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REVIEW

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GABA signalling during development: new data and old questions

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Abstract In addition to being the major inhibitory neurotransmitter, γ-aminobutyric acid (GABA) is thought to play a morphogenetic role in embryonic development. During the last decade, considerable progress has been made in elucidating the molecular mechanisms involved in GABA synthesis and biological action. The present review is an attempt to summarise recent results on the ontogeny of the different components of embryonic GABA signalling with an emphasis on the synthesis of GABA by different molecular forms of glutamic acid decarboxylase (GAD).

Keywords GAD · Embryonic development · Neuronal differentiation · Trophic action

GABA was discovered almost 50 years ago and is best known as the predominant inhibitory neurotransmitter in the adult brain. GABA is synthesised in 20–30% of all central nervous system (CNS) neurones (termed GABAergic neurones) and is therefore indispensable for the control of all CNS functions such as locomotor activity, learning, and circadian rhythm. Regulation of GABA-mediated signalling involves several mechanisms, among which modulation of GABA synthesis by the rate-limiting enzyme glutamic acid decarboxylase (GAD; EC 4.1.1.15) plays a central role. GAD and GABA are also found outside the nervous system in ovaries, testis, and insulin-producing β-cells of the pancreas (reviewed in Erdö and Wolff 1990; Tillakaratne et al. 1995; Chessler and Lernmark 2000). The precise function of GABA in these tissues and cell types has yet to be determined.

During embryonic development, GABA appears long before the onset of inhibitory synaptogenesis and has been proposed to serve as a trophic factor for differentiating neurones (Lipton and Kater 1989; Meier et al. 1991; Lauder 1993; see also Katarova et al. 2000a). In midgestation mouse and rat embryos GABAergic fibres grow near zones where neurones are being generated (Lauder et al. 1986; Del Rio et al. 2000; Katarova et al. 2000a). The spatiotemporal expression of certain $GABA_A$ receptor subunits coincides with the appearance of these GABAergic pathways (Laurie et al. 1992; Ma and Barker 1995). GABA can be released from fibres and growth cones by the reversal of membrane-bound GABA transporters (Taylor and Gordon-Weeks 1991) or exocytosis (Gao and van den Pol 2000). The concomitant presence of GABAergic fibres, GABA-releasing mechanisms and GABA receptors at early embryonic stages implies that GABA serves as a trophic factor during neurogenesis (reviewed in Barker et al. 1998).

GABA as a modulator of proliferation of neural progenitors

GABA and functional $GABA_A$ receptors have been detected in the ventricular neuroepithelium of E10 (embryonic day 10) cortex (Haydar et al. 2000) and E13 spinal cord (Ma and Barker 1995). GABA triggers signals in proliferating cells located in the telencephalic ventricular zone (VZ, LoTurco et al. 1995; Owens et al. 1999), suggesting that GABA may function as a modulator of cell proliferation (LoTurco et al. 1995; Haydar et al. 2000). Studying [^{3}H]-thymidine or bromodeoxyuridine (BrdU) incorporation in cells derived from the E16–E19 cortex has revealed that micromolar concentrations of GABA

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inhibited DNA synthesis in proliferating cells (LoTurco et al. 1995). The effect was blocked by the $GABA_A$-receptor antagonist bicuculline methiodide and could be mimicked by activating voltage-dependent ion channels by adding depolarising concentrations of KCl. A more recent study by Haydar and colleagues (2000) demonstrated a marked difference in the rate of proliferation in response to GABA between cells isolated from the ventricular (VZ) vs subventricular (SVZ) zone of embryonic mouse cortex. GABA prevented an exit from the cell cycle and reduced the cell cycle duration of cells in microdissected VZ. At the same time, application of GABA to the SVZ decreased the number of cells incorporating BrdU. Both effects seem to be mediated by functional $GABA_A$ receptors since treatment with $GABA_A$-receptor agonists mimics this action, whereas antagonists completely abolish it. The discrepancy in the results obtained by the two groups could be explained by the time shift (E13 vs E16–E19) between the embryonic ages and/or by the masking of the mitogenic response to GABA of VZ cells when using non-dissected cortex. It needs to be stressed that the parameters of these experiments, for instance the concentration of GABA (30 μM), may differ from normal physiological conditions. While high local concentrations of GABA may be available near some nerve endings at later stages (from E13 on in the mouse), it would be difficult to explain its presence at earlier embryonic stages (E10–12), given the scarce number of $GABA^+$ cells (Haydar et al. 2000) and the absence of GAD expression in the cortex (Katarova et al. 2000a). Another factor may be the heterogeneity among the cortical progenitors, even within the same germinative zone, with respect to its responsiveness to GABA (presumably based on differential expression of GABA-receptor subtypes), which has not been addressed in these studies.

In the adult brain, GABA acting on GABA receptors causes hyperpolarisation of the membrane and neuronal inhibition. During the period of active neurogenesis and until about the first postnatal week, the activation of $GABA_A$ receptors/Cl^- ion channels has been shown to induce membrane depolarisation and a rise in cytosolic Ca^{2+} (Cherubini et al. 1991; LoTurco et al. 1995; Serafini et al. 1998; Owens et al. 1996, 1999). The depolarising effect of GABA is most probably due to an elevated intracellular Cl^- concentration, which is particularly high in dividing precursors and decreases with the advance of neuronal differentiation and synaptic formation (Cherubini et al. 1991; LoTurco et al. 1995; Rivera et al. 1999; Owens et al. 1996, 1999). The activation of voltage-dependent Ca^{2+} channels (VDCC) occurring during depolarisation is thought to contribute to the elevation in intracellular Ca^{2+} (Reichling et al. 1994; Serafini et al. 1998). While elevated Ca^{2+} seems the most likely intracellular mediator of the GABA action on cell proliferation, there is no plausible explanation at present for its divergent effects on the cell cycle. Similarly, the components of the downstream cellular machinery remain largely unknown. It remains to be clarified also whether GABA modulates the cell cycle in other regions of the embryonic CNS and/or periphery, as suggested by its tissue and cellular distribution pattern (Katarova et al. 2000a).

GABA promotes and regulates the migration of neuronal precursors

Around terminal division, cortical progenitors migrate away from the VZ along radial glial cells, which guide neurones towards their target positions. The direction of cell movement is affected by environmental cues provided by surrounding cells and incoming fibres. In vivo, GABA is detected near the target destinations of migratory neurones (Lauder et al. 1986; Del Rio et al. 2000; Katarova et al. 2000a) and also in migratory neurones themselves (Taylor et al. 1990; Taylor and Gordon-Weeks 1991; Bless et al. 2000; Del Rio et al. 2000) from E10 on. Studies on the migratory responses of acutely dissociated cells derived from the VZ/SVZ of E18 embryonic cortex have revealed that femtomolar concentrations of GABA stimulate directed migration (chemotaxis), while micromolar concentrations stimulate chemokinesis (random motility) of more mature neurones derived from the cortical plate-subplate (CP/SP) region (Behar et al. 1996, 1998). Further analysis has revealed that picrotoxin-sensitive, putative $GABA_C$-like receptors regulate the migration from the VZ to the intermediate zone (IZ) while activation of saclofen-sensitive $GABA_B$ receptors contributes to the migration of cells from the IZ towards the CP (Behar et al. 2000). Bicuculline-sensitive $GABA_A$ receptor activation has been proposed to provide a “stop signal” for migrating neurones as bicuculline causes thickening of the CP due to an increase in the number of migrated neurones (Behar et al. 2000). The significance of this finding needs to be further evaluated, however, since other factors have also been reported to play a role in the arrest of cell migration at the cortical plate and establishment of the cortical layering (Ogawa et al. 1995; Supèr et al. 2000; Dulabon et al. 2000). Blocking of GABA receptors with saclofen or picrotoxin resulted only in a delay of cell movements, but not a complete arrest of migration, indicating that GABA receptor activation seems not to initiate, but only to modulate, the rate of cell migration in the developing cortex (Behar et al. 2000).

Interestingly, GABA has been reported to inhibit the migration of early LHRH (luteinizing hormone-releasing hormone) progenitors, a subpopulation of which express GABA during migration (Fueshko et al. 1998). This effect is mediated by $GABA_A$ receptors, although the underlying molecular events are largely unknown (Fueshko et al. 1998; Bless et al. 2000). Other populations of GABAergic neuronal precursors also exist in the brain that migrate, sometimes long distances, from the place of origin to their final destination – the majority of the GABAergic interneurones of the cortex, striatum and olfactory bulb (Anderson et al. 1997, 1999). It is not known yet whether GABA may influence their migration

and what receptors and/or other components of the GABAergic signalling might be involved.

The GABA-mediated migratory signals have been suggested to act through Ca^{2+} transients that alter cell movements through changing the dynamics of cytoskeletal remodelling (Gomez et al. 1995; Gomez and Spitzer 1999).

GABA accelerates neuronal maturation

The correct establishment of highly organised neuronal circuits during postnatal development depends on a variety of factors involving the guidance of pre- and postsynaptic neuronal processes and the specialisation and stabilisation of the synaptic elements. The transformation of a growth cone to a synaptic element involves the maturation of the biochemical machinery of neurotransmission. This transition from embryonic to adult GABAergic signalling may be mediated in part by switches in subunit composition of $GABA_A$ receptors (Maric et al. 1997; Owens et al. 1999) and probably involves changes in expression of components involved in GABA synthesis, storage and release (Szabó et al. 1994; Somogyi et al. 1995). Exposure of rat cerebellar granule cells to GABA agonists **(**Hansen et al. 1987) or chick cortical neurones to GABA (Spoerri 1988) increased the densities of intracellular organelles such as Golgi apparatus, rough endoplasmic reticulum, microtubules and coated vesicles (Hansen et al. 1987; Spoerri 1988), suggesting that GABA enhances the metabolic activity of neurones. GABA upregulated the expression of specific $GABA_A$ receptor subunits (α1 and β2) and increased ligand binding on pre-existing receptors in cerebellar granule cells (Kim et al. 1994). GABA has also been shown to induce the synthesis of several neuron-specific proteins including neuron-specific enolase and neural cell adhesion molecule (reviewed by Belhage et al. 1998; Meier and Jorgensen 1986; Meier et al. 1987). In the peripheral nervous system, $GABA_B$ receptor agonist baclofen induced a transient increase in the number of coated vesicles and pits in the vicinity of postsynaptic densities (Parducz et al. 1990). The present data support the idea that GABA and GABA agonists accelerate neuronal maturation and promote formation of functional synapses.

Regulation of GABA synthesis: GADs

Molecular cloning studies have shown that in the vertebrate nervous system the synthesis of GABA is catalysed by a 65-kDa and a 67-kDa form of glutamic acid decarboxylase (reviewed in Martin and Rimvall 1993; Fig. 1). The two GAD genes have derived from a single vertebrate GAD gene by a gene duplication around 400–560 million years ago (Bosma et al. 1999). The discovery that the vertebrates have two genes for GAD (Erlander et al. 1991) and previous data on the existence of two distinct GABA pools have prompted the idea that

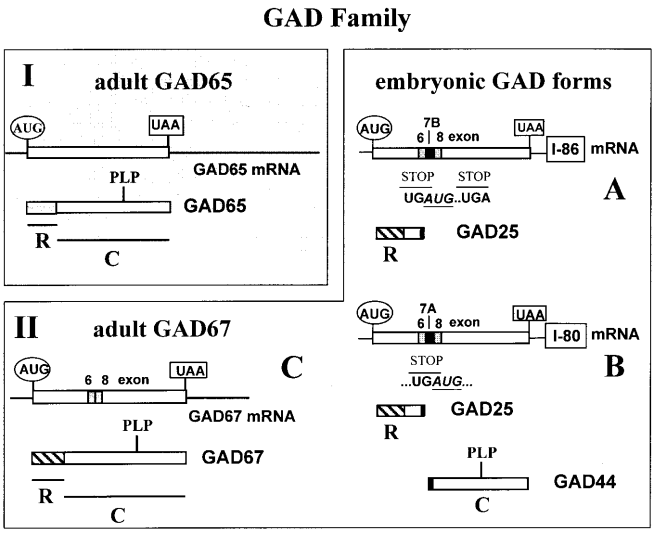

Fig. 1 Members of the GAD family. *Panel I* GAD65 mRNA codes for the 65-kDa GAD protein. GAD65 contains the N-terminal regulatory domain (*R*) and the C-terminal catalytic region (*C*) needed for co-factor binding (*PLP* pyridoxal phosphate) and glutamate decarboxylation. *Panel II* Transcripts encoded by the GAD67 gene and their protein products. *A* Embryonic transcript I-86 contains an overlapping STOP/START codon located in exon 7B followed by an additional in frame STOP codon. I-86 codes for the 25-kDa GAD retaining the N-terminal regulatory domain (*R*) of GAD67. *B* I-80 that contains exon 7A with the overlapping STOP/START signal encodes both GAD25 and GAD44. GAD44 contains the C-terminal cofactor-binding site (*PLP*) and the catalytic site (*C*) of GAD67. *C* GAD67 mRNA does not contain the embryonic exon 7A/B and codes for the adult full-length GAD67

each form may be specialised to synthesise a specific pool of GABA that serves distinct functions (reviewed in Martin and Rimvall 1993; Martin et al. 2000). Consistent with that, GAD67 and GAD65 knock-out mice show distinct phenotypes. GAD67-deficient animals show a developmental phenotype characterised by neonatal death and cleft secondary palate (Condie et al. 1997; Asada et al. 1997). GABA levels are significantly reduced in adult GAD67+/– mice, indicating that GAD65 cannot compensate for the partial loss of GAD67. In contrast, GAD65–/– mice are viable, but develop epilepsy (Kash et al. 1997), and display abnormal neural activity (Stork et al. 2000) and increased anxiety-like behaviour, suggesting that GAD65-mediated GABA synthesis may be involved in the control of emotional behaviour. Mice deficient for both GAD65 and GAD67 display the same phenotype as the GAD67–/– mice (Ji et al. 1999). Interestingly, although GABA has been scarcely detected in GAD65–/–:GAD67–/– brains, histogenesis in the neocortex, cerebellum and hippocampus seems to proceed normally until E14–P0 (Ji et al. 1999).

The two GAD enzyme forms differ in kinetic properties (Martin and Rimvall 1993; Martin et al. 2000), in intracellular distribution (Kannani et al. 1999), as well as in their interaction with the cofactor pyridoxal-phosphate (PLP) (Fig. 1; Kaufman et al. 1991; Martin et al. 2000). GAD65 and GAD67 are each composed of two major sequence domains called the N-terminal (showing only

23% sequence identity) and C-terminal (showing 73% sequence identity) domains (Fig. 1). The N-terminal domain appears to be responsible for the subcellular targeting and formation of GAD65 and GAD67 homo- and heteromers, whereas the C-terminal domain contains the cofactor-binding site and is thought to perform catalytic functions (Sheikh and Martin 1996; Soghomonian and Martin 1998; Kanaani et al. 1999). The two isoforms are synthesised as soluble enzymes, but membrane association of both forms has been demonstrated (Christgau et al. 1991; Kanaani et al. 1999; Obata et al. 1999). The 67-kDa GAD form is diffusely distributed in the cytoplasm of the cells, while the 65-kDa GAD form is mainly found attached to synaptic vesicles (Hsu et al. 2000). GAD65 protein is attached to the membrane via palmitoyl moiety added to the N-terminal region post-translationally (Christgau et al. 1992), although the palmitoylation itself is not required for membrane targeting (Shi et al. 1994). Membrane targeting of GAD67 has been shown to be independent of its dimerization with the lipophilic GAD65 (Kanaani et al. 1999), as revealed also in studies on GAD65–/– mice (Obata et al. 1999). GAD67/67 homodimers might have distinct microlocalisation within membrane compartments of nerve terminals from that of GAD65/65 or GAD65/67 dimers and therefore may be involved in different modes of GABA secretion (Kanaani et al. 1999). The preferential distribution of GAD65 in nerve terminals and its association with synaptic vesicles suggest that it could be involved in the synthesis of vesicular GABA that mediates fast-acting synaptic communications (Sheikh and Martin 1996). The mainly cytoplasmic GAD67 could be predominantly responsible for the synthesis of "non-synaptic" or "metabolic" GABA pool, which is connected to the tricarboxylic acid cycle by the "GABA shunt" (Soghomonian and Martin 1998; Waagepetersen et al. 1999).

Truncated protein forms are produced during embryonic development of the CNS

During embryonic development, alternatively spliced transcripts are produced from the GAD67 gene but not from the GAD65 (Bond et al. 1990; Szabó et al. 1994). These transcripts include two almost identical alternatively spliced exons inserted into the coding sequence of GAD67 (Bond et al. 1990; Szabó et al. 1994). The embryonic exons contain an overlapping stop/start codon (**TGA***TG*) which converts the main open frame (ORF) of GAD67 into two overlapping ORFs, coding for a 25-kDa "leader peptide" and a 44-kDa "truncated GAD" (Fig. 1; Szabó et al. 1994). An additional stop codon found at the end of exon 7B in I-86 abolishes the translation of truncated GAD (Fig. 1, IIA). Hence, I-80 codes for two truncated GAD proteins: GAD25 and GAD44 (Fig. 1, IIB), while only the 25-kDa GAD protein is generated from I-86 (Fig. 1, IIA). I-86 message is prevalent at earlier developmental stages characterised by proliferation and initial differentiation in the embryonic nervous system, whereas I-80 is more abundant at later embryonic stages (Szabó et al. 1994).

GAD25 contains the N-terminal 212 amino acids of the adult GAD and 11 amino acids derived from the embryonic exon. GAD25 form contains the putative "regulatory domain" of GAD67 and is enzymatically inactive. It is more abundant during early developmental stages (E10.5–E12.5 mouse embryos; Szabó et al. 1994), but is also detectable in adult brain regions where continuous synaptic rearrangements occur (Krizbai et al. 2000). The truncated 44-kDa form has 15 amino acids derived from the embryonic exon at its N-terminus and 381 amino acids from the COOH-terminal portion of the adult GAD67 including the PLP-binding site (Fig. 1). GAD44 is enzymatically active and is thought to represent the catalytic domain of the enzyme (Szabó et al. 1994). While a portion of adult GAD65 and GAD67 is always found in soluble brain extracts, GAD25 and GAD44 are mostly, if not exclusively, associated with the Triton X-114 insoluble membrane fraction (Z. Katarova, P. Varju, unpublished observations). The subcellular distribution of truncated GAD forms in transient expression systems depends on the cell type and on the level of expression (Katarova et al. 2000b). In cultured cells of neuronal origin, GAD25 and GAD44 sediment with the Triton X-114 insoluble membrane fraction, but show a slightly different subcellular distribution by immunocytochemistry. The 25-kDa protein form is enriched in the processes of young neurones, while the 44-kDa protein form localises mainly to perinuclear membranes (Varju et al. 2000).

The enzymatically active GAD44 is detected from E11 until P21 in the mouse. GAD67 displays the opposite tendency – it is almost undetectable at E11, rises dramatically at birth and reaches adult levels at 4 weeks postpartum, which coincides roughly with the end of inhibitory synaptogenesis (Szabó et al. 1994). This has led to the speculation that GAD44 might be responsible for the synthesis of a non-synaptic ("morphogenic") GABA (Szabó et al. 1994, 2000).

In the ventricular zone of the embryonic rat spinal cord, $GAD^+/GABA^-$ cells have been detected as early as E11 and at least some of these cells incorporated BrdU (Ma et al. 1992). At E13, the majority of cells are GAD^+, but only a small fraction produce GABA. The vast majority of the GAD^+ cells have been found to express GAD25 – the enzymatically inactive form of GAD (Behar et al. 1993). The percentage of $GABA^+$ cells has been found to increase dramatically at later embryonic stages, which coincides with the concomitant induction first of GAD67 and later of GAD65 (Behar et al. 1993). At the same time, the pattern of GABA staining changed from diffuse at embryonic and early postnatal stages to punctate beyond P21 (Behar et al. 1993). Diffuse staining has been obtained with GAD25- and GAD67-specific sera, while GAD65-specific antibodies revealed punctate staining (Behar et al. 1993). Hence, the expression of different (combinations of) GAD forms seems to correlate with the subcellular distribution of GABA. Similarly, in cultured neuroblastoma cell lines GAD67 and

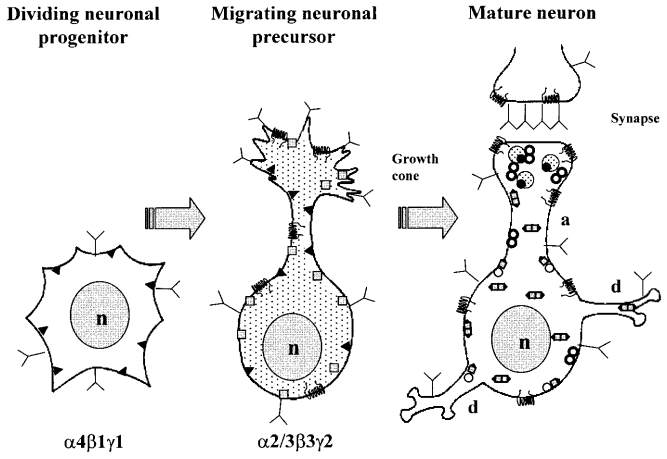

Fig. 2 Developmental changes in the GABA-signalling cascade during neural differentiation (γ GABA receptor, ▲ GAD25 protein, *grey shaded box* GAD44 protein, *double ring* GAD65 homodimer, *pointed double box* GAD67 homodimer, *pointed box-ring* GAD65/67 heterodimer, *coil* plasma membrane GABA transporter, ● vesicular GABA transporter, *dotted shading* GABA content, *a* axon, *d* dendrite, *n* nucleus)

GAD44 expressed at high levels displayed a diffuse cytoplasmic distribution, while GAD65 had a patchy or punctate appearance; a similar "patchy" appearance was observed also for GAD25 (Katarova et al. 2000b).

In the developing telencephalon, embryonic transcripts are transiently expressed in the VZ and SVZ, at sites with active neurogenesis, and also at sites containing migratory and postmigratory neuroblasts (Behar et al. 1994; see also Katarova et al. 2000a). This implies that embryonic GAD proteins may be involved in the generation, differentiation and migration of neurones in the ventricular and intermediate zones, although the molecular mechanisms are not known yet. Our recent data on a cell line derived from E9 brain vesicles of p53–/– mouse embryos (Schlett and Madarasz 1997) induced to differentiate by retinoic acid (RA) show that during initial proliferative stages only the 25-kDa GAD form is expressed, suggesting some independent, still unknown function for the shortest GAD protein (Fig. 2; Varju et al. 2000). Similar to mouse embryos, the expression of embryonic transcripts in postmitotic neurones appears to precede the expression of transcripts encoding full-length GAD proteins in the order GAD25, GAD25/GAD44, GAD67, GAD65 (Szabó et al. 1994; Somogyi et al. 1995; Varju et al. 2000). The sequential appearance of the individual GAD forms clearly indicates that they perform different developmental functions. These may be related to the differentiation of the GABAergic neurones and establishment of the GABAergic phenotype during initial stages of differentiation, as well as migration and inhibitory synaptogenesis. The variety of functions may be mediated through different combinations of GAD forms and the differential subcellular distribution (Fig. 2). The post-transcriptional regulation of embryonic GAD expression (Szabó et al. 1994) adds an additional complexity to the system, as it can be greatly influenced by external factors and ultimately provides the cell with a mechanism for fine regulation of the GABA content.

Recently, it has been shown that in single and double knock-out mutants of the homeobox genes Dlx1 and Dlx2, whose expression overlaps with that of GAD in the forebrain, the GAD expression is completely abolished, and the differentiation and migration of the GABAergic inhibitory neurones of the olfactory bulb, striatum, neocortex and hippocampus are greatly disrupted (Qui et al. 1995; Anderson et al. 1997, 1999). Whether GAD forms play specific roles in these processes remains to be verified (see Katarova et al. 2000a for discussion).

GABA release from growth cones via plasma membrane GABA transporters

The action of GABA is thought to be terminated by its rapid reuptake via membrane-bound GABA transporters (GAT1–4), which have been shown to localise on both presynaptic and postsynaptic membranes of differentiated neurones and also on the surface of glial cells (Minelli et al. 1995, 1996).

GAT-mediated GABA accumulation (Hatten et al. 1984) and release (Taylor and Gordon-Weeks 1989) was observed in the perinatal brain well before synapses are formed. GABA can be released by stimulation with high K^+ in a Ca^{2+}-independent manner from isolated growth cones due to a reversal of the plasma membrane GABA transporters (Taylor et al. 1990; Taylor and Gordon-Weeks 1991). Several reports have confirmed that GABA transporters are bidirectional and can mediate GABA efflux as well as influx, depending on the ionic environment (Nelson and Blaustein 1982). GAT1 expression can be first detected in E13 rat forebrain and spinal cord, coinciding with axonal outgrowth (Altman and Bayer 1984) and is in concert with the later overall appearance of GAT1 in selected fibre tracts, presumably growing axons. In mouse embryos the spatial and temporal expression pattern of GAT1 and GAT4 generally follows the appearance of GABAergic fibres (Liu et al. 1993; Jursky and Nelson 1996; Evans et al. 1996). The early appearance of GAT1 and GAT4 in the close vicinity of proliferative zones (Jursky and Nelson 1996; Evans et al. 1996) suggests that they might have a function in regulating the local GABA concentrations.

GABA can be secreted via multiple ways depending on the state of differentiation of the releasing cell. In early neuronal precursors where the vesicular storage of neurotransmitters has not yet developed, the release of cytoplasmic GABA can take place through the reversal of GATs. GABA release from early GABAergic axonal projections, however, may occur via exocytosis (Gao and van den Pol 2000). The normal histogenesis in the mouse CNS lacking synaptic vesicular neurotransmitter release (Verhage et al. 2000) emphasises the significance of the

non-synaptic neurotransmitter release during early stages of neural differentiation.

Ontogeny of $GABA_A$ receptor/Cl^- channels

The developmental transformation of GABAergic signals is associated with activation of specific GABA receptors. Proliferating cells in the neocortical ventricular zone, as well as migrating neural precursors, express functional $GABA_A$ receptors (LoTurco et al. 1995; Haydar et al. 2000; Behar et al. 2000). In spite of the fact that anatomically defined synaptic connections were not detected in the VZ (Balslev et al. 1996), $GABA_A$ receptor activation occurs (LoTurco et al. 1995; Haydar et al. 2000). $GABA_A$ receptors in the VZ display a relatively high affinity for GABA, little receptor desensitisation, small current magnitude and slow receptor recovery compared to neurones in the cortical plate (Owens et al. 1999; Serafini et al. 1997), supporting the notion that receptors on proliferating cells are activated by a paracrine mechanism. The density of $GABA_A$ receptor channels on VZ cells is quite low compared to postmigratory cortical neurones (Fig. 2; Owens et al. 1999) and increases with maturation. Synaptic contacts could not be detected in the SVZ/IZ (Balslev et al. 1996; Bourgeois and Rakic 1993), indicating that $GABA_A$ receptors on migrating neurones may be activated by either autocrine and/or paracrine mechanisms. During neuronal maturation in the cortical plate, $GABA_A$ receptors display a lower affinity for GABA, higher current magnitudes and higher sensitivity to desensitisation compared to proliferating progenitors (Owens et al. 1999). These changes in the pharmacological properties during neural commitment and maturation may reflect the developmental switch in the subunit composition of $GABA_A$-type receptor channels (schematically represented in Fig. 2). At earlier stages of differentiation in the neocortical proliferative zone, as well as in the spinal cord VZ, the most prominently expressed $GABA_A$ receptor subunits are the $\alpha 4$, $\beta 1$ and $\gamma 1$. These subunits are primarily present in dividing neuroepithelial cells (from E13 on) and are detected at low levels at birth, indicating that they are transiently expressed during neurogenesis (Laurie et al. 1992; Ma et al. 1993). In postmitotic neurones of the embryonic CP and spinal cord differentiated neuroepithelium $\alpha 3$, $\beta 3$- and $\gamma 2$-subunits appear to be predominantly expressed (Laurie et al. 1992; Maric et al. 1997; Serafini et al. 1998), although other subunits have also been detected (Barker et al. 1998). The $\alpha 3$, $\beta 2/3$, $\gamma 2$ combination persists throughout postnatal differentiation and new subunits are also added to this repertoire (Barker et al. 1998), which may reflect the diversification of functions mediated by GABA. Comparison between GABA receptor subunit and GAD expression reveals that the $\alpha 4$-, $\beta 1$-, and $\gamma 1$-subunits are expressed in a complementary manner, whereas $\alpha 2/3$, $\beta 3$, and $\gamma 2$ show overlapping expression with that of GAD67 in embryonic spinal cord (Ma et al. 1993; Ma and Barker 1995). It could be speculated that $GABA_A$ receptors with a distinct subunit composition mediate paracrine signals in the GAD^- cells of the VZ or autocrine signals in differentiating GAD^+ cells of the transition zone (Ma and Barker 1995). Thus, the pleiotropic functions of GABA in the development may be in part mediated by multiple classes of $GABA_A$ receptor/Cl^- channels. As mentioned before, Ca^{2+} has been implicated as an intracellular mediator of "non-synaptic" GABA response, although the downstream components of this signalling pathway are still unknown.

Concluding remarks

Recent data have strengthened the notion that GABA exerts morphogenetic functions during development in addition to its role as an inhibitory neurotransmitter. Although valuable new data have been provided with the recent advances in the molecular cloning and characterisation of the components of the GABAergic signalling pathway, the underlying molecular mechanisms remain largely a matter of speculation. The difficulties originate mainly from the widespread distribution of GABA and the variety of functions it may exert. More insights can be provided by transgenic and knock-out as well as in vitro differentiation models, which could be designed to manipulate individual components of the GABA signalling pathway.

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