Neuropharmacology 78 (2014) 75–80

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Invited review

Dynamic regulation of neurotransmitter specification: Relevance to nervous system homeostasis

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ARTICLE INFO

Article history:
Received 9 October 2012
Received in revised form
9 December 2012
Accepted 13 December 2012

Keywords:
Neurotransmitter phenotype
Activity-dependent

ABSTRACT

During nervous system development the neurotransmitter identity changes and coexpression of several neurotransmitters is a rather generalized feature of developing neurons. In the mature nervous system, different physiological and pathological circumstances recreate this phenomenon. The rules of neurotransmitter respecification are multiple. Among them, the goal of assuring balanced excitability appears as an important driving force for the modifications in neurotransmitter phenotype expression. The functional consequences of these dynamic revisions in neurotransmitter identity span a varied range, from fine-tuning the developing neural circuit to modifications in addictive and locomotor behaviors. Current challenges include determining the mechanisms underlying neurotransmitter phenotype respecification and how they intersect with genetic programs of neuronal specialization.

This article is part of the Special Issue entitled 'Homeostatic Synaptic Plasticity'.

1. Introduction

Neurotransmitters are the mediators of the most prominent form of communication in the nervous system. The identity of the neurotransmitter involved in a given chemical synapse is crucial in multiple ways. The matching of neurotransmitter in the presynaptic cell with its receptor in the postsynapse is essential to successful transmission. Whether the neurotransmitter participates in inhibitory or excitatory synapses changes the functional properties of the underlying circuit. In addition, depending on the particular pair of neurotransmitter/receptor the qualitative and quantitative features of the synapse differ due to the distinct kinetics and signaling cascades imprinted in the identity of the synaptic partners. Hence, the understanding of mechanisms that determine neurotransmitter specification is paramount. Neurotransmitter signaling is apparent before synaptogenesis arguing for a role of neurotransmitters beyond synaptic transmission. Here we review the origin of neurotransmitter phenotype determination, the ontogenesis of neurotransmitter signaling, the changes in neurotransmitter specification, and discuss the relevance of this neurotransmitter respecification to the function of the nervous system. We particularly focused the review on the studies that support the concept that neurotransmitter phenotype expression is dynamic and sensitive to changes in developmental and environmental cues.

2. Ontogeny of neurotransmitter phenotype expression and signaling

Neurotransmitters like GABA, dopamine and noradrenaline are present in the ectoderm of late blastula and early neural plate stage *Xenopus* embryos and regulate neuronal differentiation (Rowe et al., 1993). The pituitary adenylate cyclase activating peptide is also expressed in the mouse embryonic neural tube (Waschek et al., 1998). Serotonin released from notochord is uptaken by floor plate cells and regulates changes in cell shape and cell movement important for neural tube closure (Lauder, 1988; Wallace, 1982). Progenitor mitotic cells express the cholinergic phenotype in the olfactory, lateral and third ventricle of the embryonic mouse brain before the time of last cell division and onset of neuronal differentiation (Schambra et al., 1989). All these studies demonstrate the presence of neurotransmitters at early embryonic stages, before neuronal differentiation is accomplished. Because neurotransmitters are expressed in cells that are not fully differentiated neurons, the specialized structure of the synapse is not present yet at these early developmental stages but other forms of release seem to operate in the immature nervous system for neurotransmitter

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http://dx.doi.org/10.1016/j.neuropharm.2012.12.005

signaling. GABA and glutamate are released in a SNARE- and calcium-independent manner and signal in embryonic and neonatal hippocampal neurons before synapse formation (Demarque et al., 2002). Glycine is also diffusely released from radial cells in the embryonic mouse spinal cord and enhances the spontaneous calcium-mediated activity of immature neurons (Scain et al., 2010). Volume acetylcholine transmission between starburst amacrine cells mediates retinal calcium wave propagation during mouse development (Ford and Feller, 2012). These alternative modes of neurotransmitter release are not exclusive to the developing nervous system but are also apparent in progenitor niches of the adult brain. Pools of dividing neural progenitors of the postnatal subventricular zone in the mouse brain are sensitive to the nonsynaptic release of GABA, which regulates neural stem cell proliferation and migration (Liu et al., 2005; Pathania et al., 2010).

Many studies have demonstrated that neurotransmitter receptors are also expressed at early stages of nervous system development by assessing their expression and functionality. Glutamate, GABA, dopamine, serotonin, purinergic and muscarinic acetylcholine receptors are expressed in the proliferating retina of many different species (Martins and Pearson, 2008). The activation of $GABA_A$ or AMPA receptors depolarizes ventricular zone cells of the embryonic rat neocortex and regulates cell proliferation (LoTurco et al., 1995). $GABA_A$ receptors are also present in precursors of rat cerebellar granule cells and regulate their proliferation (Fiszman et al., 1999). Knockdown of the embryonic α2 glycine receptor subunit alters the proliferation rate of zebrafish spinal neuron progenitors and thus decreases the number of spinal interneurons (McDearmid et al., 2006). These studies illustrate that even at the early stages of neural progenitor proliferation the neurotransmitter signaling is functional in many different nervous system structures and species. Other developmental processes also witness the early neurotransmitter expression and are regulated by neurotransmitter signaling. Migration of granule cells is modulated by NMDA receptors in the mouse developing cerebellum (Komuro and Rakic, 1993) and GABA receptors modulate embryonic rat cortical cell migration (Behar et al., 1996) and cortical interneuron migration in mice (Bortone and Polleux, 2009).

The differentiation of neurons involves the acquisition of morphological and functional characteristics. Many of these features are specified within the neural progenitor from which the neuron emerges. In the developing spinal cord, a progressive commitment to specific neuronal features is achieved by a combinatorial transcription factor code triggered by morphogenetic proteins such as Sonic hedgehog (Shh) and Bone Morphogenetic Proteins (BMPs) (Jessell, 2000). Is the expression of neurotransmitters and neurotransmitter receptors part of the specification program? Expression of certain transcription factors in developing neurons is necessary and sufficient to drive the expression of specific neurotransmitter phenotypes (Fig. 1). For instance, the LIM homeodomain-containing transcription factor Lmx1b is required for the acquisition of the serotonergic phenotype in mice and links the Nkx2.2-mediated progenitor specification with the Pet1-dependent terminal differentiation (Ding et al., 2003). The choice of glutamatergic versus GABAergic phenotypes in the spinal cord dorsal horn is determined by the expression of the transcription factors Tlx1/3 and Pax2, respectively (Cheng et al., 2004). Moreover, Tlx knockout mice show an increase in the number of GABAergic neurons in the dorsal horn, suggesting that Tlx genes repress the expression of the GABAergic phenotype (Cheng et al., 2004). Reciprocally, the postmitotic homeobox gene Lbx1 upstream of Pax2, represses the glutamatergic phenotype and promotes the specification of GABAergic dorsal horn neurons (Cheng et al., 2005).

Despite the strong correlation between the expression of certain transcription factors and the neurotransmitter phenotype in a specific neuronal subtype, the fact that neurotransmitters are present before the specialization is achieved suggests that the acquisition of neurotransmitter identity is dynamic and may escape the deterministic genetic program of neuronal specialization.

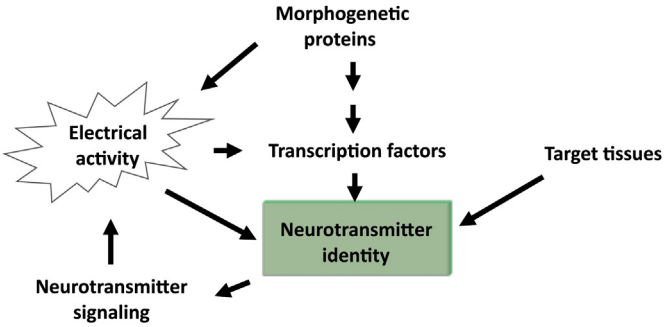

Fig. 1. Regulation of neurotransmitter phenotype expression. The specification of neural progenitors by morphogenetic proteins triggers a genetic program that results in expression of transcription factors in the emerging neurons necessary for the definition of the neurotransmitter identity. This is not a rigid program because it is intersected by other developmental and environmental cues, including pre and post-synaptogenic electrical activity and factors released from target tissues. In turn, the neurotransmitter signaling feedback loops into the process of neurotransmitter specification.

3. Interrelation between the genetic program and dynamically regulated signaling pathways

The neural fate program triggered by morphogenetic proteins is indeed modulated by intracellular signaling pathways that are in turn dynamically regulated and are sensitive to many environmental and developmental cues. In the developing mouse and chick spinal cord, the transcription factor Olig2 drives two neural cell phenotypes as different as oligodendrocytes and motor neurons depending on its phosphorylation status, presumably regulated by PKA activity (Li et al., 2011). Shh and BMPs regulate calcium-mediated electrical activity of embryonic spinal neurons and contribute to a gradient of excitability along the dorsoventral axis of the developing *Xenopus* spinal cord (Belgacem and Borodinsky, 2011; Swapna and Borodinsky, 2012). In turn, electrical activity modifies neuronal differentiation. BMPs decrease calcium-mediated electrical activity by recruiting p38 MAPK, which negatively modulates calcium spikes. Reciprocally, the higher levels of calcium spike activity in the ventral spinal cord, prevent the expansion of the BMP-induced dorsal commissural interneuron phenotype in ventral domains (Swapna and Borodinsky, 2012). In contrast, Shh enhances calcium spike activity in embryonic spinal neurons by recruiting Gαi protein and IP3 receptor-operated stores. In turn, this enhanced activity mediates the increase in number of GABAergic spinal neurons induced by ectopic Shh (Belgacem and Borodinsky, 2011). The mechanisms by which genetically-driven programs are modulated by dynamically regulated signaling pathways can be multiple but they must converge at some point in the modulation of the expression and/or activity of transcription factors that drive the neurotransmitter specification. For instance, the expression of Lmx1b, driver of the serotonergic phenotype, is regulated by levels of calcium spike activity in *Xenopus* hindbrain (Demarque and Spitzer, 2010). The expression of the GABAergic/glutamatergic transcription factor selector, Tlx3, is also regulated by levels of electrical activity in the developing *Xenopus* spinal cord (Marek et al., 2010). Ca^{2+} spikes phosphorylate cJun that binds to Tlx3 CRE site and represses its expression. This represses the specification of the glutamatergic phenotype hence promoting the prevalence of the GABAergic phenotype (Marek et al., 2010). These