\alpha$  (sAPP) [34]. sAPP has been shown to increase proliferation of embryonic and adult neural stem cells [33]. In addition, the P2 receptor-mediated activation of ectometalloproteases carries potential to induce ectodomain shedding of additional transmembrane proteins such as TGF- $\alpha$  (endogenous ligand of the EGF receptor), HB-EGF, or of Notch1 [79, 89]. Growth factor shedding could in turn result in the activation of the corresponding growth factor receptors [115].

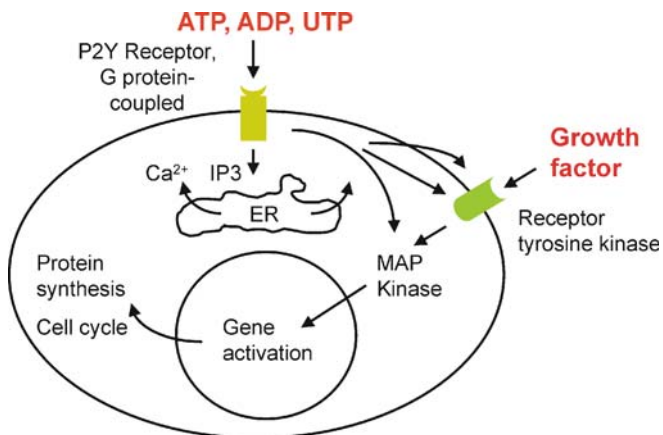
It is of interest that EGF can sensitize and potentiate the intracellular Ca<sup>2+</sup> response to ATP [49]. This opens up the possibility that the cellular release of small amounts growth factor could prime and increase the impact of nucleotidergic signaling during neural development.

#### P2X receptors

Activation of P2X receptors by ATP generally induces a robust cellular influx of Na<sup>+</sup> and Ca<sup>2+</sup>. This in turn leads to a rapid cellular depolarization and to the activation of intracellular Ca<sup>2+</sup>-dependent signaling cascades. In most cases, activation of P2X<sub>7</sub> receptors leads to the opening of large conductance channels that have been associated with the induction of apoptosis [139] and may thus be relevant for stage-specific programmed cell death. Activation of this receptor and also of other P2X receptors can also initiate ERK1/2 phosphorylation [71, 92].

#### P1 receptors

The four different receptors for adenosine (P1 receptors) couple either negatively or positively to adenylyl cyclase [146]. In addition, these receptors can couple to MAP kinases which could give them a role in cell growth, survival, death, and differentiation [163]. In astrocytes, ERK can be activated via P1 purinoceptor in addition to P2Y receptor agonists [131]. Furthermore, P1 receptors crosstalk with other metabotropic receptors, including P2Y receptors [6]. This opens up a considerable potential for an interaction of P2 receptor- and P1 receptor-mediated pathways in multiple functional contexts.



**Fig. 2** Simplified model of potential pathways for cross talk between ATP-, ADP-, or UTP-activated P2Y receptors and growth factor receptors (e.g., EGF, PDGF, and VEGFR-2). Both signaling pathways can converge at the MAP kinases Erk1/2, inducing rapid transcription and protein synthesis that could augment, e.g., cell proliferation. In addition, the G protein-coupled P2Y receptors may directly phosphorylate and thus activate the receptor tyrosine kinase (receptor transactivation). Furthermore, P2 receptors may activate plasma membrane-bound ectometalloproteases that could cleave and mobilize membrane-bound growth factor precursors

#### Developmental changes in nucleotide receptor expression

Both P1 and P2 receptors are expressed during nervous system development [31]. P2X family genes are already expressed in the developing zebrafish nervous system [140]. The early and transient expression of P2 receptors in

distinct developmental settings can serve as a first indication for an involvement of the nucleotidergic signaling cascade.

### P2X receptors

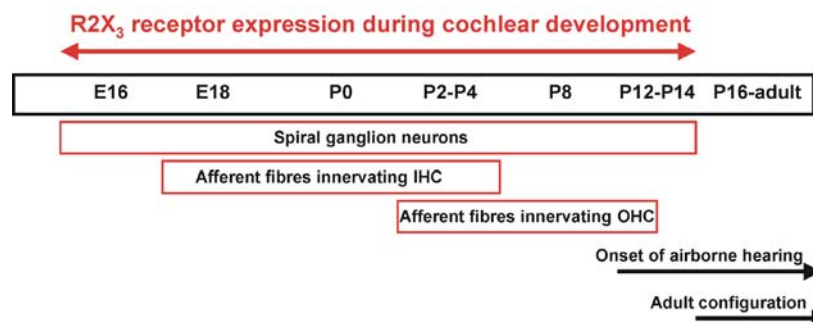
Several P2Y and P2X receptors were found by RT-PCR and immunocytochemistry to be dynamically expressed in the pre- and postnatal central and peripheral nervous system [39–41, 99, 196]. Among all the P2X receptors examined, the homomeric P2X<sub>3</sub> receptor was the first to be expressed during neurogenesis in both the rat CNS and peripheral nervous system. P2X<sub>3</sub> immunoreactivity was detected in cranial motor neurons as early as E11, when neurons exit the cell cycle and start axon outgrowth. The P2X<sub>3</sub> receptor was also identified in (the neural crest cell-derived) sensory ganglia. During further development, the P2X<sub>3</sub> receptor appeared in additional distinct brain regions and in peripheral nerves but its expression declined at later stages. From P16 onward, P2X<sub>3</sub> immunoreactivity was absent from the facial nucleus, spinal trigeminal tract, the mesencephalic trigeminal nucleus, and the vestibular nucleus. The P2X<sub>2</sub> receptors that can form heterodimers with the P2X<sub>3</sub> receptor appeared after the P2X<sub>3</sub> receptor (E14 onward). In retinal ganglion cells, the P2X<sub>3</sub> receptor was detected from E14.5 onward, whereas the P2X<sub>2</sub> receptor was not detected in the prenatal retina. P2X<sub>7</sub> receptors were detected from E14 onward. While P2X<sub>4</sub>, P2X<sub>5</sub>, and P2X<sub>6</sub> receptors were expressed from P1 onward, no expression of P2X<sub>1</sub> receptors was observed [39, 40]. It is interesting to note that both P2X<sub>4</sub> and P2X<sub>5</sub> receptors were expressed in the neurogenic subventricular zone, presumably in affiliation with separate, yet-to-be-defined cell types (see below). Similarly, P2X<sub>3</sub> receptor immunoreactivity developed transiently in the myenteric plexus of rat stomach. Immunoreactivity appeared first at E12 in the trunk and branches of the vagus nerve and was first demonstrated at P1 in intrinsic neuron cell bodies. It

declined after peaking at P14. Within the intraganglionic laminar nerve endings and intramuscular arrays, many growth cone-like structures exhibited strong P2X<sub>3</sub> immunoreactivity [195].

The P2X<sub>3</sub> receptor is also transiently expressed in the developing mouse brain, and in precursors of spinal motor neurons where it is considered to be a useful cell lineage marker for neural, crest-derived early sensory neurons, sympathetic neurons, and the adrenal medulla [22]. Based on immunohistochemistry, expression of P2X<sub>3</sub> receptors is equally transient in mouse sensory ganglia [152].

The P2X<sub>7</sub> receptor has previously been shown to induce cytotoxicity at high concentrations of ATP and to be involved in programmed cell death [105, 121]. Indeed, via the P2X<sub>7</sub> receptor, endogenous extracellular ATP triggers the death of retinal cholinergic neurons during normal development, thereby controlling the total number, local density, and regular spacing of these neurons [148]. It is possible that this receptor is also involved in apoptosis during neurogenesis in the brain.

Also in sensory organs, the expression of P2X receptors can be developmentally regulated. P2X<sub>1</sub>, P2X<sub>2</sub>, P2X<sub>3</sub>, and P2X<sub>7</sub> receptors are expressed in the developing inner ear [27, 84, 85, 87, 95, 134, 135] suggesting a contribution to the establishment of synaptic connections between the primary auditory neurons and the sensory hair cells. The time pattern of expression varies between the P2X<sub>1</sub>, P2X<sub>2</sub>, and P2X<sub>3</sub> subunits. For example, the expression of the P2X<sub>3</sub> receptor becomes apparent in neurites of spinal ganglion neurons already at E16 [87]. It is expressed to synaptic terminals and vanishes again postnatally (Fig. 3). This transient nature of expression and the association with afferent fibers implies that P2X receptor upregulation is linked to the development in particular of the innervation of the organ of Corti. P2X receptors are likely to contribute to the neurotrophic signaling that establishes functional auditory neurotransmission before the likely onset of hearing (P8–P12).



**Fig. 3** Example for the transient expression of a P2X receptor subunit (P2X<sub>3</sub>) during the development of the rat cochlear innervation, as revealed by immunohistochemistry (modified from [87]). The receptor is first detected at E16 in the spiral ganglion that provides the major primary afferent innervation to the cochlear sensory hair cells. It disappears at P14. Between E18 and P4, it can be identified in the afferent synaptic terminals that innervate the

inner hair cells (IHC). Between P2 and P13, it is associated with the afferent fibers innervating the outer hair cells (OHC). The P2X<sub>3</sub> receptor could not be immunodetected in the adult cochlea (in contrast to the P2X<sub>2</sub> receptor). The rat is born deaf and the onset of airborne hearing is around P12. The early postnatal period is thus critical for the synaptic determination of the afferent (and efferent) innervation of the cochlear outer hair cells

## P2Y receptors

A novel P2Y receptor was discovered in the *Xenopus* that is expressed at very early stages of neural plate development. It is no longer detected after neural tube closure [21]. The chick P2Y<sub>1</sub> receptor is expressed in a developmentally regulated manner in various embryonic tissues, including the brain [114]. In situ hybridization first detected the receptor at approximately stage 33. The receptor was expressed mostly in regions of postmitotic neurons, including the telencephalon, with smaller patches in the dorsal diencephalon, posterior midbrain, and anterior hindbrain. Expression in the telencephalon, dorsal diencephalon, and posterior hindbrain was strengthened by stage 36.

Using RT-PCR and immunohistochemistry, Burnstock et al. also analyzed the developmental expression of P2Y<sub>1</sub>, P2Y<sub>2</sub>, P2Y<sub>4</sub>, and P2Y<sub>6</sub> receptors during rat development [41]. Only P2Y<sub>1</sub> and P2Y<sub>4</sub> receptor proteins were identified in the developing brain. The P2Y<sub>4</sub> receptor appeared to be the dominant P2Y receptor present early in the brain. It appeared first at E14 and was identified in the olfactory system, diencephalon, amygdala, and brain stem. P2Y<sub>1</sub>, P2Y<sub>2</sub>, and P2Y<sub>4</sub> receptor proteins were all expressed in the spinal cord and the peripheral nervous system from E12. At this stage, the P2Y<sub>1</sub> receptor was detected in the floor plate of the spinal neural tube, a transient organization center that profoundly influences the development of the nervous system [52]. The P2Y<sub>2</sub> and P2Y<sub>4</sub> receptors were expressed in the ventral horns of the embryonic spinal cord and, together with the P2Y<sub>1</sub> receptor, may play a role in motor neuron development.

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### Developmental alterations in P2 receptor signaling

Several studies addressed P2 receptor-mediated signaling in development-specific states. In the chicken retina, ATP-evoked Ca<sup>2+</sup> transients were strongest as early as E3 and were drastically reduced at E11–13.5 [178]. In neonatal rats, ATP transiently affected the excitation of motoneurons of the nucleus ambiguus, which are the final output neurons of the swallowing pattern generator [28]. In medullary slices from P0–4 animals, local application of ATP produced large desensitizing inward currents whereas in slices from juvenile animals (P15–21), only negligible currents were recorded. This corresponded to a transient robust expression of the P2X<sub>3</sub> receptor and moderate and weak expression of P2X<sub>2</sub>, P2X<sub>5</sub> and P2X<sub>4</sub>, P2X<sub>6</sub> receptors, respectively. The data support the notion that the transient excitatory actions of ATP on neonatal motoneurons impact on the development and/or neuromodulation of the ambigular motor network. Similarly, ATP, operating via distinct P2X and P2Y receptors, directly contributes to modulate rat hippocampal network activity at early stages of postnatal development [154].

In presynaptic terminals of spinal cord substantia gelatinosa neurons, P2X receptors that enhance glycinergic

activity become functional late in ontogeny (later than P10–12) [94]. Similarly, in locus coeruleus neurons of the rat, inward currents could be induced by ATP antagonists at P18–23 but hardly at P10–14 [192]. It is apparent that no functional P2 receptors are present at locus coeruleus neurons right after birth but thereafter, P2 purinoceptor function increases with age.

In the rat cerebellar cortex, the neuronal network develops during the first 3 weeks of life. In a recent study, ATP was found to enhance the synaptic activity in rat cerebellar Purkinje neurons by the end of the second postnatal week. From P9/10 to P17/18, ATP was found to increase fourfold the frequency of spontaneous postsynaptic currents (sPSCs) recorded from Purkinje neurons. Functional ATP receptors appeared during P10–12, sPSC frequency was enhanced after inhibition of ecto-ATPases, and application of the P2 receptor antagonist PPADS revealed tonic stimulation of P2 receptors at P14 [37]. Similarly, immunostaining demonstrated a postnatal increase in cerebellar P2X receptor expression [196]. These data imply that purinergic systems mature during the second postnatal week in the rat cerebellum and start to contribute to the modulation of the synaptic activity in Purkinje neurons. They also reveal that the ectonucleotidase pathway is functionally developed at that developmental stage and raise the question whether mechanisms and sites of ATP release are under a comparable developmental control.

A transient expression of P2X receptor function has also been reported for the peripheral nervous system. As revealed by patch clamp recording, sympathetic neurons of the rat superior cervical ganglion are more responsive to ATP at birth and during the early postnatal period due largely to the expression of the P2X<sub>3</sub> subunit. These responses are much reduced in mature rats [54]. In the embryonic (E14) chick ciliary ganglion neurons, ATP induced a rapid inward current, inferring ATP-mediated modulation of these parasympathetic neurons [2].

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### Developmental changes in ectonucleotidase expression

Ligand availability at P2 receptors is tightly controlled by ectonucleotidases. Prolonged exposure can lead to either receptor desensitization or downregulation [146, 182]. Ectonucleotidases may hydrolyze ATP or UTP as agonists of P2 receptors. They may also generate ADP or UDP as agonists of nucleoside diphosphate-sensitive P2Y receptors and eventually produce the P1 receptor agonist adenosine [201]. Developmentally regulated transient receptor expression may be accompanied by alterations in ectonucleotidase expression. Several investigations demonstrate that the expression of individual ectonucleotidases differs in developmental onset and that ectonucleotidases can be transiently expressed in defined regions of the nervous system, including the neurogenic regions.

## Alkaline phosphatase

The four mammalian isoforms of alkaline phosphatase (AP) degrade nucleoside 5'-tri-, -di-, and -monophosphates, release inorganic phosphate from a large variety of organic compounds, including proteins, and share an alkaline pH optimum [201]. They are anchored to the cell surface via a glycosylphosphatidyl inositol (GPI) anchor. It is interesting to note that the tissue nonspecific form of alkaline phosphatase (TNAP) is expressed by mouse and human undifferentiated embryonic stem cells [43]. As revealed by enzyme histochemistry, homogeneous TNAP activity in the mouse neuroepithelium becomes apparent at E8.5 [125]. At E9.5, distinctly TNAP-positive cells appear in the brain and spinal cord. At E10.5–12.5, TNAP positivity is observed between the mesencephalon and the rhombencephalon, along the entire spinal cord and the cranial nerves emerging from the myelencephalon, suggesting an association with pioneer growth cones. At E14.5, TNAP expression is considerably reduced in brain tissue and very low in the adult brain. In the late developing rat cerebellar cortex, peak alkaline phosphatase activity was localized to the proliferative external granular cells until P7 [198]. More recent studies allocate TNAP to primary sensory areas in the adult primate sensory cortex, including axonal and dendritic processes and the synaptic cleft. It is most notable that TNAP activity is regulated by sensory experience [59]. In the postnatal marmoset (*Callithrix jacchus*), expression of TNAP is developmentally regulated both in gray and white matter [60], suggesting that TNAP is involved in the mechanisms regulating maturation of synaptic transmission and axonal conduction in the developing brain.

The functional role of the enzyme during embryonic development has not yet been investigated. In addition to its nucleotidase activity, TNAP can ectodephosphorylate proteins and it has been shown to bind to collagens [193]. Because TNAP can catalyze the entire hydrolysis chain from the nucleoside-5'-triphosphate to the respective nucleoside, it may scavenge or produce ligands of P2 receptors (comp. Fig. 1) and finally produce adenosine as the ligand of P1 receptors. Mice lacking TNAP reveal no gross abnormalities in brain development. However, at approximately 2 weeks after birth, they develop fatal seizures. These have been attributed to a defective metabolism of pyridoxal 5'-phosphate and subsequent reduced levels of the inhibitory neurotransmitter GABA [188]. Deficiency in TNAP is associated with defective bone mineralization [124]. At present, little is known concerning the mechanism controlling TNAP expression. TNAP can be induced by retinoic acid [158] and by activation of the phosphatidyl inositol 3-kinase/Akt pathway [138].

## Ectonucleotide pyrophosphatase/phosphodiesterases

Similar to alkaline phosphatases, the ectonucleotide pyrophosphatase/phosphodiesterases (E-NPPs) NPP1, NPP2,

and NPP3 share an alkaline pH optimum. They hydrolyze 5'-monodiester bonds in nucleotides and their derivatives, resulting in the release of 5'-nucleotide monophosphates. Physiological substrates include ATP, NAD<sup>+</sup>, nucleotide sugars, and also dinucleoside polyphosphates. Typically, ATP is directly degraded to AMP with the release of inorganic pyrophosphate. Whereas NPP1 and NPP3 are type II membrane proteins, NPP2 (autotaxin) is secreted [72, 172]. As revealed by in situ hybridization, autotaxin, a splice variant of NPP2, is expressed as early as E9.5 in the floor plate of the neural tube. Expression increases during the following days but is absent at E16.5 [15]. During later brain development, autotaxin has been correlated with intermediate stages of rat brain oligodendrocyte differentiation and myelin formation [68]. It becomes expressed with myelinating oligodendrocytes and participates in the myelination via a novel signaling pathway leading to changes in integrin-dependent focal adhesion assembly and consequently oligodendrocyte extracellular matrix interactions [61, 62]. The related enzyme NPP3/gp130RB<sup>13-6</sup> is transiently expressed during rat brain development by a small subset of precursor cells distributed in a specific spatio-temporal pattern in the germinal layers of the ventricular zone of the immature brain. These cells are capable of differentiating in vitro into cells with radial-gial, astrocytic or ependymal cell properties [20]. In the adult brain, immunolabeling for NPP3 is restricted to the ependyma. It is interesting to note that transfection of NPP3 into several cell lines induced cell aggregation, cell motility, and invasion into collagen I. Furthermore, it induced coexpression of both glial and neuronal markers [48]. It has been hypothesized that NPP3 may represent an important factor in the process of glial cell proliferation which is accompanied by migration of glial precursors.

The endogenous substrate relevant in the various E-NPP locations remains to be identified. In particular, NPP2/autotaxin (but not NPP1 and NPP3) also targets lipids, exerting lysophospholipase D activity. NPP2 has been shown to promote tumor cell motility [177], and this property results from the generation of lysophosphatidic acid [184]. The involvement of NPP2/autotaxin in oligodendrocyte development may thus mainly result from its lysophospholipase D activity and an additional functional domain mediating the modulation of oligodendrocyte remodeling and focal adhesion organization [50].

## Ectonucleoside triphosphate diphosphohydrolases

NTPDase1–3 and NTPDase8, four members of the ectonucleoside triphosphate diphosphohydrolase (E-NTP Dase) protein family, are firmly anchored to the plasma membrane via two transmembrane domains and thus represent typical ectoenzymes. All four enzymes hydrolyze nucleoside triphosphates including the physiologically active ATP and UTP but the hydrolysis rates for nucleoside diphosphates vary considerably. They also differ in product formation. This is of considerable relevance for the regulation of nucleotide signaling [19, 202]. NTPDase1

and NTPDase3 were found to be associated with the vasculature and neurons, respectively [17, 151]. They can also be detected by Northern hybridization in the developing nervous system (Zimmermann, unpublished results). In synaptosomes isolated from rat cerebral cortex, a postnatal increase in ecto-ATPase and ecto-ADPase activities was observed [122]. NTPDase2 protein and catalytic activity is associated with neural progenitor cells of the late embryonic and adult rodent brain [24, 168], implicating a role of purinergic signaling pathways in neurogenesis (see below). NTPDase2 is associated also with immature and nonmyelinating Schwann cells of the peripheral nervous system, with satellite glia cells of dorsal root ganglia and sympathetic ganglia, and with enteric glia [25].

### Ecto-5'-nucleotidase

Mammalian ecto-5'-nucleotidase catalyzes the hydrolysis of nucleoside monophosphates and is thus of major importance for the extracellular formation of the P1 receptor agonist adenosine [200]. In the adult CNS, ecto-5'-nucleotidase has a predominantly glial (astrocytic, oligodendroglial) location. However, there is considerable evidence that the enzyme is associated with the neural surface during development and plasticity. During postnatal ontogeny of the rat cerebellum, it is expressed to the surface of migrating neuroblasts [58, 159]. It is notable that proliferation of precursors, cell migration and differentiation, and dendritic and axonal growth and remodeling occur equally in the postnatal cerebellum. Ecto-5'-nucleotidase also becomes transiently associated with synapses during synaptogenesis and synapse remodeling [11, 58, 160]. Examples include the developing postnatal cerebellum, the adult olfactory bulb, lesion-induced sprouting within the adult dentate gyrus, and also reactive synaptogenesis in the human brain [108]. Expression of ecto-5'-nucleotidase was found to be mandatory for neurite extension *in vitro* [80].

An impressive correlation has been found between the development of the kitten visual cortex and the distribution of ecto-5'-nucleotidase [161, 162]. Within postnatal weeks 4 to 6, the enzyme exhibits a patchy distribution that is related to the terminal fields of the afferents from the two eyes that segregate during this phase to form ocular dominance columns. During this developmental phase, ecto-5'-nucleotidase is associated with synaptic contacts and shifts to a glial location only when the processes of synapse formation and remodeling have come to an end. Furthermore, the cell adhesion carbohydrate epitope HNK-1 is transiently associated with the kitten ecto-5'-nucleotidase during the period of its synaptic expression [185]. These observations support the notion that ecto-5'-nucleotidase plays a significant role in neurogenesis and in cellular contact formation within the synaptic cleft.

While it is tempting to assume that the expression of the enzyme indicates the involvement of P1 receptor-mediated mechanisms, additional functional contexts may apply. Ecto-5'-nucleotidase also binds to the extracellular matrix

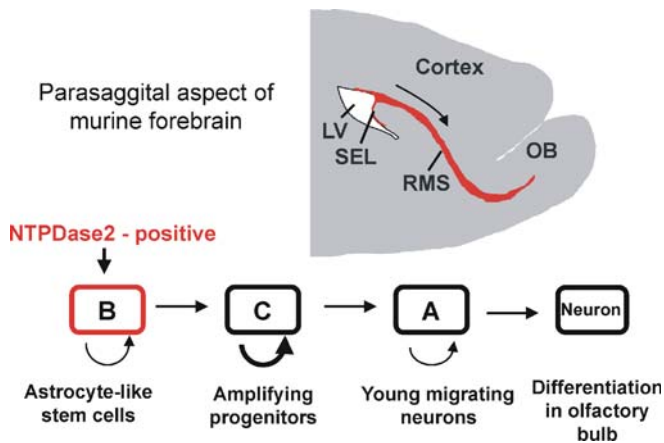
components laminin and fibronectin and may, in addition, mediate cellular adhesion [200]. Nerve growth factor and retinoic acid increase ecto-5'-nucleotidase expression [101, 150]. Wnt and  $\beta$ -catenin signaling target the expression of ecto-5'-nucleotidase and increase extracellular adenosine formation [171]. Wnt signaling is essential for neural development at various stages and also plays a role in axon guidance and neurite outgrowth [90].

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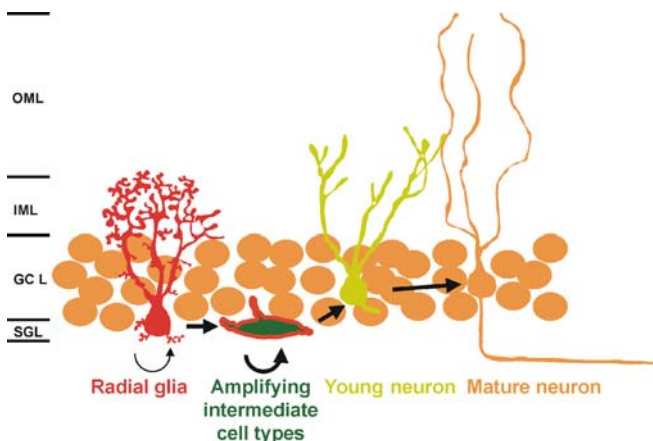
### Neural progenitors

In the mammalian nervous system, neurogenesis occurs predominantly during embryogenesis whereas glial cells are prevalently generated after birth. During prenatal and early postnatal stages, neurogenesis takes place in two actively proliferating zones, the ventricular zone (VZ) and the subventricular zone (SVZ), two relatively thin layers lining the primitive ventricular cavities [144]. After birth, these layers progressively disappear, leaving the mature nervous tissue in contact with a continuous, ventricle-lining cell monolayer, the ependyma. Both neurons and glia continue to be generated in restricted adult forebrain structures, including the subependymal layer (SEL) (or adult SVZ) of the lateral walls of the lateral ventricles [7] and the subgranular layer (SGL) of the dentate gyrus of the hippocampus [98].

The SEL and its rostral extension, originating after the occlusion of the primitive olfactory ventricle, generate neuroblasts that migrate towards the olfactory bulb (OB), constituting the rostral migratory stream (RMS) (Fig. 4). Within the RMS, the neuroblasts continue to divide and move as a network of tightly associated chains of cells ensheathed by tubes of slowly proliferating astrocyte-like cells [111, 113]. Upon reaching the OB, the neuroblasts move radially and differentiate into granular or periglomerular interneurons [111]. Within the adult SEL, three principal, closely adjacent cell types are distinguished morphologically and functionally. The glial fibrillary acidic protein- (GFAP) and nestin-expressing protoplasmic astrocyte-like type-B cells are thought to represent (at least in part) the actual stem cells. They give rise to transit-amplifying progenitors (type-C cells) that generate type-A cells, representing the neuroblasts migrating to the OB [70]. For comparison, in the hippocampus, radial glia-like cells (residual radial glia) with their cell body in the SGL are considered to function as progenitors and maintain their neurogenic potential into adulthood (Fig. 5). The GFAP- and nestin-expressing residual radial glial elements proliferate and become transformed into neurons via transit-amplifying cells [98, 173]. Cells in the transition state start to express markers of neuronal differentiation and migrate into the granule cell layer where they differentiate into interneurons [53]. There is now increasing evidence for an association of distinct functional purinergic signaling pathways with embryonic and adult neural progenitors.



**Fig. 4** Stage-specific cellular expression of NTPDase2 during adult neurogenesis in the subependymal layer (SEL) of the rodent lateral ventricles. *Top* Parasagittal section through a murine forebrain, depicting a lateral ventricle (LV) with the SEL and the corresponding rostral migratory stream (RMS), extending from the SEL into the olfactory bulb (OB). *Bottom* The nucleoside triphosphate-hydrolyzing enzyme NTPDase2 is selectively associated with the slowly proliferating type-B cells considered to represent (at least in part) adult neural stem cells. The enzyme becomes downregulated on formation of the highly proliferating type-C cells and is also absent from migrating neuroblasts (type-A cells) and mature interneurons that are formed after migration of type-A cells into the OB. Within the RMS, NTPDase2 is associated with astrocyte-like cells ensheathing the migrating neuroblasts but it is absent from cells radially migrating out from the RMS within the OB (according to [117])



**Fig. 5** Cellular association of NTPDase2 in the neurogenic adult mouse dentate gyrus. NTPDase2 is expressed by slowly proliferating residual radial glia considered to represent the neurogenic progenitors. The cell body of this cell type is situated in the subgranular layer (SGL). It extends strong radial processes throughout the granule cell layer (GCL) and forms bushy arborizations within the inner molecular layer (IML). NTPDase2 is still associated with highly multiplying intermediate cell types [166] that exhibit a horizontal extension in the SGL. Expression of NTPDase2 ceases when young neurons migrate into the GCL and differentiate into mature neurons, with long dendrites spanning the outer molecular layer (OML) and an axon innervating CA3 pyramidal neurons (according to [168])

#### Association of ectonucleotidase activity with stem cells/progenitor cells

NTPDase2 is the dominant ectonucleotidase expressed by mammalian progenitors in the late embryonic and adult murine brain [24]. The enzyme reveals a high preference for the hydrolysis of nucleoside triphosphates over nucleoside diphosphates. The enzyme thus not only inactivates ligands for nucleoside triphosphate-sensitive receptors but also generates ligands for nucleoside diphosphate-sensitive receptors. In the rat and mouse brain, NTPDase2 is first immunodetected at E18 and E17, respectively. It gradually becomes expressed along the ventricular and subventricular zone of the brain, followed by retraction to the adult expression pattern at P21. In the adult brain, NTPDase2 is selectively expressed by the GFAP, polysialylated neural cell adhesion molecule- (PSA-NCAM) and S-100-positive stem cells (type-B cells) of the SEL and the RMS (Fig. 4). It is also associated with select tanycytes of the third ventricle and by subpial astrocytes. It is interesting to note that neurogenesis has recently also been allocated to the ventricular layer of the adult third ventricle [197]. Furthermore, NTPDase2 selectively marks postnatal cortical radial glia [168]. Radial glia is considered to represent the major source of neurons during development [73]. Cortical immunostaining for NTPDase2 ceases after P5, a state when radial glia transforms into astrocytes [118].

In addition, NTPDase2 is selectively associated with progenitor cells of the mouse dentate gyrus, beginning with the early migration of progenitor cells into the dentate gyrus anlage during embryonic development [168]. The embryonic pattern of expression mirrors that of the dentate migration of neuroblasts. By P23, the immunostaining corresponds to the adult stage. At that stage, the NTPDase2-positive cells correspond to the “residual radial glia”, the presumptive adult progenitors of hippocampal granule neurons. Double immunolabeling of the adult dentate gyrus revealed that NTPDase2 is associated with subpopulations of GFAP-, nestin-, and doublecortin-positive cells and also with the amplifying horizontal D cells [166] (Fig. 5). NTPDase2 is absent from mature granule cells and S100-positive astrocytes. NTPDase2-positive cells proliferate and postmitotic cells preferentially acquire an NTPDase2-positive phenotype.

#### Association of purinergic receptors with stem cells/progenitor cells

Neural stem cells can successfully be cultured in vitro as floating cell aggregates, referred to as neurospheres. Neurospheres cultured from the adult SEL in the presence of epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF-2) contain multipotent precursors with the characteristics of neural stem cells and the ability to generate neurons, astrocytes, and oligodendrocytes [76, 149]. Neurospheres derived from the adult mouse SEL express the metabotropic P2Y<sub>1</sub> and P2Y<sub>2</sub> nucleotide receptors, resulting in the generation of rapid Ca<sup>2+</sup>



transients after the application of the agonists ATP, ADP, or UTP [117]. Agonists of these receptors and also low concentrations of adenosine augmented cell proliferation in the presence of growth factors, inferring nucleotide receptor- and growth factor receptor-mediated synergism with progenitor cell proliferation. Growth factor-stimulated neurosphere cell proliferation was attenuated after application of the P2Y<sub>1</sub> receptor antagonist MRS2179 and in neurospheres from P2Y<sub>1</sub> receptor knockout mice. These results suggest that nucleotides may be constitutively released from neurosphere cells and exert a synergistic P2 receptor-mediated effect on growth factor-mediated cell proliferation. Furthermore, neurospheres expressed the ectonucleotidases NTPDase2 and TNAP and hydrolyzed extracellular ATP to adenosine. While the data suggest that *in vitro* progenitors can directly be targeted by nucleotides, the cellular allocation of nucleotide receptors in the SEL is less defined. Using *in situ* hybridization, the P2Y<sub>1</sub> receptor could be allocated not only to striatal neurons but also to select clusters of cells in the SEL [117].

Nucleotides activate hippocampal progenitors *in situ*. In acute slices, the neural progenitors in the adult dentate gyrus of the hippocampus react with an inward current to application of ATP, implicating the expression of P2X receptors [168]. P2X receptor-mediated inward currents were also detected on individual cultured and undifferentiated adult hippocampal progenitors [82].

There is increasing evidence for a functional role of purinergic signaling pathways also in embryonic neurogenesis. Ntera-2/D1 (NT-2) cells, a pluripotent human embryonal carcinoma-derived clonal cell line, express functional P2Y<sub>1</sub> receptors resulting in an ATP- or ADP-mediated increase in intracellular Ca<sup>2+</sup> [120]. P2Y<sub>1</sub> receptor-mediated Ca<sup>2+</sup> transients could be evoked in cells (originally obtained from the embryonic striatum, E14) of connexin43-null mice that had migrated out from neurospheres shifted to growth factor-free medium [156]. In addition, progenitor migration and proliferation may be affected by the P2Y<sub>1</sub> receptor. ATP was found to induce an elevation of cytosolic Ca<sup>2+</sup> levels and proliferation of precursor cells cultured from immortalized human stem cells derived from the embryonic telencephalon as well as from mouse embryonic and adult neurospheres [153, 181]. The functional impact of nucleotides in embryonic neurogenesis is further corroborated by the observation of P2Y<sub>1</sub> receptor-mediated Ca<sup>2+</sup> waves through radial glial cells in slices of the embryonic rat ventricular zone. Disrupting Ca<sup>2+</sup> waves between these neuronal progenitors reduced ventricular zone cell proliferation during the peak of embryonic neurogenesis [191]. Similarly, ATP stimulates proliferation of neural, retinal progenitor cells in the embryonic chicken retina [143, 179]. Recent investigations using acute slices of postnatal mice further revealed the presence of ATP-induced inward currents in precursors in the SVZ and in particular in the RMS [23].

## Neurite outgrowth

Whereas coactivation of P2Y receptors and EGF leads to enhanced cell proliferation, it appears that NGF and P2Y receptor coactivation preferentially results in neurite outgrowth. The effects of nucleotides vary, however, between cell type or cell line investigated. In neural tube explants derived from E12 rat embryos, ATP (but not UTP) had an inhibitory effect on motor axon outgrowth, most likely via the P2X<sub>3</sub> receptor [40]. In contrast, ATP, several ATP analogues, and UTP were shown to enhance NGF-mediated survival and neurite outgrowth from pheochromocytoma (PC12) cells [16, 45, 46, 145] and also from cultured neonatal dorsal root ganglion neurons, apparently involving P2Y<sub>2</sub> receptor activation [9]. In accordance, P2 receptor antagonists prevented NGF-dependent neurogenesis [44]. It is interesting to note that a similar stimulatory effect on PC12 neuritogenesis has been described for guanosine and GTP [77, 78] for which no cell surface-located receptors are known to date. The effect of GTP was not mediated by P2 receptors.

In dibutyryl-cAMP-differentiated human neuroblastoma SH-SY5Y cells, a model system to study neuronal differentiation *in vitro*, UTP acting via the P2Y<sub>4</sub> receptor increased the contribution of neurite-bearing cells. Transient transfection with the P2Y<sub>4</sub> receptor facilitated neurogenesis. This was accompanied by an increased transcription of immediate early genes linked to differentiation. However, prolonged exposure to UTP induced cell death [38]. In addition, activation of adenosine A<sub>1</sub> and A<sub>2A</sub> receptors induced neurogenesis in SH-SY5Y cells in a nonsynergistic manner, apparently via differential intracellular signaling pathways [36]. A similar effect was observed with primary cultures of striatal neurons. 2-Chloroadenosine increased cell number and neurite length in cultured myenteric neurons and synergistically enhanced neurite outgrowth with FGF-2 [157]. In accordance, culturing of PC12 cells on an ecto-5'-nucleotidase substratum stimulated neurite extension of PC12 cells [81]. Inhibition of extracellular adenosine formation by inhibition of ecto-5'-nucleotidase activity via selective antibodies or by knockdown of the enzyme protein via antisense oligonucleotides inhibited neurite formation in PC12 cells and in cultured cerebellar granule neurons [80]. In contrast, A1 receptor activation was found to inhibit NGF-induced neurite outgrowth in PC12 cells and cultured cortical and hippocampal neurons [180].

There may be additional impacts of nucleotides on the interaction of neurons and on neurite outgrowth. Extracellular ATP binds to (and can be hydrolyzed by) the neural cell adhesion molecule NCAM. Neurite outgrowth from hippocampal neurons (prepared from embryonic E17–19 rats) allowing NCAM homophilic interactions was inhibited by high concentrations of ATP [169]. Autotaxin/NPP2 induced neurite retraction of PC12 cells, an effect possibly produced by its lysophospholipase D rather than by its ectonucleotidase activity [155].

## Developing glia

### Astroglia and retinal Müller cells

Multiple investigations revealed the presence of P2 and P1 receptors on all types of glial cells. During the neurogenic phase of the cerebral cortex, radial glia is considered as the relevant stem cell, generating most of the pyramidal neurons [103, 137]. Towards the end of neurogenesis, the cortical progenitor switches back to symmetric division and gives rise to astrocytes. Astrocytes, generally cultured from postnatal brain, express a wide array of P2X and P2Y receptors, and nucleotides and nucleosides have important roles in the proliferation and differentiation of these cells [63, 67, 93, 100, 132, 142]. As for other cell types, the possibility needs to be considered that the functional properties of cultured cells differ from the *in situ* situation. But nucleotide-mediated astrocyte proliferation and differentiation were demonstrated *in situ* [65]. A single cell PCR of astrocytes acutely isolated from the hippocampal CA1 region of P8-12 rats revealed the mRNA of P2Y<sub>1</sub>, P2Y<sub>2</sub>, or P2Y<sub>4</sub> receptors [199]. In cultured astrocytes, ATP or UTP increased GFAP expression and chemotactic and chemokinetic cell migration [186], and induced intracellular Ca<sup>2+</sup> transients and proliferation [130]. Astrocytes share this property with the astrocyte-like stem cells of the embryonic and adult nervous system (see above). For comparison, elevated concentrations of adenosine induce astrocyte apoptosis [51]. Differentiation of Müller cells from radial glial progenitors in the developing rabbit retina is accompanied by a decreasing capability to respond to ATP with an increase in intracellular Ca<sup>2+</sup>. Whereas postnatally most of the cells respond, the response is minor regarding cell number and amplitude in the adult retina [183].

### Oligodendroglia

Oligodendrocytes tend to arise from specific locations within the CNS, in particular the ventral region of the neural tube, and migrate into the white matter of the CNS to myelinate axons [136]. Oligodendrocytes are generated by the proliferation and differentiation of oligodendrocyte progenitors (OPs) [75]. There are multiple studies on the modulation of oligodendrocyte function by nucleotides and nucleosides [4, 5]. The effect of nucleotides and nucleosides on cultured OPs varies between investigations, possibly as a result of variations in culture conditions or source of cells. OPs cultured from the forebrain of P1 rats expressed P2X<sub>7</sub> and P2Y<sub>1</sub> as the main ionotropic and metabotropic nucleotide receptors, respectively [4]. Their activation induced intracellular Ca<sup>2+</sup> transients. P2Y<sub>1</sub> receptors were also expressed in NG2-positive OPs in P4 cerebral cortex and in the cerebellar granule cell layer. P2Y receptor activation stimulated OP random motility and to a lesser extent chemotaxis, inhibited the mitogenic response of OPs to platelet-derived growth factor (PDGF) in both purified and organotypic cultures, and promoted OP differentiation. Migration and proliferation was also

inhibited by adenosine. Other studies invoke a significant stimulation of OP migration by an A1 adenosine receptor agonist [141].

It is interesting to note that the release of nucleotides and nucleosides from electrically active axons was shown to regulate proliferation and oligodendrocyte development. OP cells cultured from embryonic rat brain were found to express adenosine receptors that were activated in response to action potential firing. Adenosine inhibited OP cell proliferation and promoted the formation of myelin [176]. Adenosine thus appears to be a primary activity-dependent signal promoting the differentiation of premyelinating progenitor cells into myelinating oligodendrocytes.

### Schwann cells

Most Schwann cells develop from the neural crest. Their generation involves the formation of two intermediates, the Schwann cell precursor and the immature Schwann cells, originating during later embryonic development. In mature nerves, individual large caliber axons are ensheathed by myelinating Schwann cells, whereas bundles of small caliber axons are loosely ensheathed by the nonmyelinating Schwann cells [96], whose sensitivity to purinergic stimulation differs [112]. Similar to oligodendrocytes, ATP and adenosine were found to act as axonally derived signals mediating activity-dependent communication between neurons and premyelinating cultured mouse Schwann cells, but their effect differed [174, 175]. ATP was found to inhibit proliferation of cultured Schwann cells (in contrast to astrocytes that are stimulated, see above) and to arrest maturation before differentiation into either the myelinating or nonmyelinating phenotypes, thus preventing the formation of myelin. For comparison, adenosine also inhibited Schwann cell proliferation albeit through a different mechanism than ATP. It did not, however, inhibit Schwann cell differentiation and myelination [175].

### Microglia

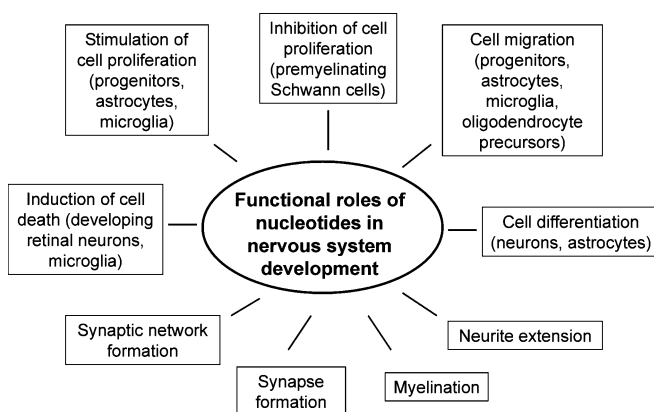
Microglial cells represent the immune effector cells of the CNS. They are derived from mesenchymal precursors that invade the brain during late embryogenesis and early postnatal periods. Several studies have identified P2X and P2Y receptors and a variety of functional responses in cultured microglia [18, 91]. These include eliciting of Ca<sup>2+</sup> transients, stimulating proliferation, inducing cell death via activation of P2X<sub>7</sub> receptors, or stimulating the release of plasminogen, IL-1 $\beta$ , or tumor necrosis factor. ATP was found to mediate rapid morphological responses in microglia to brain injury *in vivo* [47] and ATP or ADP induced chemotaxis via P2Y receptors in a microglial cell line [83]. A recent study has investigated the ontogenetic expression of the microglial P2X receptors (P2X<sub>1</sub>, P2X<sub>4</sub>, and P2X<sub>7</sub>) by immunocytochemistry in the rat brain [194]. These receptors were identified on microglial cells from late E16. The expression of individual P2X receptors varied

between microglial subtype or functional state and tissue location. At P30, microglial cells with P2X<sub>1</sub> receptors had disappeared and microglial cells with P2X<sub>7</sub> receptor-immunoreactivity were found to be widely distributed in the forebrain, suggesting that the P2X<sub>7</sub> receptor governs the actions of ATP in the resting microglial cells.

## Summary and outlook

Signaling pathways employing extracellular nucleotides and adenosine are expressed early on during embryonic development of the nervous system. The transient expression of defined receptor subtypes and of ectonucleotidases implies that nucleotides and nucleosides affect stage-specific developmental processes. Yet, much more needs to be learned concerning the developmental onset of receptor function and ectonucleotidase activity in specific cellular settings. In several cases, the transient expression of P2 receptors suggests that nucleotidergic signaling is of even greater functional significance during development than in the adult nervous system. There is increasing evidence that purinergic signaling has a direct impact on progenitor cell proliferation, cell migration, neuronal and glial cellular interaction and differentiation, and synaptic network formation (Fig. 6).

Very little is presently known concerning sites, cell types, and mechanisms of nucleotide release in the various developmental settings. While it appears possible that ubiquitous and constitutive nucleotide release becomes relevant only when the appropriate receptors are expressed, it is likely that regulated release also plays a significant role. It is now becoming clear that nucleotide receptors can induce a multiplicity of cellular signaling pathways and are involved in multiple molecular interactions. A fascinating possibility concerns the congruence and synergistic interactions of multiple signaling pathways such as those of growth factors, cytokines, and purines/pyrimidines. Preconditioning via one pathway may increase the impact of another. Thus, analyzing the functional role of single signaling pathways on defined developmental processes



**Fig. 6** Overview of developmental processes known to be targeted by nucleotides

may fall short of identifying the relevance of multiple and interactive signaling mechanisms in situ.

It can be anticipated that the focus on nucleotide signaling in nervous system development will rapidly broaden. Novel technical approaches are going to facilitate the investigations. These include not only the analysis of knockouts of specific components of the nucleotide signaling pathways but also the knockdown of individual proteins by cell-type specific viral transfection and RNA interference, or the analysis of animals in which the encoding gene can be inactivated or selectively induced in specific tissues by genetic approaches. Transgenic mice expressing fluorescent protein under the promoter of the respective receptor or ectonucleotidase will greatly facilitate the identification of the expression pattern of individual proteins during development. Purines may have several functional roles; they may act as direct functional switches or as mutual modulators or enhancers of signaling pathways mediated by other agonists. The examples provided show that nucleotides possess a broad potential to control multiple developmental cues, alone or together with other signaling substances.

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