GLIA 48:185–196 (2004)

Synaptic and Extrasynaptic Neurotransmitter Receptors in Glial Precursors' Quest for Identity

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KEY WORDS neural progenitors; channels; precursor cells; electrophysiological phenotype; development

ABSTRACT It is widely established that neurotransmitter receptors are expressed in non-neuronal cells, and particularly in neural progenitor cells in the postnatal central nervous system. The functional role of these receptors during development is unclear, but it needs to be revisited now that cells previously considered restricted to glial lineages have been shown to generate neurons. The present review integrates recent advances, to shed new light on how neurotransmitter receptors may, alternatively, serve as excitable mediators of neuron-glia and neuron-neuroblast interactions.

INTRODUCTION

It is established that neurotransmitters are present and surround neural cells during brain maturation (Miranda-Contreras et al., 1998, 1999, 2000), and functional ligand-gated ion channels (i.e., ionotropic receptors) are expressed in neuronal precursor cells before the establishment of cortical and subcortical synapses (LoTurco et al., 1995; Ma and Barker, 1995; Serafini et al., 1995; Flint et al., 1998; Haydar et al., 2000). Many experimental reports indicate that the interaction between neurotransmitters and their receptors could play an essential role during CNS development, far before they become directly involved in synaptic transmission (Barker et al., 1998; Cameron et al., 1998a; Nguyen et al., 2001). Distinct neurotransmitter-activated ionotropic receptors appear to regulate a variety of processes at specific stages of neuronal development (LoTurco et al., 1995; Haydar et al., 2000; Ben Ari, 2002; Nguyen et al., 2003a). However, the precise role of neurotransmitter receptor-mediated signaling during neural differentiation in vivo remains largely unknown, and most loss-of-function studies have failed to disclose obvious developmental defects. Hence, there might be large redundancies between functions of distinct neurotransmitter receptor subunits, as surrogate mechanisms compensate specific ionotropic receptor deficiencies during neural development.

We and others (Patneau et al., 1994; Steinhauser and Gallo, 1996; Belachew et al., 1998a,1998b, 2000; Gallo and Ghiani, 2000; Atluri et al., 2001; Rogers et al., 2001; Nguyen et al., 2001, 2002, 2003a) have recently demonstrated that type A γ-aminobutyric acid ($GABA_A$), AMPA/kainate, glycine, and nicotinic acetylcholine ionotropic receptors are also expressed in glial progenitors, particularly in oligodendrocyte progenitor cells (OPCs). Until very recently, extrasynaptic neurotransmitter receptors expressed in immature glial progenitor cells have been considered to be transducers of putative neurotransmitter-mediated neuron-glia interactions (Fields and Stevens-Graham, 2002). However, we and other investigators have now demonstrated that OPCs represent a subset of bipotential neuron-oligodendrocyte progenitor cells in specific locations of the postnatal brain (Belachew et al., 2003; Nunes et al.,

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Received 29 March 2004; Accepted 28 April 2004

DOI 10.1002/glia.20077

Published online 16 June 2004 in Wiley InterScience (www.interscience.wiley.com).

2003; Aguirre et al., 2004). It is possible that neurotransmitter-receptor interactions may regulate the development of these precursor cells. In the present review, we shall discuss the hypotheses that (1) neurotransmitter receptor expression by postnatal glial progenitors in neurogenic regions might modulate their neurogenic potential, and (2) these receptors represent instructive signaling pathways that may be involved in the regulation of specific stages of neuronal differentiation. Are neurotransmitter receptors constitutively expressed in postnatal glial progenitors that retain the ability to give rise to neurons in the appropriate extracellular environment? We will summarize the available evidence demonstrating neurotransmitter receptor expression in OPCs and in other glial precursors, to discuss whether these receptors might mediate developmental cues that regulate glial proliferation and differentiation, and/or that instruct terminal neuronal maturation.

EXPRESSION OF NEUROTRANSMITTER RECEPTORS AND VOLTAGE-GATED ION CHANNELS IN POSTNATAL NEURAL PRECURSOR CELLS

Current experimental evidence demonstrating that neurotransmitter receptors are expressed in neuron-committed and multipotent or glia-oriented progenitor cells is comprehensively summarized in Tables 1 and 2, respectively. These tables are restricted to ionotropic receptors studied in postnatal neural tissue.

Focus on a Newcomer: Glycine Receptors in Perinatal CNS Precursor Cells

Strychnine-sensitive glycine receptor chloride channels (GlyRs) are members of the nicotinic ligand-gated ion channel family (Le Novere and Changeux, 2001). They mediate postsynaptic inhibition by increasing the chloride permeability of the postsynaptic cell membrane in adult spinal cord, brainstem, and some higher brain regions (Betz, 1991; Kuhse et al., 1995; Schofield et al., 1996). GlyR is a pentameric transmembrane protein composed of glycosylated integral transmembrane α-(48-kDa) and β-subunits (58-kDa) (Schofield et al., 1996). Four α-subunit variants (α1, α2, α3, and α4) (Kuhse et al., 1990; Matzenbach et al., 1994) and one β-subunit variant (Schofield et al., 1996) have been described so far. In newborn rats, the GlyR is α2-homomeric and differs from the adult stoichiometry, which is classically made of three α1- and two β-subunits (Langosch et al., 1988). In contrast to the adult brain, where glycine is inhibitory, the activation of GlyR in immature neural cells causes excitation that results from a depolarized chloride equilibrium potential in these cells during their early developmental period (Ben-Ari, 2002). GlyR-mediated depolarization of immature neurons induces a calcium entry through voltage-gated calcium channels (VGCC) (Reichling et al., 1994; Flint et al., 1998), which appears to modulate cell proliferation, migration, and differentiation in neural precursors (Ben Ari, 2001, 2002; Nguyen et al., 2001) and, later, maturation of fully differentiated neurons bearing inhibitory synapses (Legendre, 2001; Moss and Smart, 2001).

We recently demonstrated that GlyRs are expressed in three distinct types of early postnatal neural precursor cells in vitro (Belachew et al., 1998b, 2000; Nguyen et al., 2002, 2004). Functional GlyRs have been described in newborn rat astrocytes, oligodendrocytes, and OPCs in spinal cord slices (Pastor et al., 1995; Kirchhoff et al., 1996; Belachew et al., 1998a). During oligodendroglial lineage progression, GlyR expression (α2- and β-subunits) culminates at the OPC stage and decreases thereafter (Belachew et al., 1998b). We demonstrated that glycine-induced chloride currents recorded from newborn rat cortical oligosphere-derived OPCs are mediated by GlyRs that seem to be not only pharmacologically, but also structurally distinct from the homologous neuronal receptor (Belachew et al., 1998b). Furthermore, glycine application on cultured OPCs induces a rise of $[Ca^{2+}]_i$ via the opening of VGCC. This effect is mediated by a depolarization related to the concurrent activation of GlyR and Na^+-dependent glycine transporters GLYT1 and/or GLYT2 (Belachew et al., 2000).

Using the classical neurosphere culture model from early postnatal striatum (Reynolds et al., 1992; Reynolds and Weiss, 1992), we also demonstrated the expression of GlyR α1-, α2- and β genes, and could detect GlyRα subunit proteins in multipotent nestin-expressing stem/progenitor cells (Nguyen et al., 2002). Consistent with these findings, we observed that, in these multipotent progenitor cells, GlyR subunits forms functional chloride channels gated by glycine and blocked by the GlyR antagonists strychnine and picrotoxin (Nguyen et al., 2002).

Similarly, immunocytochemical analysis showed that GlyRs are expressed in PSA-$NCAM^+$ neurogenic progenitors from perinatal rat striatum both in vitro and in situ (Nguyen et al., 2004). Glycine triggers inward strychnine-sensitive currents in most of these neurogenic striatal precursors (Nguyen et al., 2004). Finally, neurogenic PSA-$NCAM^+$ progenitors from the perinatal striatum express GlyRs that display electrophysiological characteristics (Hill coefficient and EC_{50} mean effective concentration) intermediate between nestin-expressing neural stem cells (Nguyen et al., 2002) and mature interneurons (Sergeeva, 1998; Sergeeva and Haas, 2001).

Consistent with the role of GlyR α2 subunit during retinal development (Young and Cepko, 2004), GlyRs and their agonists, in concert with other neurotransmitter receptors and voltage-gated ion channels, may

TABLE 1. Ionotropic Neurotransmitter Receptors Expressed in Neuron-Committed Precursor Cells

Age	Receptor	Progenitor cell phenotype	Model	Species	Results and Effects of NT	References
			GABA			
P6–P8	$GABA_A$	Cerebellar granule cell progenitors	In vitro cell culture	Rat	MAPK-dependent ↑ proliferation	Fiszman et al., 1999
P6–P8	$GABA_A$	Immature cerebellar granule cells	In vitro cell culture	Rat	Ca^{2+}-dependent ↓ of neurite outgrowth	Borodinsky et al., 2003
P0–P3	$GABA_A$	Striatal PSA–$NCAM^+$ precursors	In vitro cell culture and slices	Rat	MAPK- and Ca^{2+}-dependent ↓ of proliferation	Nguyen et al., 2003a
P13–P70	$GABA_A$	anterior SVZ-RMS-olfactory bulb neuronal precursors	Ex vivo tissue slices	Mice	Neuronal progenitors of the SVZ/RMS/OB contain GABA and are depolarized by GABA-mediated activation of first extrasynaptic and then later synaptic $GABA_A$ receptors	Belluzzi et al., 2003; Carleton et al., 2003; Gallo and Haydar, 2003; Wang et al., 2003
P0–P1	$GABA_A$	Anterior SVZ neuronal precursors	In vitro cell culture	Rat	SVZa neuronal precursor cells are GABAergic and express functional $GABA_A$ receptors	Stewart et al., 2002
			Glutamate			
P0–P3	ND	Striatal PSA-$NCAM^+$ precursors	In vitro cell culture	Rat	Glutamate induces inward currents	Nguyen et al., 2003b
P0–adult	NMDA	Dentate gyrus progenitor cells	In vivo	Rat	Blockade of NMDA receptor increases cell death and [^{3}H]thymidine incorporation, particularly in granule cells	Cameron et al., 1995, Cameron et al., 1998b; Gould et al., 1994
P7–P11	NMDA	Immature cerebellar granule cells	In vitro and in vivo	Rat	Activation of NMDA receptor ↑ survival	Balazs et al., 1988a, 1988b; Monti et al., 2002; Monti and Contestabile, 2000
P70	AMPA	Tangentially migrating olfactory bulb neuroblasts	Ex vivo tissue slices	Mice	Expression of first extrasynaptic and later synaptic AMPA receptors	Carleton et al., 2003
P70	NMDA	Radially migrating olfactory bulb neuroblasts	Ex vivo tissue slices	Mice	Expression of extrasynaptic functional NMDA receptors	Carleton et al., 2003
			Glycine			
P0–P3	GlyR	Striatal PSA-$NCAM^+$ precursors	In vitro cell culture	Rat	Functional GlyR expressed in these neonatal neuronal precursor cells	Nguyen et al., 2004
			Acetylcholine			
P0–P3	ND	Striatal PSA-$NCAM^+$ precursors	In vitro cell culture	Rat	Acetylcholine induces inward currents	Nguyen et al., 2003b
P1–P3	Nicotinic (α7) AChR	Olfactory bulb neuronal precursors	In vitro cell culture	Rat	Activation of nAChR ↑ neurite outgrowth	Coronas et al., 2000
P5	Nicotinic (α3) AChR	Cerebellar external granule layer (EGL) neuronal precursors	In vitro cell culture	Mouse	Activation of nAChR ↑ proliferation and survival in EGL neuroblasts	Opanashuk et al., 2001

also control the development of postnatal neural precursors, i.e. (1) cell cycle kinetics in different types of immature progenitors; (2) cell fate choice and differentiation from nestin-expressing stem cells, (3) neurogenesis from PSA-$NCAM^+$ neuronal precursors, and (4) maturation of OPCs into myelinating cells.

OPCs Express Not Only Neurotransmitter Receptors, But Also Voltage-Gated Potassium Channels

Similar to the widespread array of neurotransmitter receptors that are displayed by CNS precursor cells, voltage-gated ion channels, and in particular voltage-

TABLE 2. Ionotropic Neurotransmitter Receptors Expressed in Multipotent and Glia-Oriented Precursor Cells

Age	Receptor	Progenitor cell phenotype	Model	Species	Results and effects of NT	References
				GABA		
Adult	$GABA_A$	Astrocytes and astrocyte precursors from and hippocampus	In vitro tissue slices	Rat	GABA induces depolarizing chloride-mediated inward currents	MacVicar et al., 1989
P0–P3	$GABA_A$	Spinal cord and cerebral OPCs	In vitro cell culture and slices	Rat and moketteruse	GABA induces depolarizing chloride-mediated inward currents and ↑ $[Ca^{2+}]$:	Belachew et al., 1998a; Gilbert et al., 1984; Kettenmann et al., 1984b, Kirchhoff and Kettenmann, 1992, Pastor et al., 1995
				Glutamate		
P1	AMPA and kainate	Whole brain and hippocampus	In vitro cell culture and slices	Rat	Ca^{2+}-permeable functional AMPA/kainate receptor expression; their activation induces cell depolarization	Bowman and Kimelberg, 1984; Jabs et al., 1994; Kettenmann et al., 1984a; Porter and McCarthy, 1995; Sontheimer et al., 1988; Steinhauser et al., 1994
Juvenile adult	AMPA and kainate	Hippocampus	In vitro tissue slices	Mouse	Glial inwardly rectifying K^+ channels (Kir) are rapidly blocked upon activation of AMPA/kainate-type glutamate receptors, most probably due a receptor-mediated influx of Na^+, which plugs the channels from the intracellular side	Jabs et al., 1994; Robert and Magistretti, 1997; Schroder et al., 2002
P1	AMPA and kainate	Cortical astrocytes and mixed glial cells from optic nerve	In vitro cell culture and slices	Rat	Glutamate receptor-mediated regulation of immediate early gene expression (e.g., c-fos, c-jun, jun-B)	Mack et al., 1994; McNaughton and Hunt, 1992
P1	AMPA and kainate	Cortical and optic nerve OPCs	In vitro cell culture	Rat	Functional AMPA and kainate-specific subtypes of glutamate receptors are expressed by OPCs	Barres et al., 1990; Gallo et al., 1994a,1994b; Jensen and Chiu 1993; Patneau et al., 1994
P1	AMPA and kainate	Cortical OPCs	In vitro cell culture and slices	Rat	AMPA/kainate receptor activation: (1) triggers Ca^{2+}-dependent early gene expression; (2) induces a blockade of voltage-dependent K^+ channels (Kvl) that is mediated by depolarization and Na^+ entry; (3) inhibits proliferation at G1/S transition by regulation of cyclinE/cdk2 complex activity	Belachew et al., 2002; Chittajallu et al., 2002; Gallo et al., 1996; Gallo and Ghiani, 2000; Ghiani et al., 1999a,1999b; Ghiani and Gallo, 2001; Knutson et al., 1997; Liu and Almazan, 1995; Pende et al., 1994, 1997
P1	AMPA	Cortical OPCs	In vitro cell culture	Rat	Overactivation of AMPA receptors causes apoptosis in oligodendrocyte progenitors through mechanisms involving Ca^{2+} influx, depletion of glutathione, and activation of JNK, calpain, and caspase-3	Liu et al., 2002
				Glycine		
P0–P3	GlyR	Nestin-expressing striatal stem cells	In vitro cell culture	Rat	Functional GlyR expressed in these stem cells	Nguyen et al., 2002
P0–P3	GlyR	Cortical OPCs	In vitro cell culture	Rat	Activation of functional GlyR induces depolarizing chloride-mediated inward currents and ↑ $[Ca^{2+}]$	Belachew et al., 1998, 2000
				Acetylcholine		
P1	Nicotinic (7) AChR	Hippocampal astrocytes	In vitro cell culture	Rat	Activation of nAChR ↑ intracellular calcium concentration	Sharma and Vijayaraghavan, 2001
P0–P3	Nicotinic AChR	Cortical OPCs	In vitro cell culture	Rat	Functional nAChR expressed by cultured OPCs	Belachew et al., 1998a
P1	Nicotinic AChR	OPCs from corpus callosum	Ex vivo and in vitro cell culture	Rat	Activation of nAChR ↑ intracellular calcium concentration; expression of 3, 4, 5, 7, 2, and 4 subunits.	Rogers et al., 2001

dependent K^+ (Kv) channels, are expressed not only in neuron-committed, but also in multipotent and glia-oriented precursor cells. The prototypical Kv channels have been divided into four families (Kv1–Kv4), based on sequence homology to single gene orthologues in *Drosophila* (name given in italics): Kv1 (*Shaker*), Kv2 (*Shab*), Kv3 (*Shaw*), and Kv4 (*Shal*) (Trimmer and Rhodes, 2004). Importantly, there is a direct correlation between the expression of delayed outward-rectifying voltage-gated K^+-currents (I_k) and the proliferative state of different types of cells (DeCoursey et al., 1984; Sobko et al., 1998). Furthermore, pharmacological block of such potassium channels interferes with cell proliferation (DeCoursey et al., 1984; Sontheimer et al., 1989; Barres et al., 1990; Berger et al., 1991; Kettenmann et al., 1991; Dubois and Rouzaire-Dubois, 1993; Wonderlin and Strobl, 1996; Knutson et al., 1997; MacFarlane and Sontheimer, 1997; Sobko et al., 1998). Proliferative OPCs express large I_k, whereas nondividing OPCs and mature oligodendrocytes display much smaller outward currents (Knutson et al., 1997). These large I_k currents occur in G1 phase of the OPC cell cycle and have been correlated to an RNA synthesis-dependent selective increase of expression of K^+ channel subunit proteins Kv1.3 and Kv1.5 (Gallo et al., 1996; Knutson et al., 1997; Ghiani et al., 1999b; Chittajallu et al., 2002).

The inhibition of outward K^+ channels by the blocker tetraethylammonium or by cell membrane depolarization with the Na^+ channel opener veratridine inhibits OPC proliferation (Knutson et al., 1997; Ghiani et al., 1999b). The use of subunit-specific pharmacological blockers first suggested that outward K^+ channels containing the Kv1.3 subunit play a critical role in cell cycle progression in proliferating OPCs (Chittajallu et al., 2002). We recently performed gain-of-function experiments providing evidence that overexpression of Kv1.3–1.4 increases OPC proliferation in the absence of mitogen, whereas Kv1.6 overexpression prevents mitogen-induced OP cell cycle progression (Vautier et al., 2004). Kv1.3–1.6 subunit overexpression does not interfere with oligodendrocyte differentiation (Vautier et al., 2004). This study demonstrates that the activity of potassium channels comprising distinct Kv1 subunit proteins not only correlates with specific stages of oligodendrocyte lineage progression, but directly controls oligodendroglial proliferation in mitogenic or in growth factor-free conditions.

ESTABLISHED ROLE OF NEUROTRANSMITTER RECEPTORS IN POSTNATAL CNS PRECURSOR CELL BIOLOGY

AMPA/Kainate Receptors: A Matter of Cell Cycle or Cell Death

In the postnatal and adult brain, glutamate is released from neurons (either synaptically or axonally) (Chiu and Kriegler, 1994; Bergles and Jahr, 1997) and reaches effective concentrations that can activate different classes of glutamate receptors (GluRs) in glia. This event can cause (1) modulation of transmitter uptake, thereby affecting termination of synaptic transmission (Vernadakis, 1996; Bergles and Jahr, 1997); (2) modulation of K^+ conductance, with consequences on the extracellular ionic environment (Muller et al., 1992; Vernadakis, 1996); and (3) release of neuroactive substances from glia that can feedback and modulate synaptic transmission in neurons (Araque et al., 1999).

Glutamate receptor function is not limited to mature glial cells, because during late embryonic and early postnatal development, astrocytes and oligodendrocytes arise from CNS precursor cells of the subventricular zone that also express glutamate-activated channels (LoTurco et al., 1995; Yuan et al., 1998). Glutamate activates ionotropic GluRs in OPCs in purified cultures or in tissue slices, to inhibit their proliferation and prevent differentiation (Gallo et al., 1996; Yuan et al., 1998). Furthermore, glutamate inhibits SVZ progenitor proliferation in cortical slices (Haydar et al., 2000). The antiproliferative effects of glutamate are Ca^{2+}-independent and arise from an increase in intracellular Na^+ and subsequent block of outward K^+ currents (Gallo et al., 1996; Knutson et al., 1997). Agonists specific for AMPA/kainate receptors or blockers of outward K^+ channels cause a reversible G1 cell cycle arrest in OPCs. This is associated with accumulation of the cyclin-dependent kinase inhibitors $p27^{Kip1}$ and $p21^{CIP1}$, selective decrease in cdk2 activity, and impairment of cyclin E-cdk2 complex formation (Ghiani et al., 1999a, 1999b; Ghiani and Gallo, 2001). Overexpression of cdk2 partially prevents G1 arrest induced by AMPA/kainate agonists or K^+ channel blockers (Belachew et al., 2002), indicating that regulation of cdk2 activity causally underlies cell cycle arrest. It still needs to be determined whether GluR activation interferes with cell cycle progression in CNS precursor cells from adult brain, and whether gray matter vs. white matter $NG2^+$ precursor cells express distinct receptor and channel subtypes with area-specific functions (see below).

Several laboratories have also demonstrated that AMPA/kainate receptor activation triggers excitotoxic cell death in cells of the oligodendroglial lineage, although most of this work was performed using cultures of postmitotic $O4^+$/$GalC^+$ pre-oligodendrocytes (Oka et al., 1993; Yoshioka et al., 1995; Matute et al., 1997; Sanchez-Gomez and Matute, 1999; Alberdi et al., 2002; Sanchez-Gomez et al., 2003). In contrast, it appears that both proliferating OPCs and mature myelinating oligodendrocytes are not sensitive to excitotoxic insults (Gallo et al., 1996; Rosenberg et al., 2003).

The mechanism of AMPA/kainate receptor-mediated excitotoxic lesions in white matter oligodendroglial cells in vivo remains a subject of intense debate (Matute, 1998; McDonald et al., 1998; Follett et al., 2000; Li and Stys, 2000; Matute et al., 2001; Pitt et al., 2000; Smith et al., 2000; Tekkok and Goldberg, 2001), since surrounding microglia (Noda et al., 2000) and astro-

cytes (Jabs et al., 1994; Verkhratsky and Steinhauser, 2000; Schroder et al., 2002; Matthias et al., 2003) also express such receptors, and therefore may be directly or indirectly involved in the excitotoxic cascade. It is important to mention that the only in situ electrophysiological demonstration of functional AMPA/kainate receptor expression in adult cells identified as OPCs (based on NG2 expression; see below) was not provided in the white matter, but in the hippocampus (Bergles et al., 2000). It is also of interest to note that the demonstration of excitotoxicity in adult white matter oligodendroglial cells is not by itself evidence for the presence of AMPA/kainate receptors in these cells. This issue can be better defined in future experiments by recording astrocytes and oligodendroglial cells from the adult white matter of transgenic mice expressing reporter genes under cell-specific promoters (Zhuo et al., 1997; Belachew et al., 2001; Nolte et al., 2001; Mallon et al., 2002; Yuan et al., 2002). In particular, in the case of demyelinating disorders, this approach will reveal whether excitotoxic GluRs overactivation, which occurs primarily or secondarily to auto-immune activation, triggers direct oligodendroglial and axonal damages, and/or modulates astroglial/microglial components of the inflammatory reaction, which would then cause the final toxic effects (Pitt et al., 2000; Smith et al., 2000; Matute et al., 2001).

$GABA_A$ Receptors

In the embryonic brain, the neurotransmitter γ-aminobutyric acid (GABA) partially prevents bFGF-induced proliferation of VZ progenitor cells (Antonopoulos et al., 1997). However, GABA was shown to increase the number of proliferating cells and to accelerate cell cycle kinetics in VZ progenitors analyzed in organotypic cultures under basal conditions (LoTurco et al., 1995; Haydar et al., 2000). In contrast, GABA decreases SVZ progenitor cell proliferation (Haydar et al., 2000). It appears, therefore, that neural progenitors in the SVZ display a differential physiological response to GABA, as compared with VZ progenitors, indicating that neural progenitors can rapidly modulate their sensitivity to the inhibitory neurotransmitter as development proceeds.

Consistent with these prior prenatal studies, GABA was found to inhibit cell proliferation in the postnatal SVZ. We recently analyzed the expression and function of $GABA_A$ receptors ($GABA_A$Rs) in SVZ/striatal neural precursor cells during postnatal development. We developed an isolation procedure that allowed us to purify in a buoyant density gradient proliferative neural precursor cells from early postnatal rat striatum and adjacent SVZ, based on their isopyknic properties. These cells expressed the polysialylated form of the neural cell adhesion molecule (PSA-NCAM) (Nguyen et al., 2003a). We investigated whether GABA might control the proliferation of these postnatal PSA-$NCAM^+$ precursors and reported that (1) epidermal growth factor (EGF)-responsive PSA-$NCAM^+$ precursors generate neurospheres mostly committed to a neuronal fate; (2) postnatal striatal/SVZ PSA-$NCAM^+$ precursors express functional $GABA_A$Rs and the GABA-synthesizing enzymes glutamate decarboxylase (GAD)-65 and GAD-67 in vitro and in situ ; (3) proliferation of PSA-$NCAM^+$ precursors is inhibited by GABA produced by these cells under the control of EGF; and (4) $GABA_A$R-dependent inhibition of PSA-$NCAM^+$ cell proliferation is mediated by a complex intracellular signaling involving an increase of intracellular calcium concentration by opening of voltage-gated calcium channels and inhibition of the mitogen-activated protein kinase (MAPK) pathway (Nguyen et al., 2003a). The demonstration that early postnatal PSA-$NCAM^+$ striatal/SVZ neuronal precursors synthesized and released GABA suggests that an autocrine/paracrine GABA/$GABA_A$R-mediated mechanism might regulate EGF-driven proliferation of these progenitors.

Similarly, receptor activation by exogenous application of $GABA_A$ agonists to perinatal OPCs in cultured organotypic cerebellar slices also inhibited proliferation of these precursor cells (Yuan et al., 1998). However, treatment of the slices with the $GABA_A$ receptor antagonist bicuculline did not cause any effect (Yuan et al., 1998), suggesting that, at least in cerebellum, endogenous activation of $GABA_A$ receptors is not a major mechanism of control of cell cycle progression in postnatal OPCs in vivo.

Altogether, these findings strongly indicate that $GABA_A$ receptors may be directly involved in the regulation of proliferation and differentiation of perinatal precursor cells. Like early postnatal cells, also adult (P13–P70) neuronal progenitors of the SVZ/RMS/olfactory bulb pathway synthesize GABA and are depolarized by activation of first extrasynaptic and later synaptic $GABA_A$ receptors (Carleton et al., 2003; Gallo and Haydar, 2003; Wang et al., 2003). Thus, $GABA_A$ receptor-mediated signaling might be part of the complex array of extrinsic signals that regulate adult neurogenesis.

SYNAPTIC INPUTS ON NEURAL PROGENITORS: NEURON-GLIA OR NEURON-NEUROBLAST SIGNALING?

NG2-Expressing Progenitor

In recent studies, Bergles and colleagues (Bergles et al., 2000; Lin and Bergles, 2003) have suggested that OPCs receive glutamatergic and GABAergic synaptic inputs from interneuronal collaterals in the CA1 region of the postnatal/adult hippocampus. The criteria used for identifying the cells analyzed electrophysiologically as OPCs was the immunohistochemical detection of the chondroitin sulfate proteoglycan NG2. NG2 has been widely used as an excellent marker of OPCs in vivo by many investigators (Levine et al., 2001). Although it cannot be disputed that $NG2^+$ cells are capable of differentiating into myelinating oligodendrocytes in

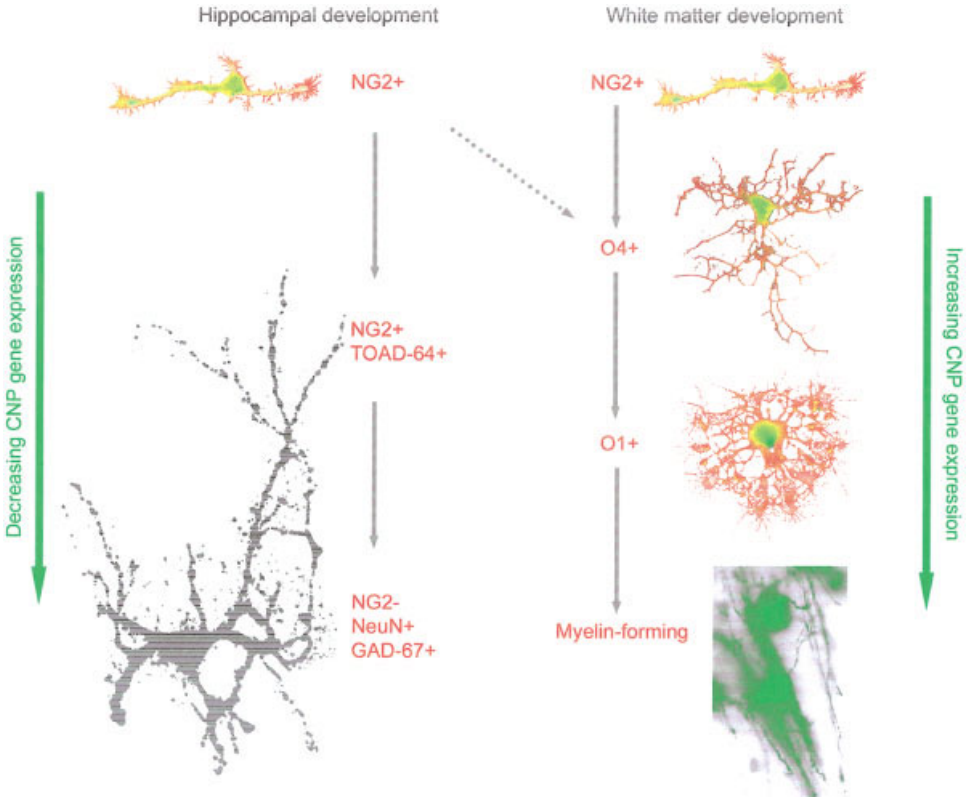

Fig. 1. Schematic representation of white matter- and hippocampal-specific fate of NG2-expressing cells in the postnatal brain. In white matter regions, $NG2^+$ cells generate myelinating oligodendrocytes through the formation of $O4^+$ pre-oligodendrocytes and postmitotic $O1^+$ oligodendrocytes. In the hippocampus, immature CNP-$EGFP^+$ neurons express TOAD-64 (Cameron and McKay, 2001), are $NG2^+$, and display high levels of EGFP, whereas differentiated $NeuN^+$/CNP-$EGFP^+$ neurons are NG2-negative and exhibit lower levels of EGFP fluorescence (Belachew et al., 2003). These findings indicated that NG2 proteoglycan expression and CNP gene promoter activity, as assessed by EGFP fluorescence intensity, are inversely correlated with the progression of neuronal differentiation in vivo. Hence, altogether these results delineated a possible developmental link between a defined class of adult progenitor cells expressing NG2 chondroitin proteoglycan and the CNP gene, and newborn postnatal GABAergic hippocampal neurons. Left: we proposed that $NG2^+$ cells can escape their restricted glial commitment in the hippocampus, and undergo the following developmental changes: $NG2^+$/CNP-$EGFP^{+++}$ $\rightarrow$ TOAD-64^+/$NG2^+$/CNP-$EGFP^{++}$ $\rightarrow$ GAD-67^+/$NeuN^+$/NG2-negative/CNP$EGFP^+$. It remains to be established whether this differentiation potential of $NG2^+$ cells may be observed in other CNS location, particularly in the subventricular zone.

white matter regions, it is debatable whether this developmental program occurs for all CNS cells that express NG2, particularly in those brain regions that display relatively less oligodendrogenesis, but more neurogenesis in the postnatal and adult brain. We and others have now demonstrated that precursor cells which were thought to be restricted to an oligodendroglial fate, i.e., so-called OPCs, were able to generate neurons under certain conditions both in vitro and in vivo (Belachew et al., 2003; Kondo and Raff, 2000; Nunes et al., 2003) (Fig. 1). These precursor cells express NG2 or A2B5, and the 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP) gene (Belachew et al., 2003; Nunes et al., 2003). Furthermore, although neural stem cells are thought to possess an undifferentiated phenotype, cells with properties of the astroglial lineage, i.e., radial glia and astrocytes, act as neural stem cells in the embryonic and adult CNS across species (Doetsch et al., 1999; Malatesta et al., 2000; Noctor et al., 2001; Doetsch, 2003).

Importantly, these studies have also led to a redefinition of the neural stem cell nomenclature, to include glial progenitors as stem cells capable of generating neurons (Goldman, 2003). Based on these recent findings, it is premature to define functional synaptic connections onto all NG2-expressing cells in the hippocampus as directly involved in neuron-oligodendroglial signaling, until it is proved that all $NG2^+$ cells that receive excitatory and inhibitory synapses in this particular region are restricted to an oligodendroglial fate. The synapses analyzed and characterized by Bergles and colleagues might, in fact, be synaptic contacts between mature resident interneurons and early postmitotic progenitors of adult GABAergic neurons. As previously described in the olfactory bulb (Carleton et al., 2003), this would also imply that the establishment of synaptic inputs occurs before progenitors for GABAergic neurons are able to propagate action potential spikes during the course of interneuron renewal in the hippocampus (Liu et al., 2003).

Unlike in the developing brain, where neurogenesis usually precedes the maturation of neural circuits, in

the adult newborn neurons must integrate into a preexisting neuronal network.

During SVZ-RMS-OB (subventricular zone-rostromigratory stream-olfactory bulb) neurogenesis, it was shown that tangentially migrating neuroblasts in the RMS first express extrasynaptic $GABA_A$ receptors and then AMPARs (Carleton et al., 2003). Again, this differs from the developing mammalian brain, where most often NMDARs are first detected, and functional AMPAR recruitment is induced by NMDAR activation (Durand et al., 1996). Adult-generated OB granule neurons receive synaptic inputs shortly after completing their radial migration, as they begin to develop their dendritic arbor, and GABAergic synaptic events appear before AMPAR-mediated excitatory postsynaptic potentials (Carleton et al., 2003). In sharp contrast with prenatal development, OB granule neurons developing in the adult brain receive both GABAergic and glutamatergic synaptic inputs before they acquire the property of propagating action potentials (Carleton et al., 2003). Unlike newborn OB granule neurons, however, in adult-generated OB periglomerular neurons the maturation of voltage-dependent sodium currents appears to precede the formation of synaptic contacts (Belluzzi et al., 2003). Thus, the sequential events that result in the functional integration of newly formed adult neurons appear to be age-specific. It remains to be elucidated whether GABA and glutamate, through the activation of extrasynaptic and synaptic $GABA_A$ and AMPA receptors, are instructive signals required for subsequent OB neuronal maturation, including the emergence of voltage-gated channels.

In contrast to SVZ-RMS-OB neurogenesis, the migration pathway and the time course of electrophysiological maturation of adult-born hippocampal neurons have not yet been precisely defined. Since it is widely accepted that neurogenesis in the adult intact brain is restricted to the SVZ and the dentate gyrus, it could be speculated that the $NG2^+$ cells analyzed by Bergles and colleagues in the adult CA1 may be immature neuronal precursors with the potential to become GABAergic interneurons (Gage, 2000; Lie et al., 2004). Recordings from $NG2^+$ cells were obtained exclusively in the stratum radiatum region of CA1 in the hippocampus of young (P6–16) and adult (>P33) rats (Bergles et al., 2000; Lin and Bergles, 2003). To our knowledge, there is no evidence that neurogenesis occurs in this region of the normal hippocampus, a fact confirmed in a recent report (Liu et al., 2003). Furthermore, it is unlikely that synaptically integrated neuronal precursor cells could still be migratory. Thus, if CA1 $NG2^+$ cells indeed represent neuronal precursor cells, they could be arrested in their differentiation process to become mature neurons, but still available to be recruited and terminally differentiate, as observed under pathological conditions such as ischemia (Nakatomi et al., 2002).

In conclusion, although it still needs to be established what percentage of $NG2^+$ cells in the hippocampus give rise to neurons and oligodendrocytes, respectively, a more detailed cellular and developmental characterization of the cell population studied is necessary to unequivocally conclude that they represent OPCs.

Recent data also emphasize that we can no longer rely on a single antigenic marker to identify a specific cell phenotype. In particular, there is now a crucial need for revisiting the definition of an OPC with region- and context-specific functional and antigenic criteria, with respect to a cell that would be restricted to a glial vs. a neuronal fate. The precise characterization of the developmental potential of $NG2^+$ cells in distinct brain regions will go beyond issues of mere nomenclature, to define whether glutamate- and GABA-mediated signaling on these progenitors is an exclusive form of neuron-oligodendroglia communication or if a proportion is in fact representative of neuron to neuroblast signaling. Besides the white matter, where it is well defined that $NG2^+$ cells do express AMPA/kainate receptors that are likely involved in neuron-oligodendroglial interactions (Yuan et al., 2002; Chittajallu et al., 2002), the physiological role of GluR-mediated signaling for $NG2^+$ cells located in the olfactory bulb, hippocampus, striatum, and cerebral cortex is by far more elusive. Further studies that will analyze a variety of antigenic markers in combination with detailed electrophysiological analysis will elucidate the importance of synaptic and extrasynaptic GluRs in $NG2^+$ cells located in gray matter regions of the perinatal and adult brain.

Astrocytes

The term neuroglia, or "nerve glue," was coined in 1859 by Rudolph Virchow, who conceived it as an inactive connective tissue in the CNS. Many years later, we now fully recognize that glial cells play many active roles in brain function, metabolism and development. In the glial kingdom, astrocytes display sheet-like or lamellate processes enwrapping the brain surface, capillaries, neuronal cell bodies, dendrites, and synapses. The astrocytic processes contain a broad range of ion channels and neurotransmitter receptors that can trigger $[Ca^{2+}]_i$ elevation in response to synaptic activity (Araque et al., 2001; Haydon, 2001). Calcium signaling in astrocytes is thought to influence glial morphology, function and gene expression, and in turn to feed back onto neurons (Verkhratsky and Kettenmann, 1996). In particular, much attention has been paid to GluRs on astrocytes as possible mediators of activity-dependent neuron-glia interplay (Steinhauser and Gallo, 1996), in terms of both neuron-to-glia and glia-to-neuron signaling (Gallo and Ghiani, 2000; Haydon, 2001). However, until recently there was little experimental evidence that supported this idea or investigated possible mechanisms involved.

Cerebellar Bergmann glial cells ensheathe Purkinje cell dendrites and synapses, and express high levels of Ca^{2+}-permeable glutamate receptors (GluRs) (Burna-

shev et al., 1992; Muller et al., 1992). The GluR2 subunit controls Ca^{2+}-permeability of AMPA receptors, i.e., receptors containing this subunit are impermeable to Ca^{2+} ions (Hollmann et al., 1991; Verdoorn et al., 1991). Suppression of Ca^{2+} permeability by virus-mediated GluR2 transfer in Bergmann glia has revealed that GluR-mediated Ca^{2+} signaling is essential to maintain both anatomical and functional connections between Bergmann glial cells and the glutamatergic synapses (Iino et al., 2001). The finding that a structural interaction between Bergmann glial processes and synapses on Purkinje cells is mediated by the glial Ca^{2+}-permeable GluRs further suggests that glia-synapse interaction could undergo structural modifications in response to synaptic activity (Gallo and Chittajallu, 2001; Watanabe, 2002). Astrocytes also express high-affinity glutamate transporters, and particularly GLAST and GLT-1, which play a major role in controlling the rapid clearance of glutamate released from neurons from the synaptic cleft (Chaudhry et al., 1995; Rothstein et al., 1996; Tanaka et al., 1997; Oliet et al., 2001). This process is central in terminating the effects of released neurotransmitters, and contributes to the fine tuning of excitatory synaptic transmission in the mature brain.

Both astrocytic glutamate uptake and homeostasis of appropriate connections between Bergmann glia and Purkinje cell synapses that depend on GluR2-containing AMPA receptors represent unequivocal examples of neuron-glia signaling and interaction. In future studies, it will be important to assess similar functional and structural relationships between astrocytes and neighboring neurons or neuronal precursors in brain regions such as the hippocampus, where GFAP-expressing cells (defined as astrocytes) have been suggested to give rise to neurons (Doetsch, 2003; Seri et al., 2001). It is striking that, together with recent studies showing that astrocytes can regulate synapse formation and synaptic transmission (Mauch et al., 2001; Nagler et al., 2001; Ullian et al., 2001), other reports have demonstrated that hippocampal astrocytes can instruct neurogenesis from neural stem cells and/or from committed neuronal precursor cells in the adult brain (Song et al., 2002a,b). It will be important to establish to what extent neurotransmitter-mediated signaling may be involved in these specific developmental properties of astrocytes in neurogenic regions.

CONCLUSIONS

The studies reviewed in this article indicate that expression of ligand- and voltage-gated channels is not an exclusive property of mature neural cells fully integrated in synaptic circuits. The exciting finding that receptors and channels are found in neural progenitors is somewhat counterbalanced by the issue of defining the identity of these precursor cells and their fate, i.e., the type of differentiated progeny they can generate in distinct brain regions. The availability of new lineage tracing approaches and transgenic mouse strains will greatly help in associating specific precursor cells to a defined electrophysiological phenotype, and to a particular progeny. Similar approaches, combined with gain- and loss-of-function studies will also contribute to clarifying the functional role of receptors and channels at specific stages of neural development.

ACKNOWLEDGMENTS

S.B. is a Research Associate of the Belgian National Fund for Scientific Research (F.N.R.S.).

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