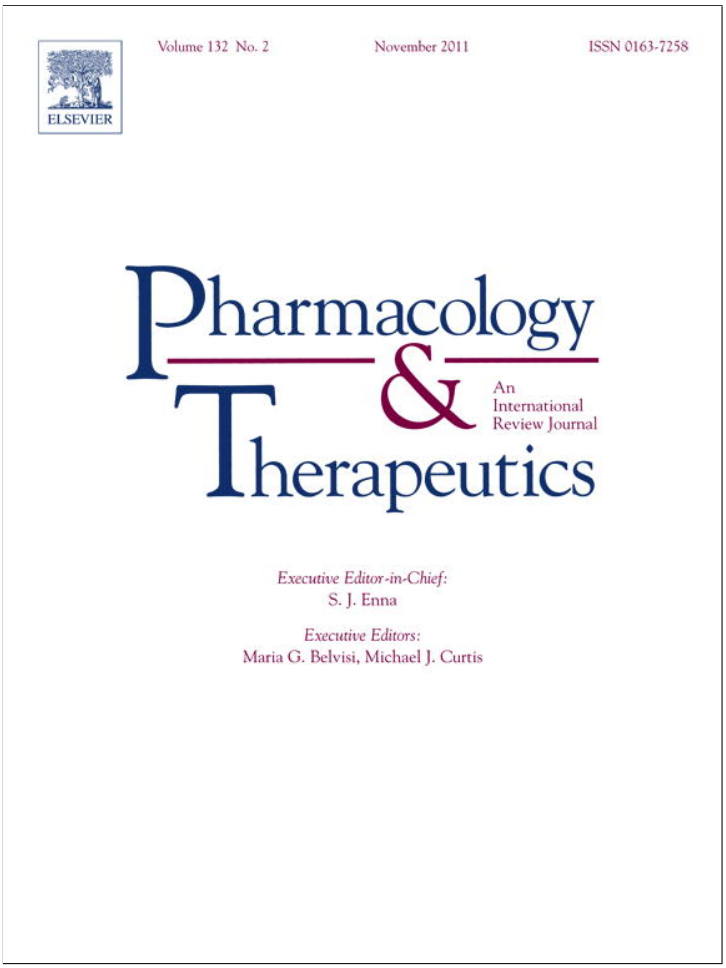

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Contribution of metabotropic $GABA_B$ receptors to neuronal network construction

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ABSTRACT

In the 1980s, Bowery and colleagues discovered the presence of a novel, bicuculline-resistant and baclofen-sensitive type of GABA receptor on peripheral nerve terminals, the $GABA_B$ receptor. Since this pioneering work, $GABA_B$ receptors have been identified in the Central Nervous System (CNS), where they provide an important inhibitory control of postsynaptic excitability and presynaptic transmitter release. $GABA_B$ receptors have been implicated in a number of important processes in the adult brain such as the regulation of synaptic plasticity and modulation of rhythmic activity. As a result of these studies, several potential therapeutic applications of $GABA_B$ receptor ligands have been identified. Recent advances have further shown that $GABA_B$ receptors play more than a classical inhibitory role in adult neurotransmission, and can in fact function as an important developmental signal early in life. Here we summarize current knowledge on the contribution of $GABA_B$ receptors to the construction and function of developing neuronal networks.

Contents

1. Introduction

Neuronal network formation is the result of a complex and precise sequence of regulated events including cell migration, differentiation and the establishment of synaptic connections. It is now well known that, in addition to genetic programs, the level and pattern of synaptic activity generated in the nervous system contributes to neuronal network development and remodeling (Katz & Shatz, 1996; Zhang & Poo, 2001). Rhythmic synaptic activity is a hallmark of the developing embryonic and postnatal nervous system, both in vivo and in vitro, and several studies suggest that these activities are important for the maturation of young neuronal networks (Ben Ari, 2001; Khazipov & Luhmann, 2006). Moreover, abundant evidence has shown that neurotransmitters are more than simple mediators of synaptic activity, and can play an important role in the coordination of network construction. γ-Amino-Butyric Acid (GABA), the main inhibitory transmitter in the adult brain, has been reported to modulate cell proliferation and migration, increase protein synthesis, promote neuronal differentiation, and to control neurite outgrowth and synapse formation (Owens & Kriegstein, 2002; Represa & Ben Ari, 2005; Ben Ari et al., 2007; Huang et al., 2007).

The two families of GABA receptors in the mammalian brain are the ionotropic $GABA_{A/C}$ receptors and the metabotropic $GABA_B$ receptors ($GABA_{A/C}$Rs and $GABA_B$Rs). $GABA_{A/C}$Rs are ligand-gated

Abbreviations: CNS, Central Nervous System; CREB, cAMP Response Element Binding protein; BDNF, Brain-Derived Neurotrophic Factor; GIRK, G-protein activated inwardly rectifying potassium channel; GABA, γ-Amino-Butyric Acid; GAD, Glutamic Acid Decarboxylase; IP_3, Inositol tri-Phosphate; LSO, Lateral Superior Olive; MNTB, Medial Nucleus of the Trapezoid Body; PKA, Protein Kinase A; PKC, Protein Kinase C; PLC, Phospho-Lipase C; TrkB-R, tropomyosin receptor kinase (type B); RGC, Retinal Ganglion Cell; VHM, Ventromedial Nucleus of the Hypothalamus.

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channels permeable to chloride that mediate fast inhibition in the CNS. $GABA_B$Rs are coupled to different effectors via GTP binding protein (Bowery, 1993; Couve et al., 2000) and mediate slow and prolonged inhibition both post and presynaptically (Bowery et al., 1980; Dutar & Nicoll, 1988). Most of the trophic actions of GABA have been attributed to the activation of the ionotropic $GABA_A$Rs, which induce excitatory, rather than inhibitory, responses at early stages of embryonic and postnatal development (Ben Ari, 2001). Accordingly, early conversion of GABA-induced depolarization into hyperpolarization impairs synapse formation and dendritic development of the target neurons both in vitro (Chudotvorova et al., 2005) and in vivo (Ge et al., 2006; Cancedda et al., 2007; Reynolds et al., 2008; Wang & Kriegstein, 2008).

Recent studies have revealed developmental functions of the metabotropic $GABA_B$Rs as well. Indeed, $GABA_B$Rs are expressed and functional at embryonic stages of development, even before synapses are formed. They can be activated by endogenous GABA released during synaptic activity, and control neuronal network activity. Activation of $GABA_B$Rs also modulates early developmental events such as migration, neurite outgrowth, axon guidance and synaptogenesis. In the present review we summarize our recent advances in establishing the impact that $GABA_B$Rs have on neuronal network construction and function during development.

2. Development of $GABA_B$ receptor mediated inhibition

2.1. Morphological studies

Functional $GABA_B$Rs are formed by heterodimeric assembly of two subunits, the $GABA_{B1}$ and $GABA_{B2}$ subunits (Bowery, 1997; Kaupmann et al., 1997, 1998a; Jones et al., 1998; White et al., 1998). The $GABA_{B1}$ subunit exists under two isoforms ($GABA_{B1a}$ and $GABA_{B1b}$) generated by alternative promoters (Steiger et al., 2004). The differential control of $GABA_{B1}$ isoforms by alternative promoters could provide an explanation for their differential developmental and tissue-specific regulation. The two isoforms have very similar pharmacological and biophysical properties, precluding determination of the functional significance of this molecular diversity. However, by using a knocking down approach, it has been demonstrated that $GABA_{B1}$ isoforms localize to distinct synaptic sites and convey separate functions (Perez-Garci et al., 2006; Vigot et al., 2006), with $GABA_{B1a}$ predominantly found at presynaptic terminals and $GABA_{B1b}$ mostly expressed at the postsynaptic level. Information on the expression and distribution of the $GABA_B$ receptor subunits is therefore a prerequisite to estimate a possible contribution of $GABA_B$Rs to developmental processes.

In situ hybridization and immunohistochemical studies show that the expression of each subunit is developmentally regulated with the expression of the $GABA_{B1}$ subunit exceeding that of the $GABA_{B2}$ during embryonic development (Kim et al., 2003; Fritschy et al., 2004; Lopez-Bendito et al., 2004; Martin et al., 2004; Panzanelli et al., 2004; Bianchi et al., 2005). Moreover, among the main $GABA_{B1}$ subunit isoforms, the $GABA_{B1a}$ isoform dominates during postnatal development, while the $GABA_{B1b}$ isoform is the most abundant in adult brain (Fritschy et al., 1999, 2004). An overlap between the distribution of $GABA_{B1}$ and $GABA_{B2}$ immuno-reactivity is observed in the embryonic cortical marginal zone, subplate, and in the dendritic fields of hippocampal pyramidal cells throughout development, supporting the idea that functional $GABA_B$Rs are present at early stages of development. $GABA_{B1}$ and $GABA_{B2}$ subunits are expressed by Purkinje cell, striatal neurons and immature cortical neurons in the marginal zone (Fritschy et al., 2004), while other cells express only the $GABA_{B1}$ subunit, as documented for tangentially migrating neurons in the cortical intermediate zone and Cajal–Retzius cells in cortical layer I (Lopez-Bendito et al., 2002). Astrocytes positive for $GABA_{B1a,b}$ are observed at birth in the cerebellum and brainstem, and at postnatal day 5 in the hippocampal formation and cerebral cortex (Fritschy et al., 2004; Lopez-Bendito et al., 2004). $GABA_{B1}$ and $GABA_{B2}$ immuno-reactivity is also observed in the Ventromedial Nucleus of the Hypothalamus (VMH) at embryonic stages (Davis et al., 2002).

At the electron microscopic level, the $GABA_{B1a/b}$ and $GABA_{B2}$ subunits are located postsynaptically in both pyramidal and nonpyramidal cells, similar to the adult distribution (Lopez-Bendito et al., 2002, 2004). At postsynaptic locations, the $GABA_B$ subunits are found on dendritic spines and shaft, at the edge of the synapse (peri-synaptic) as well as at extrasynaptic sites. $GABA_{B1}$ and $GABA_{B2}$ subunits are also found presynaptically along axon terminals and the presynaptic active zone of both symmetric and asymmetric synapses. A similar pre- and postsynaptic distribution of $GABA_{B1}$ and $GABA_{B2}$ subunits is observed in the developing cerebellum (Lujan & Shigemoto, 2006).

These studies therefore show that $GABA_B$Rs are widely expressed in the brain throughout development, at both pre- and post-synaptic sites, supporting a potential role of these receptors in neuronal network formation and function.

2.2. Functional studies

The above studies show that $GABA_B$Rs are expressed in the developing brain. Further studies have investigated whether these receptors are functional. In the adult nervous system, the metabotropic $GABA_B$Rs mediate slow and prolonged inhibition through activation of GTP binding protein. Postsynaptically located $GABA_B$Rs activate potassium conductances, thereby hyperpolarizing neurons (Gahwiler & Brown, 1985; Kaupmann et al., 1998b), while presynaptically located $GABA_B$Rs inhibit transmitter release by inhibiting activation of voltage-gated calcium channels (Scholz & Miller, 1991; Mintz & Bean, 1993).

Electrophysiological studies reveal that the functional maturation of postsynaptic and presynaptic $GABA_B$R-mediated inhibition is developmentally regulated. In the developing rat hippocampus, postsynaptic $GABA_B$R-mediated inhibition is not yet functional at early stages of development on either pyramidal cells (Gaïarsa et al., 1995; Caillard et al., 1998; Nurse & Lacaille, 1999; Verheugen et al., 1999) or interneurons (Nurse & Lacaille, 1999), while presynaptic $GABA_B$R-mediated inhibition is already functional at birth and controls both GABA and glutamate release. A similar delayed maturation of postsynaptic compared to presynaptic $GABA_B$R-mediated inhibition has also been observed in the rat somatosensory cortex (Fukuda et al., 1993) and neocortex (Luhmann & Prince, 1991). Similarly, bath application of the $GABA_B$R agonist baclofen (Bowery, 1993) fails to induce an outward current or change in membrane resistance of Cajal–Retzius cells in the postnatal mouse visual cortex, while the same treatment presynaptically decreases ongoing GABAergic synaptic activity (Kirmse & Kirischuk, 2006). The absence of $GABA_B$R-induced postsynaptic potassium outward currents is also observed in developing chick retina (Catsicas & Mobbs, 2001). However in this preparation postsynaptic $GABA_B$Rs participate to the modulation of Ca^{2+} transients triggered by synaptic activity through a down regulation of postsynaptic N- and L-type Ca^{2+} channels on ganglion and amacrine cells. These results therefore show that postsynaptic $GABA_B$Rs are present but not yet linked to the potassium channels. In the developing rat hypothalamus, glutamate and $GABA_A$R agonist elicits postsynaptic Ca^{2+} increases that can be depressed by co-application of baclofen, suggesting that postsynaptic $GABA_B$Rs are present and functional at the earliest stage studied, i.e. E15 neurons cultured during 3 days in vitro (Obrietan & Van den Pol, 1998, 1999). It remains however to determine whether this suppressive effect is due to the opening of a postsynaptic potassium conductance or to the inhibition of postsynaptic voltage dependent Ca^{2+} channels.

The mechanism underlying the absence of functional postsynaptic $GABA_B$R-mediated inhibition in the neonatal rat hippocampus and cortex has not yet been clearly established. It is unlikely to be due to

an absence of $GABA_BRs$. Autoradiographic (Turgeon & Albin, 1994), in situ hybridization (Kim et al., 2003) and immunohistochemical studies (Fritschy et al., 1999; Lopez-Bendito et al., 2002, 2004) have shown that $GABA_{B1a/b}$ and $GABA_{B2}$ subunits are expressed in the hippocampal primordium and cortex by embryonic day E14 and during postnatal development. In hippocampal neuronal cultures one study has suggested that the absence of postsynaptic $GABA_BR$-mediated responses in the developing cortex and hippocampus results from a delayed expression of the inwardly rectifying potassium channel (GIRK or Kir3) that underlies the late phase of the inhibitory postsynaptic potential (Ehrengruber et al., 1997). At 4 days in vitro (DIV), hippocampal neurons lack both GIRK proteins and $GABA_BR$-mediated postsynaptic outward currents, and a typical outward current could be induced by $GABA_BR$ activation only after transfection of GIRK proteins (Ehrengruber et al., 1997). Thus the absence of functional postsynaptic $GABA_BR$-mediated inhibition in the developing rat hippocampus likely results from a delayed expression or maturation of GIRK channels.

3. $GABA_B$ receptors are activated by ambient GABA early in development and control network-driven synaptic activity

Because $GABA_BRs$ are located at perisynaptic or extrasynaptic locations (Lopez-Bendito et al., 2002, 2004; Kulik et al., 2003), their activation requires GABA spillover. Such spillover becomes substantial during high frequency stimulation (Isaacson & Nicoll, 1993; Xu et al., 2008), concomitant activation of several interneurons or block of GABA uptake (Scanziani, 2000). In the developing brain, most of the ongoing synaptic activity is provided by primitive patterns of network-driven activity both in vitro and in vivo (Ben Ari, 2001; Khazipov & Luhmann, 2006). During this patterned synaptic activity GABAergic interneurons fire synchronously, such that $GABA_BRs$ are likely to be activated by endogenously released GABA. Accordingly, bath application of the $GABA_BR$ antagonist, CGP 35348 (Olpe et al., 1990), prolongs the duration of the network-driven synaptic activity generated by the developing rat hippocampus during the first postnatal week of life (McLean et al., 1996b). A similar lengthening of network-driven activity is observed in $GABA_{B1}$-KO mice that lack functional $GABA_BR$ mediated inhibition (Fiorentino et al., 2009) or after desensitization of presynaptic $GABA_BR$-mediated inhibition by prolonged and sustained application of baclofen (Tosetti et al., 2004, 2005). In contrast, when network-driven synaptic activity is blocked, $GABA_BR$ antagonism has no effect on spontaneous GABAergic and glutamatergic synaptic activities (McLean et al., 1996b; Fiorentino et al., 2009). Thus, as in the adult, the activation of $GABA_BRs$ by ambient GABA requires the synchronous activation of several interneurons, which in the developing brain is provided by ongoing network-driven synaptic activity. A similar control of patterned synaptic activity has been observed in the developing retina (Catsicas & Mobbs, 2001), hypothalamus (Obrietan & Van den Pol, 1998, 1999) and cortex (Obrietan & Van den Pol, 1999; Kirmse & Kirischuk, 2006). In chick retina, between E8 and E12, CGP35348 induces a fivefold increase in Ca^{2+} wave duration, and blockade of the GABA transporter GAT-1 decreases the frequency of the Ca^{2+} waves, an effect prevented by CGP35348 (Catsicas & Mobbs, 2001). In the developing hypothalamus and cortex, administration of the $GABA_B$-R antagonist alone elicits a significant increase in activity-dependent rise in intracellular Ca^{2+}, showing that synaptically released GABA exerts a tonic inhibitory control of ongoing synaptic activity (Obrietan & Van den Pol, 1999). Similarly, in the developing mouse visual cortex, ambient GABA presynaptically reduces the strength of GABAergic inputs to Cajal–Retzius cells as illustrated by the increase in the frequency of spontaneous Ca^{2+} transient and spontaneous synaptic GABAergic activity induced by bath applied $GABA_BR$ antagonist (Kirmse & Kirischuk, 2006).

From these studies it has become clear that in development, $GABA_BRs$ play a widespread and critical role in modulating neuronal network excitability. These receptors are activated by ambient GABA and dynamically reduce ongoing synaptic activity, at a time when the activation of $GABA_ARs$ excites, rather than inhibits (Ben Ari et al., 2007), developing neurons in many parts of the brain.

4. $GABA_B$ receptors contribute to neuronal network development

The above studies show that functional $GABA_BRs$ are present in the developing brain, activated by endogenous GABA and provide an important inhibitory drive for network-driven synaptic activity. Recent advances in understanding how neuronal activity influences brain development have further shown that the $GABA_BRs$ act beyond their classical inhibitory roles and can function as an important development-promoting signal early in life. Neuronal network construction follows a sequence of events during which neurons migrate, differentiate and establish appropriate synaptic connections. Pharmacological studies indicate that $GABA_BR$ activation modulates nearly all key steps of network construction.

4.1. $GABA_B$ receptors and cell migration

Neuronal migration is a critical step in brain construction. Once generated in the germinal zone, postmitotic neurons migrate to their final positions. Several factors influence cell movement and GABA is one of these. In the embryonic rat cerebral cortex, GABA has been shown to act as a chemoattractant via mechanisms involving $GABA_{A/C}Rs$ but also $GABA_BRs$.

In vitro studies on dissociated embryonic cortex report two different dose-dependent actions of GABA (Behar et al., 1996, 1998): fentomolar concentrations of GABA promote chemotaxis (i.e. directed migration along chemical gradients) of immature GAD-negative neurones dissociated from the ventricular zone (VZ), while micromolar concentrations of GABA induce chemokinesis (i.e. random movement) of more mature GAD-positive neurons dissociated from the cortical plate (CP). Pharmacological manipulations show that the enhancement of cell movement and migration by GABA involves, at least in part, the activation of $GABA_BRs$. The chemotropic action of GABA is mimicked by application of the selective $GABA_BR$ agonist baclofen, and prevented by treatment with $GABA_BR$ antagonists and pertussis toxin showing that a $G_{o,i}$ protein is involved (Behar et al., 1996, 1998). A similar approach showed that $GABA_ARs$ and $GABA_BRs$ modulate the migration of spinal neuroblasts during cord development, through pertussis toxin sensitive mechanisms (Behar et al., 1995). The influence of $GABA_BRs$ on migration does not appear to be a unique property of neurons, but is also a property of non-neuronal cell types such as oligodendrocyte precursor cells (Luyt et al., 2007).

Studies using dissociated neurons have thus convincingly demonstrated that exogenously applied GABA affects neuronal migration. Organotypic slice cultures have been used to reveal that activation of $GABA_BRs$ by ambient GABA can stimulate radial migration of postmitotic cortical neurons destined for layer II/III (Behar et al., 2000, 2001). In this study, bromodeoxyuridine (BrdU) was used to label pre-migratory cells in the ventricular zone as they underwent terminal mitosis. Following overnight exposure to BrdU, organotypic slices maintained in the presence $GABA_BR$ antagonist phaclofen had fewer BrdU-positive cells in the cortical plate compared to control, but showed more BrdU-positive cells in the intermediate zone compared to control. These observations therefore show that $GABA_BR$ activation promotes the migration of cells from the intermediate zone into the cortical plate (Behar et al., 2000, 2001). Lopez-Bendito and co-workers have further studied the role of $GABA_BRs$ in tangential migration by combining pre-embedding immunocytochemistry with cell tracking in embryonic brain slice cultures (Lopez-Bendito et al., 2003). They show that $GABA_BR$ antagonist produces an accumulation of tangentially migrating neurons in the ventricular/subventricular zones of the cortex as well as a shortening of their leading process.

These findings therefore show that $GABA_BRs$ exert an important role in both radial and tangential migration during cortical histogenesis.

Brain slice preparation was also used to examine the contribution of $GABA_BR$ activation on cell movement in the embryonic Ventromedial Nucleus of the Hypothalamus (VMH) (Davis et al., 2002; McClellan et al., 2008). $GABA_BRs$ are expressed in this region as early as at E13 (Davis et al., 2002) and $GABA_BR$-mediated response is detectable in cultured hypothalamic neurons at E18 (Obrietan & Van den Pol, 1998, 1999). Application of the $GABA_BR$ agonist baclofen reduces the rate of VMH cell movement in slices placed in vitro at E13–14 (Davis et al., 2002; McClellan et al., 2008). Conversely, the $GABA_BR$ antagonist saclofen enhances the rate of VMH cell movement, showing that endogenous GABA controls cell migration in this structure. Accordingly, differences in VMH cell position are observed in $GABA_{B1}$-KO mice (McClellan et al., 2008) that lack $GABA_BR$-mediated inhibition (Prosser et al., 2001).

If $GABA_BR$ activation by ambient GABA controls cell migration, an endogenous source of GABA must exist at these ages. Outward currents are induced in ventricular zone neurons in response to the application of the $GABA_AR$ antagonist bicuculline, demonstrating that ambient GABA exerts a tonic action on immature neurons even before the formation of functional GABAergic synapses (LoTurco et al., 1995). To determine the GABA source responsible for directing cell migration into the cortical plate, Behar and collaborators developed an in vitro assay in which dissociated ventricular zone cells were placed in the upper half of a chemotaxis chamber (see Behar et al. (1994) for a description of the chemotaxis chamber), and dissociated cortical plate cells in the lower half (Behar et al., 2001). The authors show that the cortical plate cells induced migration of ventricular zone cells, an effect prevented by the $GABA_BR$ antagonist saclofen. They further show that cortical plate neurons release GABA and taurine, both acting as chemoattractants for neurons through the activation of $GABA_BRs$. These findings therefore suggest a model in which cortical plate neurons release GABA and/or taurine, creating a gradient of chemoattractant signals that direct the migration of ventricular zone cells via $GABA_BR$ dependent mechanisms (Behar et al., 2001).

The precise location and the mechanisms by which $GABA_BRs$ modulate cell migration remain to be determined. Even though immunohistochemical studies show that $GABA_BR$ subunits are present at early embryonic stages on immature neurons (Lopez-Bendito et al., 2002), direct evidence showing a functional as well as causal link between their activation and migration is lacking. Moreover, baclofen has no detectable effects on the resting membrane potential and input resistance of tangentially migrating neurons that only express the $GABA_{B1}$ subunit (Lopez-Bendito et al., 2002). It is however possible that the $GABA_BRs$ couple to signaling pathways that are not detectable with electrophysiological recordings (see Chapter V and Table 1). Accordingly, baclofen induces intracellular Ca^{2+} rise in some dissociated embryonic rat cortical neurons (Behar et al., 1996). Such variations in intracellular Ca^{2+} concentration may account for the chemotropic effect of $GABA_BR$ activation (Behar et al., 1996, 1998). Alternatively, GABA could act on presynaptic $GABA_BRs$, thereby controlling the release of neurotransmitters, which in turn modulate cell migration (Komuro & Rakic, 1998). Finally, an indirect effect on glial cells cannot be excluded.

4.2. $GABA_B$ receptors and cell differentiation

Neuronal differentiation has many components, one of which is the specification of neurotransmitter phenotype. In the embryonic *Xenopus* spinal cord, this process is controlled by synaptic activity (Borodinsky et al., 2004; Spitzer, 2006) and by endogenous GABA and glutamate acting on metabotropic receptors (Root et al., 2008). When the neuronal tube of *Xenopus* embryos is implanted with agarose beads loaded with the $GABA_A$ and $GABA_B$ receptor antagonists, the incidence of neurons expressing excitatory or inhibitory transmitters

Table 1
$GABA_B$ receptors, effector systems and developmental functions. $GABA_BRs$ couple to different effector systems that may support their trophic actions to neuronal network construction. It should be pointed however that in most studies the type of interactions (direct or indirect) between the $GABA_BRs$ and effectors remained to be clarified.

Structure	Preparation	Effector system	Effect	Reference
Rat cortical neurons	Neuronal cultures (E17–18, 9–11 DIV)	PLC/IP_3/↗$[Ca^{2+}]_i$	Unknown	New et al., 2006
Mouse Purkinje cell	Slices (4–5 weeks)	PLC/IP_3/↗$[Ca^{2+}]_i$	Potentiation of mGluR responses	Hirono et al., 2001
Xenopus spinal neuron growth cones	Neuronal cultures (P1, 5–10 h after plating)	PLC/PKC/IP_3/internal Ca^{2+} stores PLC/IP_3/internal Ca^{2+} stores	Growth cone repulsion Growth cone attraction	Xiang et al., 2002
Rat hippocampal neurons	Slices (P1–14) Slices (P21–60)	↗PKCα,β,ε activity and immuno-reactivity ↙PKCα,β,ε activity and immuno-reactivity	Unknown Unknown	Tremblay et al., 1995
Salamander retinal neurons	Freshly dissociated retina	PKC facilitates L-type Ca^{2+} channel	Unknown	Shen and Slaughter, 1999
Xenopus spinal neuron	In vivo (stage 18–20)	PKC and PKA/↗$[Ca^{2+}]_i$	Neurotransmitter phenotype specification	Root et al., 2008
Rat visual cortex	Slices (P15–25)	PLC/IP_3/↗$[Ca^{2+}]_i$	BDNF-dependent potentiation of GABA synapses	Komatsu, 1996
Rat embryonic cortical cells	Dissociated from E14–21 cortex	↗$[Ca^{2+}]_i$	Promotion of cell movement and migration	Behar et al., 1996
Rat CA1 pyramidal neurons	Slices (P9–16)	CaMKII phosphorylation	Long term potentiation of GABA synapses	Xu et al., 2008
Rat hippocampal neurons retinal amacrine cells	Neuronal cultures (E17–20 DIV)	Direct interaction of the $GABA_{B1}$ subunit with the activating transcription factor 4	Unknown	Nehring et al., 2000; Vernon et al., 2001; White et al., 2000
Mouse CA1 hippocampal neuron	Slices (6–11 week)	Phosphorylation of $ERK_{1/2}$	Unknown	Vanhoose et al., 2002
Mouse cerebellar neurons	Neuronal cultures (P7–3 DIV)	Phosphorylation of CREB	Unknown	Tu et al., 2007
Rat cortical neurons	Neuronal cultures (E18, 20 DIV)	Modulates gene expression Up regulation of BDNF expression	Unknown	Ghorbel et al., 2005
Rat cerebellar granular cells	Neuronal cultures	Inhibits neuronal transcription	Unknown	Barthel et al., 1996
Mouse hippocampal neurons	Neuronal cultures (P0, 12–14 DIV)	Ca^{2+}-dependent release of BDNF	Maturation of GABAergic synapses	Fiorentino et al., 2009
Mouse Cerebellar granule cells	Neuronal cultures (1 week old–3 DIV)	Transactivation of IGF-1 receptor	Protection of neurons from apoptosis	Tu et al., 2010

is increased or decreased, respectively, two days later (Root et al., 2008). To explore how $GABA_BRs$ operate, the authors have tested the effect of the $GABA_BR$ antagonist phaclofen on Ca^{2+} spikes and found that the antagonist decreases their incidence in embryonic *Xenopus* spinal cord (Table 1). This effect is rescued by the administration of Protein Kinase A or C (PKA or PKC) activators and mimicked by PKA or PKC inhibitors (Root et al., 2008). The authors further tested the presence of a sensitive period for GABA-mediated transmitter specification by implanting loaded beads with GABA receptor antagonists (bicuculline and phaclofen) at different stages of development. They found that blocking GABA signaling alters transmitter specification only during a restricted period of development. Moreover, comparable effects during a similar restricted sensitive period are found when blocking Ca^{2+} spikes. Although it is not clear whether the effects are cell autonomous, these results suggest that $GABA_BRs$ activate PKA and PKC signaling, which stimulate generation of Ca^{2+} spikes to drive neurotransmitter specification in the embryonic *Xenopus* spinal cord during a critical period of development (Root et al., 2008).

A second important aspect of neuronal differentiation is the growth and targeting of axons to establish appropriate synaptic connections. Different spatially and temporally regulated mechanisms control neurite growth and guidance (Tessier-Lavigne & Goodman, 1996; Song & Poo, 1999). $GABA_BR$ activation is one such mechanism that has been reported to modulate the shape and behavior of advancing growth cones.

Several studies show that $GABA_AR$ activation acts as a "stop growth" signal for neurite outgrowth of cultured neurons from embryonic chick tectum (Michler, 1990), mouse spinal cord (Bird & Owen, 1998) and mouse olfactory bulb (Priest & Puche, 2004). In these cultures, GABA or baclofen decreases neuritic outgrowth, an effect prevented by a concurrent application of $GABA_BR$ antagonist (Michler, 1990; Bird & Owen, 1998; Priest & Puche, 2004). The cellular mechanisms underlying the inhibition of neurite growth are not known. An opposite effect has been observed in the developing *Xenopus* visual system, where both GABA and baclofen stimulate neurite outgrowth from Retinal Ganglion Cells (RGCs) (Ferguson & McFarlane, 2002). In the same study, the authors show that RGC axons grow alongside GABAergic cells in vivo, suggesting that ambient GABA may control the growth and targeting of RGC axons to the optic tectum. Accordingly, they show that the $GABA_BR$ antagonist CGP54626 applied in vivo to the developing optic projection causes a shortening of the optic nerve (Ferguson & McFarlane, 2002).

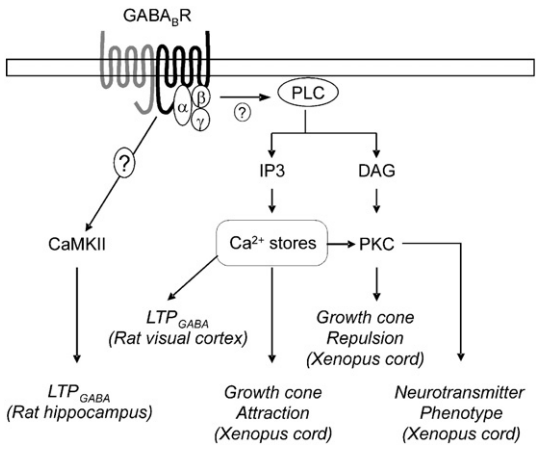

Fig. 1. $GABA_BRs$ increase intracellular Ca^{2+} concentration. $GABA_BRs$ activate PLC via $G_{\beta\gamma}$ subunits via an unknown mechanism. Activated PLC leads to IP_3-induced Ca^{2+} release and PKC activation. Activated PKC is involved in the repulsive turning response of spinal growth cones (Xiang et al., 2002) and in the specification of neurotransmitter phenotype of spinal neurons (Root et al., 2008) induced by baclofen in *Xenopus* embryos. Postsynaptic IP_3-induced Ca^{2+} release is involved in the attraction turning response of *Xenopus* spinal growth cones induced by baclofen (Xiang et al., 2002) and in the induction of GABAergic long term potentiation (LTPGABA) in the developing visual cortex (Komatsu, 1996). $GABA_BR$ activation leads to the phosphorylation of CaMKII though an unknown signaling pathway. The $GABA_BR$-dependent activation of CaMKII is required to trigger the long term potentiation of GABAergic synapses (LTP_{GABA}) in the developing rat hippocampus (Xu et al., 2008).

Using cultures obtained from embryonic *Xenopus* spinal neurons, both repulsive and attractive actions of baclofen on axon guidance have been reported (Xiang et al., 2002). Thus, when a gradient of baclofen is applied to *Xenopus* spinal growth cones a marked repulsion is observed in control conditions. However, in the presence of PKC inhibitors, growth cone repulsion induced by baclofen is converted to attraction. Both attraction and repulsion are antagonized by bath applied saclofen, a selective $GABA_BR$ antagonist, or by pre-treatment with pertussis toxin (Xiang et al., 2002). The molecular mechanisms underlying the repulsive and attractive turning of the growth cones are not yet entirely clear (Table 1 and Fig. 1). In the same study, Xiang and colleagues showed that the activation of PLC–PKC/IP_3 pathways and the release of Ca^{2+} from internal Ca^{2+} stores are required to induce the turning responses (Xiang et al., 2002). Under control conditions, the PLC–PKC pathway dominates over the PLC–IP_3 pathway and a repulsive response is observed. When PKC activity is inhibited, the PLC–IP_3 pathway induces attractive turning of the growth cones. The signaling pathways downstream of PKC activation or IP_3 formation are presently unknown.

4.3. $GABA_B$ receptors and formation of GABAergic synapses

A final step in the assembly of neuronal networks involves the establishment of functional synaptic connections. This process occurs during a period extending well into postnatal ages. Of the many signals involved, synaptic activity and neurotransmitter release are important regulators of synapse development. Glutamate, the major excitatory transmitter in the vertebrate CNS, is probably the most well-documented example (Zhang & Poo, 2001; Manent & Represa, 2007), but GABA signaling through the activation of $GABA_ARs$ also contributes to synapse formation (Chudotvorova et al., 2005; Ben Ari et al., 2007; Cancedda et al., 2007; Chattopadhyaya et al., 2007; Wang & Kriegstein, 2008). Recent data indicate that $GABA_B$ receptors could also participate in this process.

Several studies have shown that the development of inhibitory synapses is delayed by the suppression of synaptic activity (Seil & Drake-Baumann, 1994; Kilman et al., 2002; Chattopadhyaya et al., 2004; Colin-Le Brun et al., 2004). To explore the role of GABA in regulating the maturation of cortical GABAergic innervation, GABA levels were reduced or increased in basket interneurons by manipulating the expression of its major synthesis enzyme, the Glutamic Acid Decarboxylase (GAD67) (Chattopadhyaya et al., 2007). Knockdown of GAD67 in single basket GABAergic interneurons results in a cell autonomous deficit in the number of perisomatic synapses formed by these interneurons on pyramidal neurons in cortical organotypic slice cultures. The beauty of this approach is that a single cell manipulation minimally impacts the overall level of synaptic activity in the slice, in contrast to pharmacological approaches that add GABA receptor antagonists to the extracellular medium. These deficits in GABAergic perisomatic innervation are partially rescued by suppressing GABA reuptake to increase ambient endogenous GABA, or adding the $GABA_B$ receptor agonist baclofen (Chattopadhyaya et al., 2007). Conversely, over-expression of GAD67 in single basket interneurons leads to an opposite effect, namely a cell autonomous increase in the formation of perisomatic synapses (Chattopadhyaya et al., 2007). These findings therefore indicate that GABA, acting on $GABA_A$ and $GABA_B$ receptors, contributes to the development of perisomatic innervation.

A similar decrease in the density of peri-somatic GABAergic synapses is observed in the hippocampus of $GABA_{B1}$ subunit knockout mice that lack functional $GABA_BR$-mediated inhibition (Fiorentino et al., 2009). Earlier studies have shown that $GABA_BRs$ are activated by ambient

GABA, thereby controlling the ongoing synaptic activity generated by the developing network (McLean et al., 1996b; Obrietan & Van den Pol, 1998, 1999; Catsicas & Mobbs, 2001; Kirmse & Kirischuk, 2006). Consequently, the deficit in GABAergic synaptic transmission observed in the $GABA_{B1}$-KO mouse could have been accounted for by alterations in the level or pattern of spontaneous synaptic activity. To rule out this hypothesis, intact hippocampi (Khalilov et al., 1997) obtained from newborn wild type mice were incubated in vitro for several hours in the presence of tetrodotoxin to suppress action potential-dependent synaptic activity, and the synaptic activation of $GABA_B$-Rs (Fiorentino et al., 2009). A deficit in GABAergic synaptic transmission, similar to that observed in the $GABA_{B1}$-KO, is observed in TTX-treated intact hippocampi (Fiorentino et al., 2009). This deficit is rescued by baclofen, thus demonstrating that the synaptic activation of $GABA_B$-Rs per se is required for the functional maturation of GABAergic synapses in the developing rat hippocampus.

The mechanism by which $GABA_B$-Rs promote the formation of hippocampal GABAergic synapses in vivo likely involves the secretion of Brain-Derived Neurotrophic Factor (BDNF) and subsequent activation of the tropomyosin receptor kinase (TrkB) signaling pathway (Table 1 and Fig. 2). Indeed, a deficit in GABAergic synaptic transmission, similar to that observed in $GABA_{B1}$-KO mice, is found in the intact hippocampi of newborn mice, incubated with a $GABA_BR$ antagonist (Fiorentino et al., 2009). This deficit is rescued by treatment with BDNF, and occluded by the absence of endogenous BDNF, i.e. in BDNF KO mice or in wild type mice in the presence of TrkB-IgG, a BDNF/NT4 scavenger. Moreover, time lapse fluorescence imaging and immunohistochemical studies show that $GABA_B$-R activation triggers a dendritic Ca^{2+}-dependent secretion of BDNF in hippocampal cultures (Fiorentino et al., 2009).

Regulated activity-dependent release of BDNF is crucial for many different aspects of GABAergic and glutamatergic synapse development (Lu et al., 2005; Gottmann et al., 2009). At least three distinct signals regulating dendritic BDNF secretion have been directly identified in neuronal cultures (Kuczewski et al., 2009): i) tetanic stimulation of presynaptic glutamatergic fibers (Hartmann et al., 2001), ii) action potentials that propagate backwards into the dendrites (Kuczewski et al., 2008b), and iii) prolonged depolarization of the postsynaptic neuron in the absence of action potentials (Magby et al., 2006). This dendritic release of BDNF has been proposed to play a critical role in the formation and plasticity of glutamatergic synapses (Gottmann et al., 2009). BDNF-TrkB signaling is necessary for the full development of inhibitory GABAergic circuitry (Gottmann et al., 2009; Kuczewski et al., 2009). The observation that synaptic activation of $GABA_B$-Rs also triggers BDNF release provides a novel and unexpected mechanism by which synaptic activity can promote the development of functional GABAergic synapses. Activation of $GABA_B$-Rs by ambient GABA has been reported in several brain structures (Obrietan & Van den Pol, 1998, 1999; Catsicas & Mobbs, 2001; Kirmse & Kirischuk, 2006). It is therefore possible that this mechanism is important for synaptic maturation throughout the nervous system.

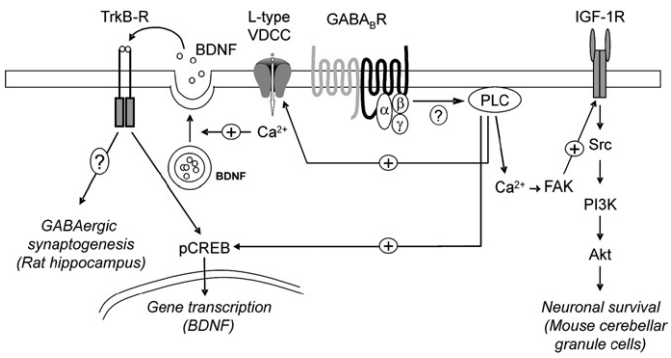

Fig. 2. $GABA_B$Rs interact with neurotrophic factors. $GABA_BR$ activation leads to the release of the $G_{\beta\gamma}$ subunits to activate the PLC/Ca^{2+}-dependent FAK pathway. FAK in turn transactivates the IGF-1 receptor to induce neuro-protection of cerebellar granule cells from apoptosis (Tu et al., 2010). $GABA_BR$-dependent activation of PLC also leads to the postsynaptic release of BDNF through the activation of L-type dependent Ca^{2+} channels. Once released, BDNF activates specific TrkB receptors and triggers the phosphorylation of CREB (Fiorentino et al., 2009). $GABA_B$Rs activation also leads to the phosphorylation of CREB via an unknown mechanism (Tu et al., 2007). Phosphorylation of CREB may be one of the mechanism by which $GABA_B$Rs modulate gene expression (Ghorbel et al., 2005).

4.4. $GABA_B$ receptors and plasticity of GABAergic synapses

Synaptic plasticity represents the capacity of individual synapses to adjust their strength in response to modifications in the level or pattern of intrinsic or sensory-driven activity. Due to the involvement of GABA in brain development, synaptic function and cognition, interest in GABAergic synaptic plasticity has intensified in recent years (Gaïarsa et al., 2002).

Long term potentiation and long term depression of GABAergic and glycinergic synaptic transmission have been described in several developing brain regions including the rat hippocampus (McLean et al., 1996a; Gubellini et al., 2001), visual cortex (Komatsu, 1994; Lien et al., 2006) and auditory system (Kotak & Sanes, 2000; Xu et al., 2010). Not surprisingly, several different mechanisms contribute to their induction and expression. However, a rise in intracellular Ca^{2+} concentration appears to be a common key signal to shape the strength of inhibitory synapses, even if the source and location of the Ca^{2+} rise could differ depending on the structures and conditioning protocols used (Gaïarsa et al., 2002). Another common requisite for the induction of long-term plasticity at developing inhibitory synapses is the activation of $GABA_B$Rs, either pre- or postsynaptic, during the conditioning protocol. Thus, low frequency stimulation of the inhibitory projection from the Medial Nucleus of the Trapezoid Body (MNTB) to the Lateral Superior Olive (LSO) induces a long-term depression of evoked inhibitory postsynaptic currents in newborn gerbils until the third postnatal week of life (Kotak & Sanes, 2000). This depression is prevented by the application of a $GABA_BR$ antagonist during the conditioning protocol (Kotak et al., 2001) and mimicked by baclofen applied in the bath (Kotak et al., 2001; Chang et al., 2003). In the developing rat visual cortex, a long term potentiation of inhibitory synaptic transmission is induced in layer V pyramidal neurons after high frequency stimulation of layer IV (Komatsu, 1994). This potentiation is prevented when the activation of $GABA_BR$ is antagonized during the conditioning protocol (Komatsu, 1996). Finally, repetitive coincident stimulation of presynaptic GABAergic fibers and postsynaptic CA1 pyramidal neurons induces a potentiation of GABAergic synaptic transmission in the developing rat hippocampus (Xu et al., 2008). This potentiation is also prevented by the application of antagonists for $GABA_B$Rs (Xu et al., 2008).

The mechanism by which activation of $GABA_B$Rs contributes to the induction of long term changes in the strength of GABAergic synapses has been explored, but remains largely speculative. Several findings suggest a possible connection between $GABA_B$Rs and neurotrophins, in particular BDNF. The neurotrophin–Trk signaling pathway contributes to the induction of $GABA_BR$-dependent GABAergic synaptic plasticity in the developing LSO (NT3-TrkC) (Kotak et al., 2001), hippocampus (BDNF-TrkB) (Gubellini et al., 2005; Kuczewski et al., 2008a) and visual cortex (BDNF-TrkB) (Inagaki et al., 2008). Furthermore, in the developing rat hippocampus, the induction of the $GABA_BR$-dependent potentiation of GABAergic synapses is prevented by the L-type voltage dependent Ca^{2+} channel blocker nifedipine, by depleting intracellular Ca^{2+} stores with a thapsigargin protocol or by intracellular administration of CaMKII inhibitors (Xu et al., 2008) (Fig. 1). This result is particularly interesting in light of reports that the dendritic release of BDNF from pyramidal neurons requires a postsynaptic rise in calcium through the activation of L-type voltage dependent Ca^{2+} channel and intracellular Ca^{2+} stores, and a subsequent activation of CaMKII (Lessmann et al., 2003; Kolarow et al.,

2007; Lessmann & Brigadski, 2009). Similarly, in the developing rat visual cortex, the induction of $GABA_BR$-dependent potentiation of GABAergic synapses requires a postsynaptic rise in Ca^{2+} via IP_3 receptor-mediated Ca^{2+} elevation (Komatsu, 1996) (Fig. 1). IP_3 receptor-mediated Ca^{2+} elevation also initiates BDNF release (Canossa et al., 2001; Balkowiec & Katz, 2002; Gartner & Staiger, 2002). Although correlational results do not demonstrate a causal link, the above observations support a model in which activation of $GABA_BRs$ induces a Ca^{2+}-dependent postsynaptic release of BDNF that in turn triggers long lasting changes in the strength of inhibitory synapses. In agreement with this hypothesis, baclofen has been reported to increase the level of phosphorylation of αCaMKII in the developing rat hippocampus (Xu et al., 2008) and to trigger a Ca^{2+}-dependent dendritic secretion of BDNF from hippocampal neurons in culture (Fiorentino et al., 2009). Further experiments are however required to directly address this hypothesis.

Long term changes in synaptic efficacy have been proposed to participate in the refinement of initially coarse patterns of synaptic connections (Kandler & Gillespie, 2005); long term depression could support synapse elimination and conversely, long term potentiation could contribute to synapse stabilization (Kandler & Gillespie, 2005). In this context, the observation that $GABA_BRs$ participate in both phenomena further strengthen their contribution to the development of neuronal networks.

5. $GABA_B$ receptors and effector systems

$GABA_BRs$ couple to different effector systems via GTP binding protein to produce postsynaptic membrane hyperpolarization and modulate presynaptic transmitter release. Most of the effects of $GABA_BRs$ have been attributed to the activation of potassium conductances (GIRK), inhibition of voltage-dependent Ca^{2+} channels, and modulation of cAMP production (Bowery et al., 2002). Developmental studies reveal a delayed functional maturation of postsynaptic GIRK-mediated inhibitory responses (Luhmann & Prince, 1991; Fukuda et al., 1993; Gaïarsa et al., 1995; Caillard et al., 1998; Nurse & Lacaille, 1999; Verheugen et al., 1999; Kirmse & Kirischuk, 2006), compared to presynaptic control of transmitter release (Luhmann & Prince, 1991; Fukuda et al., 1993; Caillard et al., 1998; Kirmse & Kirischuk, 2006) and modulation of cAMP production (Martin et al., 2004). Several studies show that in addition to their classic signaling pathways involving adenylate cyclase, GIRK and Ca^{2+}, $GABA_BRs$ can couple to other effector systems that may at least in part clarify some of the trophic actions of these receptors. These effector systems are described in the following section.

5.1. $GABA_B$ receptors increase intracellular calcium concentration

$GABA_BRs$ increase intracellular Ca^{2+} concentration in several neuronal cell types including mouse Purkinje cells (Hirono et al., 2001), *Xenopus* spinal neurons (Root et al., 2008), rat neocortical neurons (Behar et al., 1996; New et al., 2006), rat visual cortical neurons (Komatsu, 1996) and salamander retinal neurons (Shen & Slaughter, 1999) (Table 1). The mechanism by which $GABA_BRs$ increase intracellular Ca^{2+} concentration appears to involve multiple signaling intermediaries (Table 1 and Fig. 1). Pertussis toxin treatment and U73122 (Komatsu, 1996; Hirono et al., 2001; Xiang et al., 2002; New et al., 2006) both inhibit Ca^{2+} increases indicating that the first intermediaries are $G_{i/o}$ proteins, followed by subsequent activation of PLC. To date, however, there is no evidence of $G\alpha_{i/o}$ proteins directly activating PLC. Nevertheless, several studies do report that $G_{\beta\gamma}$ dimers, released after $G_{i/o}$ protein activation, can activate PLC (Blank et al., 1992; Selbie & Hill, 1998; Rebecchi & Pentyala, 2000). How $G_{\beta\gamma}$ and PLC interact is presently unknown.

Activation of PLC leads to IP_3 formation and the subsequent release of Ca^{2+} from internal stores, as well as the generation of diacylglycerol (DAG) and the subsequent activation of PKC (Berridge, 1993). Both pathways are activated by $GABA_BRs$ (Table 1 and Fig. 1). In cortical neuronal cultures, the increase in intracellular Ca^{2+} concentration induced by baclofen is prevented by IP_3 receptor antagonists (New et al., 2006). In another study, baclofen has been reported to enhance mGluR-mediated responses in mouse Purkinje cells (Hirono et al., 2001). This enhancement is prevented by intracellular infusion of the Ca^{2+} chelator BAPTA and by the IP_3 receptor antagonist heparin. In the developing rat visual cortex, the $GABA_BR$-dependent potentiation of GABAergic synaptic transmission is not observed when PLC is inhibited or when the postsynaptic neuron is loaded with heparin or BAPTA (Komatsu, 1996). Finally, the attraction turning response of embryonic *Xenopus* spinal growth cones induced by a gradient of a baclofen is prevented by the IP_3 receptor antagonist Xestospongin C (Xiang et al., 2002). In the developing rat hippocampus, GABA induces a rapid increase of PKC activity in the membrane fraction, and a decrease in the cytosolic fraction (Tremblay et al., 1995). This effect of GABA is mimicked by baclofen and antagonized by saclofen. $GABA_BR$ activation also increases the membrane immunoreactivity of some PKC isoforms (α-, β- and ε-PKC). Interestingly, the baclofen-induced increase in PKC activity is developmentally regulated. Starting in the third postnatal week of life, $GABA_BRs$ activation inhibits membrane PKC activity and immunoreactivity (Tremblay et al., 1995). In the embryonic *Xenopus* spinal cord $GABA_BRs$ activate PKC, triggering a repulsive turning response of growth cones (Xiang et al., 2002) and Ca^{2+} spike activity (Root et al., 2008). $GABA_BR$-induced activation of PKC also enhances or activates L-type Ca^{2+} currents in respectively the salamander retina (Shen & Slaughter, 1999) and hippocampal neuronal cultures (Kuczewski et al., in press).

5.2. $GABA_B$ receptors regulate gene expression

To gain insight into the pathways by which $GABA_BR$ activation can mediate long term changes in synaptic plasticity and neuronal growth, cDNA micro array technology was used to determine the profiles of genes regulated by baclofen in neuronal cultures. With this approach, baclofen application has been reported to alter the transcription (up- or down-regulation) of 20 of the approximately 1000 genes studied (Ghorbel et al., 2005).

How baclofen can alter neuron transcription was not addressed in this study. However, such control could result from modulation of the cytosolic level of cAMP and Ca^{2+} by $GABA_BRs$ (Ito et al., 1995; Barthel et al., 1996; Ren & Mody, 2006; Tu et al., 2007) or from a direct interaction of $GABA_BRs$ with transcription factors (Nehring et al., 2000; White et al., 2000; Vernon et al., 2001) (Table 1 and Fig. 2). Yeast two-hybrid screens of rat libraries and immunohistochemistry on hippocampal neurons show that $GABA_{B1}R$ subunits are engaged in a direct and specific interaction with activating transcription factor 4 (ATF4) a member of the cAMP response element-binding protein (CREB) family (Nehring et al., 2000; White et al., 2000; Vernon et al., 2001). Agonist stimulation of $GABA_BRs$ leads to a translocation of AT4 from the cytoplasm to the nucleus (White et al., 2000, but see Vernon et al., 2001). ATF-4 can act as a transcriptional activator (Liang & Hai, 1997) or repressor (Karpinski et al., 1992). It has been reported that the selective activation of $GABA_BR$ leads to CREB phosphorylation via an extracellular signal-regulated protein kinases 1/2 ($ERK_{1/2}$)-dependent pathway in cultured cerebellar granular neurons (Tu et al., 2007) and hippocampal slices (Vanhoose et al., 2002). This effect occurs via the $GABA_{B2}$ coupling to $G_{i/o}$ proteins by releasing $G_{\beta\gamma}$ subunits and activation the phosphatidyl inositol 3 kinase (PI-3K). The $GABA_BR$/ATF4 and/or $GABA_BR$/CREB interactions may therefore represent important signaling pathways for activity-dependent regulation of gene expression and may underlie some of the developmental roles of $GABA_BRs$ in neuronal network construction (Fig. 2).

5.3. $GABA_B$ receptors interact with growth factors

An interaction between $GABA_BRs$ and growth factor signaling is another mechanism by which $GABA_BRs$ may influence the

construction of neuronal networks. Indeed, growth factors play a pivotal role in brain development by influencing cell survival and differentiation, as well as synaptic structure, function and plasticity (Thoenen, 1995; Poo, 2001; Huberman & McAllister, 2002). Different interactions between growth factors and $GABA_BRs$ have recently been described, specifically an up-regulation of BDNF expression (Ghorbel et al., 2005), an induction of BDNF release (Fiorentino et al., 2009; Kuczewski et al., in press) and a trans-activation of the insulin growth factor receptor (Tu et al., 2010) (Table 1 and Fig. 2).

A control of BDNF expression by $GABA_BRs$ has also been reported in vivo, where injection of $GABA_BR$ antagonists enhances the levels of both mRNA and protein levels of BDNF (Heese et al., 2000; Enna et al., 2006). It is however likely that this enhancement resulted from an indirect effect on synaptic activity, rather than from the blockade of $GABA_BR$ per se. To address this issue, the effect of baclofen on BDNF expression has been investigated in neuronal culture in the presence of tetrodotoxin to block spontaneous synaptic activity (Ghorbel et al., 2005). This study shows that the direct activation of $GABA_BR$ leads to an up-regulation of BDNF expression (Ghorbel et al., 2005).

Time lapse fluorescence imaging experiments show that baclofen triggers a dendritic Ca^{2+}-dependent secretion of BDNF-GFP from transfected hippocampal neurons in culture (Fiorentino et al., 2009). Moreover, baclofen also induces the phosphorylation of CREB in neuronal cultures, an effect prevented by TrkB-IgG and mimicked by BDNF, showing that $GABA_BR$ activation also induces the secretion of endogenous BDNF and subsequent activation of the TrkB receptor. How $GABA_BRs$ lead to dendritic release of BDNF is presently unknown, but preliminary results obtained in hippocampal neuronal cultures implicate the involvement of PLC and L-type voltage-dependent Ca^{2+} channels (Kuczewski et al., in press) (Fig. 2). With the observation that baclofen up-regulates BDNF expression in neuronal cortical cultures (Ghorbel et al., 2005), these findings nevertheless reveal an important signaling pathway by which $GABA_BRs$ can influence neuronal network construction.

$GABA_BR$ activation induces a trans-activation of the insulin growth factor receptor (IGF-1R) (Tu et al., 2010). The intracellular events that promote this cross talk involve the activation of $G_{i/o}$ protein and the PLC/Ca^{2+}-dependent activation of the focal adhesion kinase (FAK1), which in turn leads to IGF-1R trans-activation (Fig. 2). More importantly, the authors show that $GABA_BR$ activation protects cerebellar granule neurons from apoptotic cell death and that this protective effect results from the functional trans-activation of IGF-1R. Thus, $GABA_BR$-mediated trans-activation of IGF-1R induces the phosphorylation of Akt, also known as protein kinase B, and the subsequent activation of pro-survival signals. These findings reveal a novel cellular target and new function for $GABA_BRs$ in regulating neuronal survival.

6. Conclusions: more questions than answers

Considerable progress has been made in the identification of developmental functions of $GABA_BRs$. A large number of convincing studies show that $GABA_BRs$ contribute to cell survival, migration, differentiation, as well as to the maturation and plasticity of developing synaptic connections. These receptors therefore participate to the diversity of actions mediated by GABA in the developing nervous system. These results expand our knowledge of the mechanism by which synaptic activity contributes to brain development. Yet, several questions remain to be answered, including the source and mode of GABA release activating $GABA_BRs$, the signaling pathways involved and the path by which activation of these signaling pathways leads to specific outcomes.

We have reviewed how $GABA_BRs$ participate to neuronal network construction. Because impairments in network wiring have been associated with the emergence of a variety of neurological disorders, $GABA_BRs$ represent possible targets for the treatment of pathological conditions. It will be therefore important in future studies to translate this knowledge into a physiological context to evaluate the importance of $GABA_BRs$ to developmental pathology.

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