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$GABA_B$-receptor splice variants GB1a and GB1b in rat brain: developmental regulation, cellular distribution and extrasynaptic localization

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Abstract

$GABA_B$ (γ-aminobutyric acid)-receptors have been implicated in central nervous system (CNS) functions, e.g. cognition and pain perception, and dysfunctions including spasticity and absence epilepsy. To permit an analysis of the two known $GABA_B$-receptor splice variants $GABA_B$-R1a (GB1a) and $GABA_B$-R1b (GB1b), their distribution pattern has been differentiated in the rat brain, using Western blotting and immunohistochemistry with isoform-specific antisera. During postnatal maturation, the expression of the two splice variants was differentially regulated with GB1a being preponderant at birth. In adult brain, GB1b-immunoreactivity (-IR) was predominant, and the two isoforms largely accounted for the pattern of $GABA_B$-receptor binding sites in the brain. Receptor heterogeneity was pronounced in the hippocampus, where both isoforms occurred in CA1, but only GB1b in CA3. Similarly, in the cerebellum, GB1b was exclusively found in Purkinje cells in a zebrin-like pattern. The staining was most pronounced in Purkinje cell dendrites and spines. Using electron microscopy, over 80% of the spine profiles in which a synaptic contact with a parallel fibre was visible contained GB1b-IR at extrasynaptic sites. This subcellular localization is unrelated to GABAergic inputs, indicating that the role of $GABA_B$-receptors *in vivo* extends beyond synaptic GABAergic neurotransmission and may, in the cerebellum, involve taurine as a ligand.

Introduction

γ-Aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system (CNS), exerts its effects on different time scales depending on whether its action is mediated by $GABA_A$- or $GABA_B$-receptors. The former are fast-acting GABA-gated chloride channels, while the latter are G-protein-coupled receptors that modulate multiple signal transduction pathways, e.g. inhibiting adenylate cyclase, stimulating phospholipase A2, activating K^+ channels, inhibiting voltage-dependent Ca^{2+} channels and regulating inositol phospholipid hydrolysis (for review see, Misgeld *et al*., 1995; Bowery, 1996). In line with this multiplicity of signal transduction, pharmacological evidence points to $GABA_B$-receptor heterogeneity with regard to the regulation of spasticity, nociception, cognition and absence epilepsy (Brugger *et al*., 1993; Snead, 1994; Castro-Lopes *et al*., 1995; Misgeld *et al*., 1995; Caddick & Hosford, 1996). On the structural level, two $GABA_B$-receptor isoforms, called $GABA_B$-R1a (GB1a) and $GABA_B$-R1b (GB1b), have recently been identified, whereby the first 147 residues in GB1a are replaced by alternative splicing of the N-terminal region with 18 different residues in GB1b (Kaupmann *et al*., 1997).

A functional differentiation of $GABA_B$-receptors *in situ* has not been achieved, which is, to a large part, due to the lack of information on the cellular localization of $GABA_B$-receptor isoforms. Presently, only a low-resolution map of $GABA_B$-receptor distribution is available based on autoradiography and *in situ* hybridization (Bowery *et al*., 1987; Chu *et al*., 1990; Kaupmann *et al*., 1997), showing the presence of the receptor mainly in the thalamus, cerebral cortex, molecular layer of the cerebellum and spinal cord dorsal horn. However, a distinctive localization of the $GABA_B$-receptors isoforms has not been accomplished. Therefore, in the present study, the cellular and subcellular localization of the GB1a and GB1b isoforms was investigated in the rat brain using specific antibodies in combination with confocal laser scanning microscopy and electron microscopy. The results are in line with the view that the two receptor isoforms represent the entire complement of $GABA_B$-receptors. The two isoforms differ in their developmental regulation and regional distribution, with GB1b predominating in adulthood. Most strikingly, the GB1b isoform, in addition to its synaptic localization, was localized extrasynaptically, as shown in Purkinje cell dendrites and spines. This subcellular localization is unrelated to GABAergic input and points to $GABA_B$-receptors as being activated by spillover of GABA released from adjacent synapses or even as targets for mediating the action of taurine.

Materials and methods

Antibodies

Antisera were raised in rabbits and guinea-pigs against synthetic peptides derived from rat cDNA sequences selected to be either isoform specific (antisera $gb1a_{(83–107)}$; $gb1b_{(1–18)}$) or common to both

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isoforms (antiserum gb1a,b$_{(922–944)}$) (Kaupmann *et al*., 1997). The polyclonal antisera were purified by affinity chromatography using the peptide antigen coupled to thiopropyl-Sepharose 6B (Benke *et al*., 1991). For immunohistochemistry, both affinity-purified antibodies and crude serum were used, with the same results. Double-immunofluorescence staining experiments were performed with antibodies against the GABA$_A$-receptor β2,3-subunits (Fritschy & Mohler, 1995), glutamic acid decarboxylase (GAD; GC 3008, Affiniti Research, Exeter, UK), and zebrin II (Brochu *et al*., 1990).

Western blotting

Crude membrane fractions were prepared from Sprague–Dawley rats (Institute for Laboratory Animal Sciences, University of Zürich, Switzerland) and subjected to Western blotting as described (Benke *et al*., 1996). Immunoreactivity (IR) was detected by the chemoluminescence method (DuPont NEN) and quantified by densitometry with a high-resolution image analysis system (MCID M2, Imaging Research, St-Catherines, Ontario). For calibration, X-ray films were exposed to Western blots of increasing protein concentrations (5–40 μg) for varying times.

Immunohistochemistry

Adult rats were anaesthetized with pentobarbital (50 mg/kg, i.p.) and perfused with 2–4% paraformaldehyde in 0.15 M phosphate buffer (pH 7.4) containing 0.2% picric acid. For light microscopy, brains were postfixed for 3–6 h and then pretreated with microwave irradiation for enhancing the detection of synaptic receptor proteins (Fritschy *et al*., 1998). Sections were cut at 40 μm with a freezing microtome and processed for immunofluorescence and immunoperoxidase staining with conventional protocols (Fritschy & Mohler, 1995). As a control, primary antibodies were replaced with non-immune serum, or were preabsorbed with their respective peptide antigen, as described (Fritschy & Mohler, 1995). No specific staining was observed in either case. Immunofluorescence staining experiments were analysed by confocal laser scanning microscopy (Leica TCS 4D, Heidelberg, Germany), using the Imaris software (Bitplane AG, Zürich, Switzerland) for image processing.

For electron microscopy, tissue was processed for pre-embedding immunoperoxidase staining with the gb1b$_{(1–18)}$ antiserum, as described (Meskenaite, 1997). Serial ultrathin sections throughout the thickness of the molecular layer were cut from selected areas at 2–4 μm from the surface. Synapses and dendrites were identified with the criteria of Palay & Chan-Palay (1974). Quantification of immunoreactive structures was performed on six blocks of cerebellar cortex from three rats, using photographs at a final magnification of 33 170 ×. A total of 471 spine profiles was sampled over an area of 662 μm^2. No statistical difference (chi-square test) in sampling frequency was found between any pair of the three sampled animals.

Results

Identification of the GABA$_B$-receptor splice variants GB1a and GB1b in adult and developing rat brain

Antisera were raised against N-terminal sequences specific for either GB1a or GB1b (Kaupmann *et al*., 1997), called gb1a$_{(83–107)}$ and gb1b$_{(1–18)}$. In addition, a pan-antiserum was raised against a C-terminal sequence common to both isoforms, called gb1a,b$_{(922–944)}$. The specificity of the antisera was confirmed by Western blotting of brain membranes, in which proteins of 130 and 100 kDa were identified as GB1a and GB1b, respectively, while the pan-antiserum recognized both of these protein species (Fig. 1). These molecular sizes correspond to the size of the two native GABA$_B$-receptor isoforms identified by photoaffinity labelling with the antagonist [^{125}I]CGP 71872 (Kaupmann *et al*., 1997). The amino acid sequences deduced from cDNA predict lower values for the size of GB1a and GB1b (106 and 92 kDa, respectively), in line with *N*-glycosylation of both isoforms *in situ* (Kaupmann *et al*., 1997). Furthermore, the specificity of the three antisera was verified by Western blotting of recombinant GABA$_B$-receptors. Following transfections of HEK 293 cells with GB1a and/or GB1b cDNAs, only the expected single protein band was detected, while both protein bands were stained with the pan-antiserum (not shown).

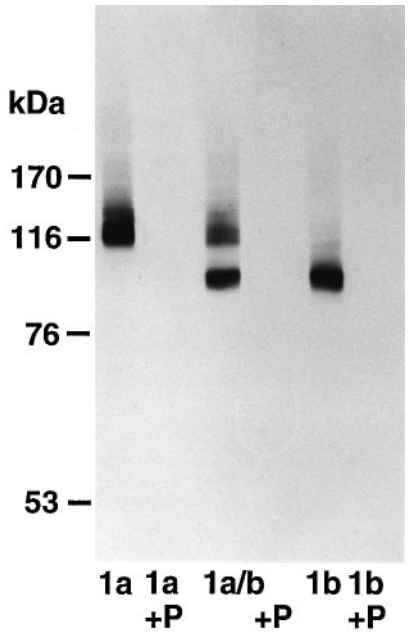

FIG. 1. Identification of the GABA$_B$-receptor GB1a and GB1b isoforms by Western blotting of adult rat brain membrane preparations. Lanes 1–2: GB1a-IR detected with the gb1a$_{(83–107)}$ antiserum; lanes 3–4: doublet representing the GB1a- and GB1b-IR detected with the gb1a,b$_{(922–944)}$ antiserum raised against the C-terminus common to both splice variants; lanes 5–6: GB1b-IR detected with the gb1b$_{(1–18)}$ antiserum. + P indicates competition of the antibody reaction in the presence of 10 μg/mL of the corresponding peptide antigen.

During postnatal development, a distinct expression pattern of the GB1a and GB1b isoforms was revealed by densitometric analysis of Western blots performed at defined ontogenetic stages (Fig. 2A). The intensity of the GB1a-IR was highest within the first postnatal days (P0–P5) and decreased rapidly within 2 weeks to almost adult levels. By contrast, the GB1b-IR increased after P5 and reached its maximum around P10 before decreasing gradually in juvenile rats (Fig. 2A). The relative abundance of GB1a and GB1b was determined by directly comparing the intensity of their staining with the gb1a,b$_{(922–944)}$ antiserum, which recognizes a common epitope. At P0, the GB1a-IR was five times more intense than the GB1b-IR. Both proteins were expressed at equal levels at P10 (exceeding adult values by 1.5- and 3-fold, respectively), and GB1b was twice as abundant as GB1a in the adult brain (Fig. 2A). Thus, during brain maturation, the overall expression of GABA$_B$-receptors decreased. However, this process is the result of opposite regulation of the prevalence of the two isoforms, with GB1a being preponderant at birth and GB1b in the adult brain.

Differential regional distribution of the GB1a and GB1b splice variants

In most regions of the adult brain, the GB1b-IR exceeded the GB1a-IR, the difference being highest in the cerebral cortex, thalamus and

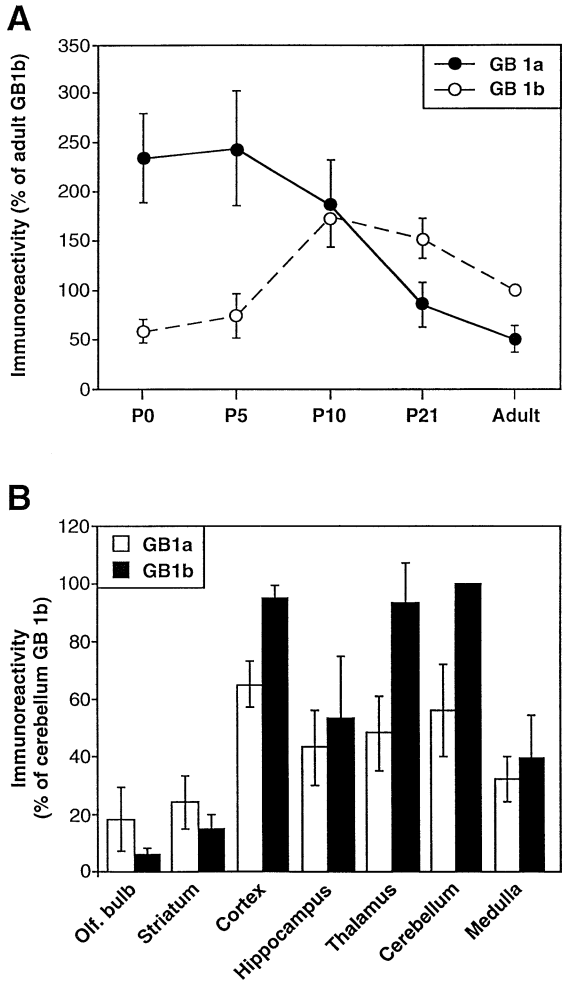

FIG. 2. (A) Expression pattern of GB1a and GB1b during postnatal development. Densitometric analysis of six independent Western blots stained with the gb1a,$b_{(922–944)}$ antiserum. Data are normalized relative to the intensity of the GB1b-IR in adult tissue. The relative expression levels of the GB1a and GB1b isoforms can be compared directly at each age, as the antiserum was raised against a peptide sequence common to both isoforms. (B) Differential regional distribution of GB1a and GB1b in adult rat brain. The relative abundance of the two isoforms in each region was determined by densitometry in five independent Western blots stained with the gb1a,$b_{(922–944)}$ antiserum. Data are normalized relative to the intensity of the GB1b-IR in the cerebellum. For both studies, similar results were also obtained with the isoform-specific antisera.

cerebellum, as shown by Western blotting with the pan-antiserum gb1a,$b_{(922–944)}$ (Fig. 2B). It is only in the olfactory bulb and striatum that the inverse ratio of the two isoforms was found (Fig. 2B). These results were confirmed immunohistochemically, though only the gb1$b_{(1,18)}$ and gb1a,$b_{(922–944)}$ antisera were suitable for use in fixed tissue. Nevertheless, a distinct and highly selective distribution could be identified for each of the two isoforms by subtractive analysis (Fig. 3), with the GB1b-IR essentially corresponding to the distribution of $GABA_B$-receptor binding sites revealed autoradiographically in the adult brain (Bowery *et al.*, 1987; Chu *et al.*, 1990). Thus, a very intense GB1b staining was detected in the medial habenula and interpeduncular nucleus, with distinct axonal labelling present in the fasciculus retroflexus, which connects these two nuclei, suggesting anterograde axonal transport of the receptor protein. The GB1b-IR was also prominent in the molecular layer of the cerebellum, thalamus, superficial cortical layers and spinal cord dorsal horn (Fig. 3). Moder-

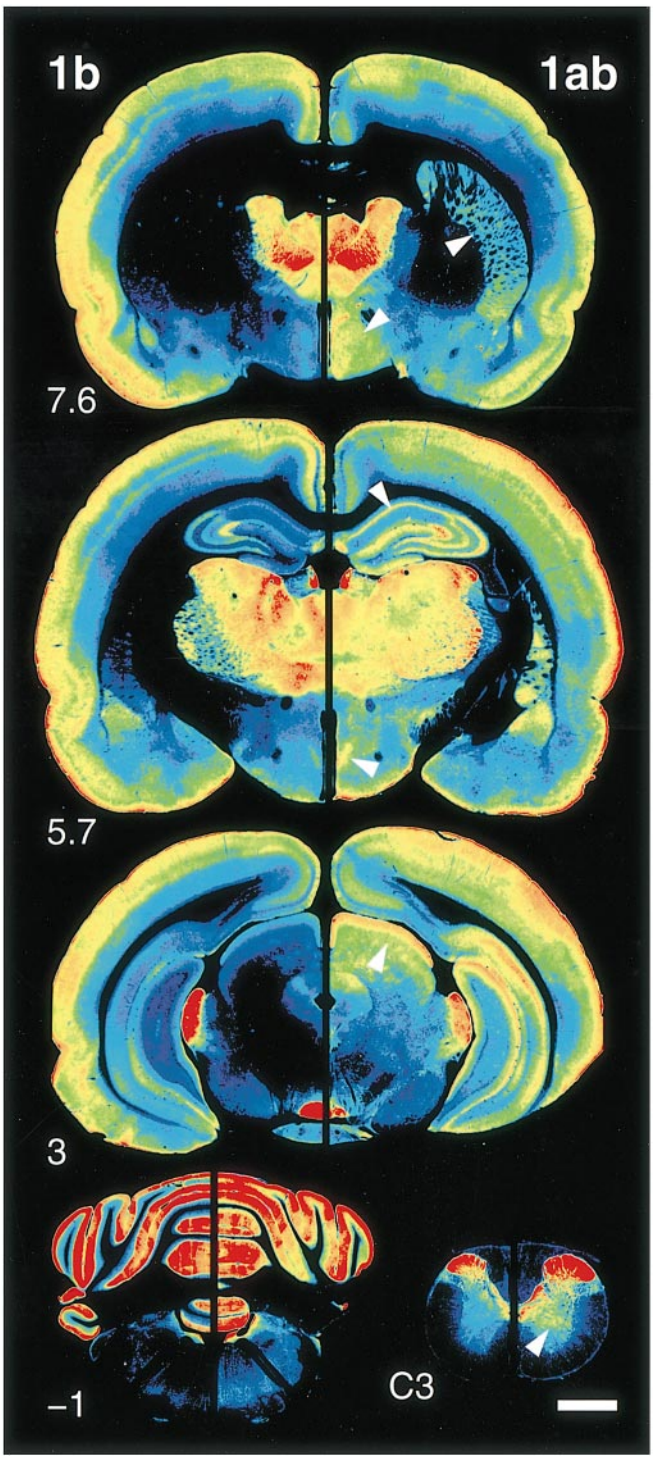

FIG. 3. Comparative regional distribution of GB1b-IR (left hemisection) and GB1a,b-IR (right hemisection) in adult rat brain and cervical spinal cord. Colour-coding of sections processed for immunoperoxidase staining ranges from dark blue for background, to blue, green, yellow, orange and red, for maximal staining intensity. At each level, pairs of matching hemi-sections are arranged as mirror images to facilitate the comparison. Arrowheads point to regions expressing the GB1a,b-, but not GB1b-IR, suggesting the selective presence of the GB1a isoform. Numbers indicate the approximate location of the five levels relative to standard stereotaxic coordinates (Paxinos & Watson, 1986). Scale bar, 1 mm.

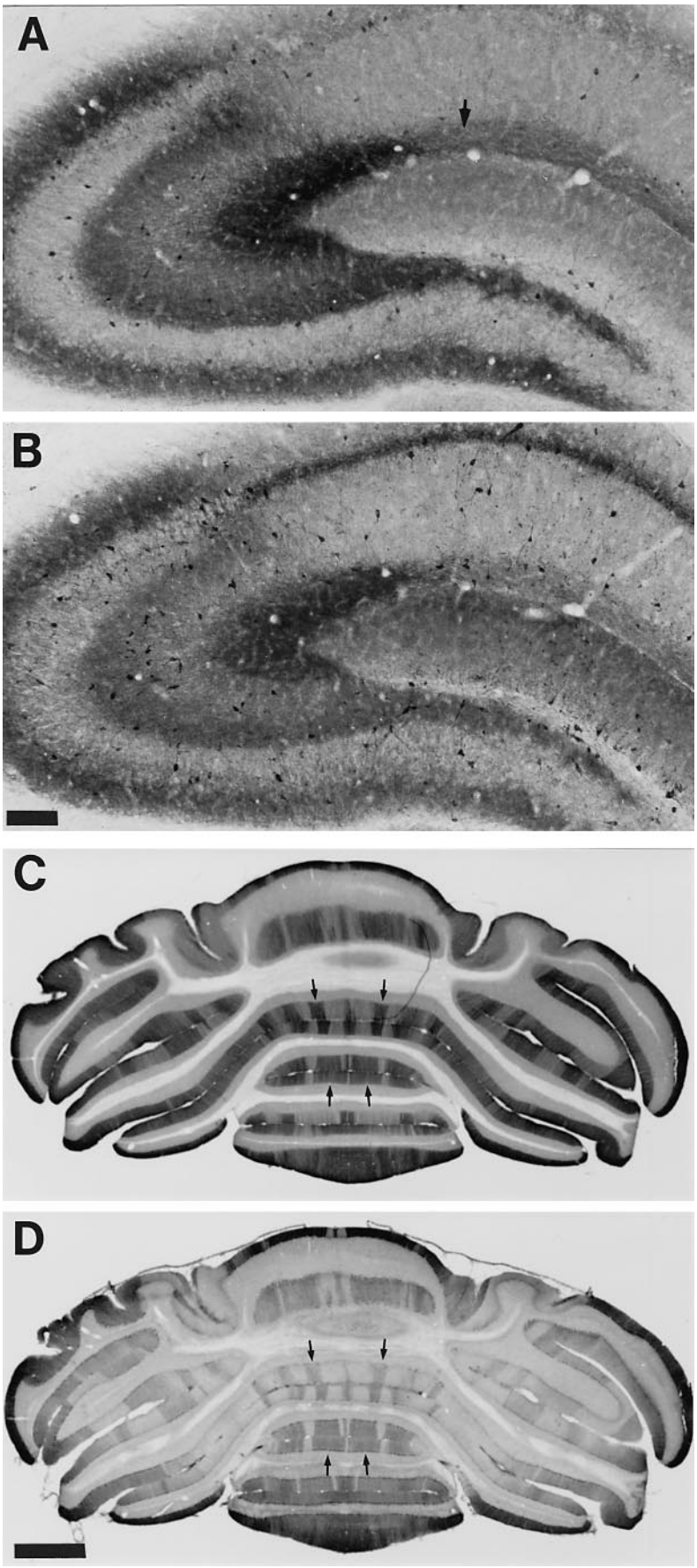

FIG. 4. (A,B) Comparative distribution of the GB1b- and GB1a,b-IR in the hippocampal formation of adult rats, as seen in transverse sections processed for immunoperoxidase staining. Note the selective distribution of the GB1b-IR in CA3, in the stratum lacunosum-moleculare of CA1 (arrow), and in a subset of non-pyramidal cells. By comparison, the prominent staining seen in the pyramidal cell layer of CA1 and in the dentate gyrus with the $gb1a,b_{(922–944)}$ antiserum (B) suggests the selective presence of GB1a in these regions. (C,D) 'Zebrin-like' distribution of the GB1a,b-IR in the molecular layer of the cerebellum. The alternation of bands strongly and weakly stained for GB1a,b (C) corresponds precisely to the distribution of zebrin II-IR (arrows), as seen in an adjacent section (D). Scale bars, (A,B) 100 μm; (C,D) 500 μm.

ate levels were detected in the hippocampus and amygdala, while GB1b-IR was of low abundance in the olfactory bulb, basal ganglia, mesencephalon and pons. White matter was free of IR, except for the fasciculus retroflexus mentioned above. Staining with the $gb1a,b_{(922–944)}$ antiserum was widespread throughout the brain. By subtractive analysis (Fig. 3), the presence of the GB1a isoform was evident in the striatum, hippocampus CA1 field, dentate gyrus, preoptic area, hypothalamus, tectum, central grey, granule cell layer of the cerebellum, and brainstem (Fig. 3). These findings indicate that GB1a- and GB1b-IR correspond to distinct subtypes of $GABA_B$-receptors, with largely different distribution patterns in the CNS.

$GABA_B$-receptor heterogeneity was most remarkable in the hippocampal formation, with the CA1 and CA3 fields differing in relative abundance and cellular distribution of the two isoforms (Fig. 4A,B). In CA1, the $gb1a,b_{(922–944)}$ antiserum revealed a prominent staining of the pyramidal cell layer and a weak to moderate staining in the dendritic layers, whereas the $gb1b_{(1–18)}$ antiserum produced only a diffuse signal in the stratum lacunosum-moleculare (Fig. 4A,B). GB1a and GB1b are therefore present in different circuits in this region. In CA3, both antisera produced identical staining patterns, suggesting the selective presence of GB1b only. The dentate gyrus exhibited a weak GB1b-IR and a moderate GB1a,b-IR, pointing to the preferential expression of GB1a in the molecular layer (Fig. 4A,B). Finally, a subset of non-pyramidal cells throughout the hippocampal formation was strongly labelled with both antisera. Since numerous interneurons are known to express high levels of $GABA_A$-receptors, the presence of both types of receptors within single interneurons was tested by double-immunofluorescence staining, with either the $gb1b_{(1–18)}$ or $gb1a,b_{(922–944)}$ antiserum combined with the monoclonal antibody bd-17, which recognizes both the β2- and β3-subunits of the $GABA_A$-receptor. This analysis revealed that $GABA_A$- and $GABA_B$-receptors are markers of mutually exclusive populations of interneurons in the dentate gyrus (Fig. 5A) and hippocampus. Functional and pharmacological heterogeneity of $GABA_B$-receptors might thus have a morphological basis in the distinct regional and cellular localization of these two isoforms.

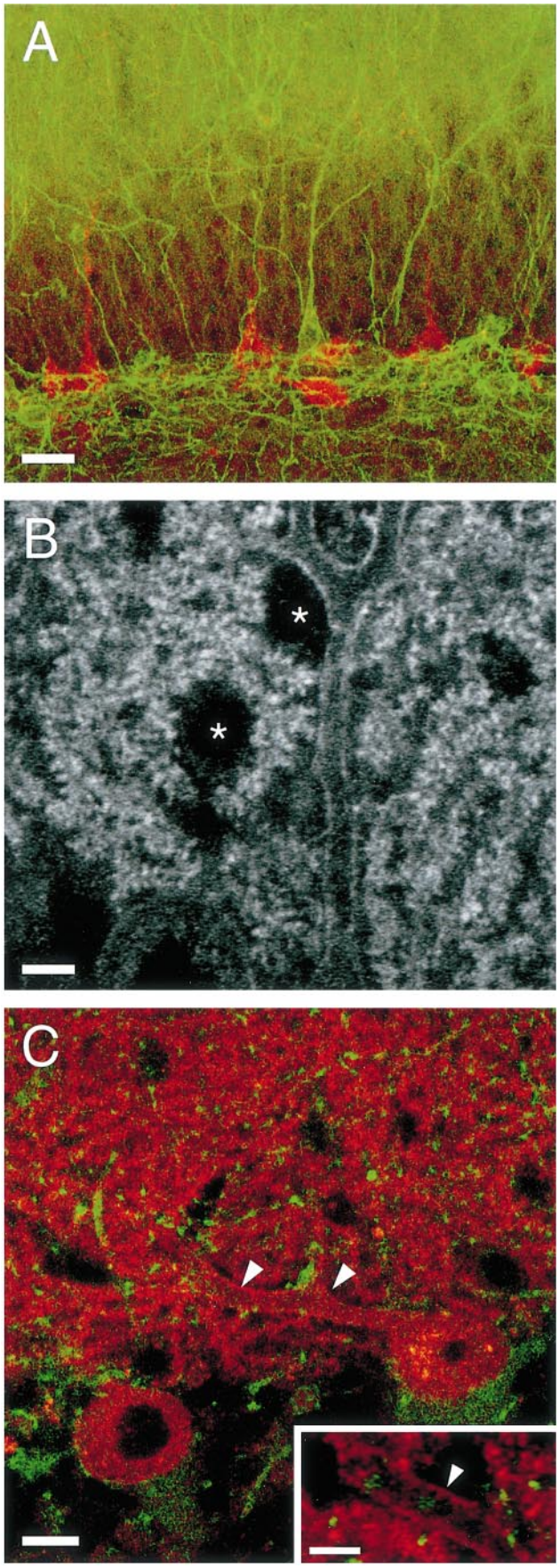

FIG. 5. (A) Double-immunofluorescence staining with the $gb1b_{(1–18)}$ antiserum (red) and the monoclonal antibody bd-17 (recognizing the $GABA_A$-receptor β2,3 subunits; green) in the dentate gyrus. Visualization by confocal laser scanning microscopy reveals that interneurons positive for gb1b are distinct from those stained with bd-17, although both populations are intermingled in the granule cell layer. Images from the green and red channels were sampled simultaneously and merged for display (colocalization would appear yellow). The image was generated by superposition of 18 confocal images spaced by 300 nm. (B) Subcellular distribution of the GB1b-IR in Purkinje cells, as seen in a single confocal plane through the molecular layer of the cerebellum; note the immunoreactivity around the Purkinje cell bodies and along dendritic shafts and in presumptive spines. Unstained oval structures (stars) represent cell bodies of stellate or basket cells (superposition of three confocal images spaces by 250 nm). (C) Double-immunofluorescence staining with the $gb1b_{(1–18)}$ antiserum (red) and a GAD antibody (green) in the molecular layer of the cerebellum. Notice that while GAD-positive fibres are scattered through the molecular layer, the GB1b-IR along the main dendritic shaft of the Purkinje cells (arrowheads) or in the dendritic spines is not apposed to sites of GAD-IR. The image represents the superposition of eight confocal images spaced by 250 nm. The inset shows a portion of the main dendritic shaft of another Purkinje cell, as seen in a single confocal image, to illustrate that the immunoreactivity is concentrated along the surface of the dendrite. Images from the green and red channels were sampled simultaneously and merged for display (colocalization would appear yellow). Scale bars: (A) 20 μm; (B,C) 5 μm.

Extrasynaptic localization of $GABA_B$-receptors in the cerebellum

$GABA_B$-receptors have been postulated to presynaptically modulate transmitter release in parallel fibre synapses in the cerebellar molecular layer (Misgeld *et al*., 1995; Bowery, 1996). Yet, Purkinje cells appeared to be by far the most intensely immunoreactive neurons in the cerebellum, irrespective of whether they were stained with the $gb1b_{(1–18)}$ or $gb1a,b_{(922–944)}$ antiserum. A weak labelling of granule cell somata and glomeruli was seen only with the pan-antiserum ($gb1a,b_{(922–944)}$). Other cells of the cerebellar cortex (basket and stellate cells, Golgi type II cells and Bergman glia) were distinctly immunonegative. The cell type-specific distribution of the GB1b receptor isoform was therefore investigated in more detail. On the regional level, profound differences in labelling intensity of Purkinje cells were observed in transverse sections, with 'zebrin-like' bands (Brochu *et al*., 1990) of strong- and weak-IR alternating irregularly in the molecular layer (Fig. 4C). The abrupt transitions between intensely and weakly stained Purkinje cells indicated that even neighbouring neurons can express strikingly different amounts of $GABA_B$-receptor-IR. Staining of adjacent sections with an antibody against zebrin II (Brochu *et al*., 1990) revealed the complete overlap between the two markers, with zebrin-positive Purkinje cells being intensely immunoreactive for GB1b (Fig. 4D). Co-localization of both markers within individual cells was confirmed by double-immunofluorescence staining (not shown).

The prominent staining of Purkinje cells was mainly present on cell bodies and along dendritic trees, as shown by confocal laser scanning microscopy (Fig. 5B). Intracellular staining was also detected, but it varied considerably from cell to cell, irrespective of the intensity of dendritic immunoreactivity. To determine whether the GB1b-IR on dendritic shafts was located at sites of GABAergic input, double-immunofluorescence staining was performed with an antiserum against GAD. This analysis revealed that the vast majority of GAD-positive puncta in the molecular layer were not closely apposed to sites of GB1b-IR (Fig. 5C). Thus, the GB1b staining on Purkinje cell dendrites was apparently not associated with GABAergic synapses.

The ultrastructural distribution of GB1b in the molecular layer was therefore investigated further by pre-embedding electron microscopy. Purkinje cell somata and dendrites exhibited a distinct labelling along the plasma membrane (Fig. 6A) and intracellularly in close association with the endoplasmatic reticulum. Spines were even more intensely labelled, although the neck was frequently only weakly positive (Fig. 6B,C). A semiquantitative analysis revealed that 81.3% of spine profiles in which a synaptic contact from a parallel fibre was visible were positive for the $gb1b_{(1–18)}$ antiserum. Remarkably, however, both the pre- and postsynaptic densities were always immunonegative (Fig. 6B,C). All other synaptic profiles in the molecular layer, in particular those contacting presumed basket cells dendrites, were also consistently devoid of immunoreactivity. The lack of synaptic labelling was not due to methodological limitations, since synaptic profiles were frequently labelled in sections of the cerebral cortex. These findings corroborate the results from light microscopy and indicate that the GB1b isoform can be selectively localized at extrasynaptic sites.

Discussion

Mammalian neurotransmitter receptors largely exist in multiple isoforms, either encoded by distinct genes or generated by alternative mRNA splicing (Zifa & Fillion, 1992; Seeburg, 1993; Galzi & Changeux, 1995; Pin & Duvoisin, 1995). With the recognition of two $GABA_B$-receptor isoforms (Kaupmann *et al*., 1997), metabotropic

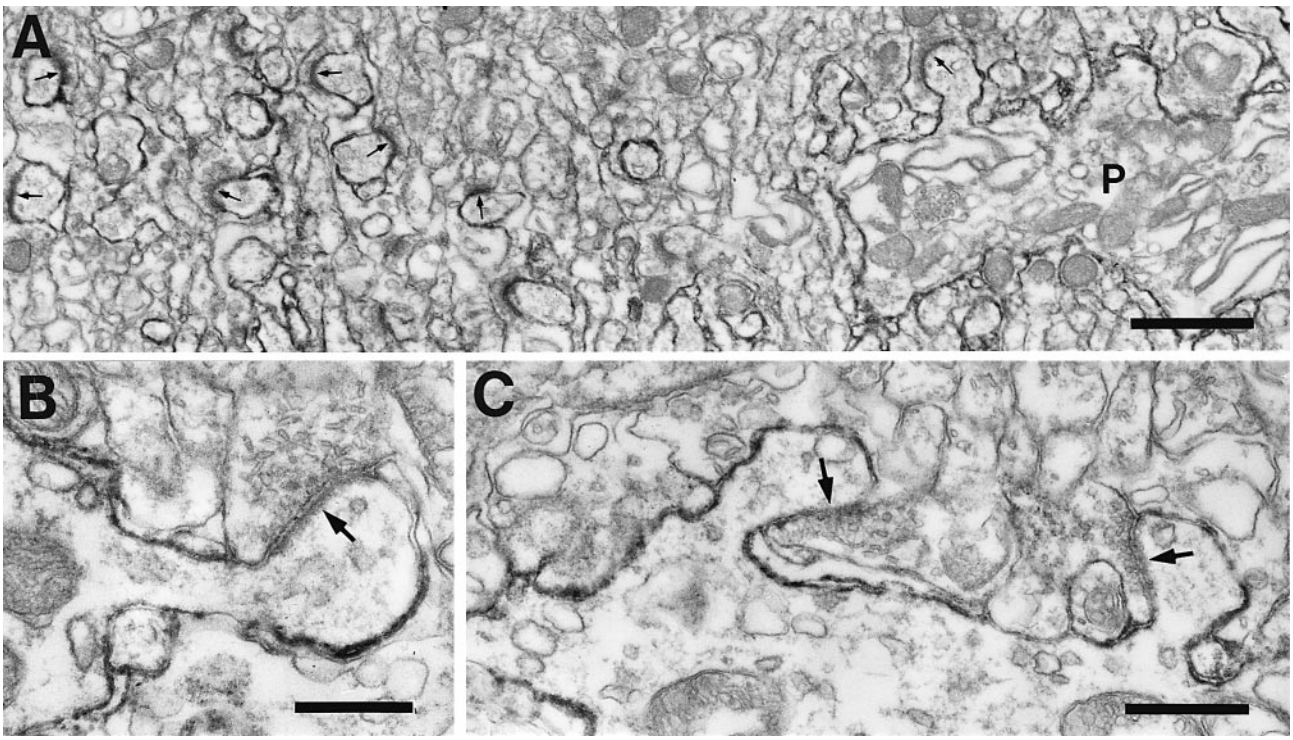

FIG. 6. Ultrastructural distribution of the GB1b-IR in the molecular layer of the cerebellum. (A) Selective labelling of the plasma membrane outlining a Purkinje cell dendrite (P) and numerous spine profiles. Those with a visible synapse are labelled with arrows. Note that the neuropil is otherwise devoid of GB1b-IR. (B,C) Higher magnification micrographs of Purkinje cell spines, demonstrating the lack of GB1b-IR in the synaptic specialization (arrows). Scale bars: (A) 1 μm; (B,C) 0.5 μm.

GABAergic transmission has to be analysed in terms of receptor heterogeneity. While additional $GABA_B$-receptor isoforms cannot be ruled out, the immunohistochemical distribution of GB1a and GB1b closely matches that of $GABA_B$-receptor binding sites in adult and developing brain (Bowery *et al*., 1987; Chu *et al*., 1990; Turgeon & Albin, 1993, 1994), suggesting that the two isoforms represent the major $GABA_B$-receptor entities in the brain. The two receptor populations are differentially regulated during postnatal development, and exhibit distinct regional and cellular distribution patterns in adult rat brain.

Heterogeneity of $GABA_B$-receptors

Since the two $GABA_B$-receptor isoforms are generated by alternative splicing of a single gene, their distinct developmental and regional expression pattern may be accounted for by differential regulation of mRNA splicing. A preliminary *in situ* hybridization analysis indicated that GB1a mRNA is expressed at higher levels than GB1b mRNA in adult rat brain (Bischoff *et al*., 1997), which is the reverse of the ratio of immunoreactivity of the two receptors detected by Western blotting and immunohistochemistry (Figs 2 and 3). Since this ratio was determined quantitatively with the pan-antiserum gb1a,$b_{(922–944)}$ recognizing the same epitope on the two isoforms, the discrepancy cannot be due to a differential affinity for GB1a and GB1b. Rather, the mismatch between the respective mRNA and protein levels suggests a differential translational control, or mRNA stability, of the two isoforms. On the protein level, GB1a is most prevalent in neonatal brain and remains expressed at moderate levels throughout the adult rat brain. In contrast, GB1b represents receptors arising to a large part during the second and third postnatal weeks, i.e. during the peak of synaptogenesis, and having a more selective regional distribution. These receptors are about twice as abundant as GB1a in adult brain (Fig. 2A), suggesting that they represent the majority of $GABA_B$-receptors. However, an important contribution of GB1a to $GABA_B$-receptor-mediated mechanisms can be expected in those areas, e.g. the striatum, hippocampus CA1 pyramidal cell layer, hypothalamus or superior colliculus, where GB1b is virtually absent.

Our results suggest a distinct functional role of the two isoforms, as best seen in the CA1 region of the hippocampus, where GB1a predominates in the pyramidal cell layer, whereas GB1b is restricted to the stratum lacunosum-pyramidale. Furthermore, $GABA_B$-receptors are selectively present in a subset of non-pyramidal cells that does not express high levels of $GABA_A$-receptors (Fig. 5A). A cell type-specific expression of a $GABA_B$-receptor isoform was also evident in the cerebellum (see below). It remains unclear whether, in certain brain regions, GB1a and GB1b can be expressed in the same cell.

It is evident that the extensive pharmacological heterogeneity attributed to $GABA_B$-receptors (Bonanno & Raiteri, 1993; Lanza *et al*., 1993; Cunningham & Enna, 1996; Deisz *et al*., 1997) cannot be accounted for solely by the differential distribution of the GB1a and GB1b isoforms. For example, Calabresi *et al*. (1990) postulated various distinct receptor subtypes in the neostriatum, in which GB1a appears to be virtually the only isoform expressed. Post-translational modifications of $GABA_B$-receptor isoforms, e.g. protein phosphorylation and coupling to differential effector systems, are likely to add to their functional and pharmacological heterogeneity.

Extrasynaptic localization of $GABA_B$-receptors in the cerebellum

The distribution of $GABA_B$-receptor isoforms in the cerebellum is most remarkable based on their specific cellular and subcellular localization.

1 GB1b is selectively present in Purkinje cells, while low levels of GB1a are found in granule cells. This finding is in line with the *in situ* hybridization analysis of Bischoff *et al*. (1997) and strongly supports previous indirect evidence for the presence of $GABA_B$-receptors in Purkinje cells (Martinelli *et al*., 1992; Turgeon & Albin, 1993; Vigot & Batini, 1997). The possibility that $GABA_B$-receptors play a major role in presynaptic modulation of transmitter release from parallel fibre terminals can therefore be excluded.

2 The level of $GABA_B$-receptor expression in the molecular layer is not uniform, as first suggested by Turgeon & Albin (1993). The pattern of intensely stained bands of Purkinje cells coincides with anatomical and functional compartments previously defined by zebrins (Brochu *et al*., 1990; Hawkes *et al*., 1992). Several hypotheses may be advanced to account for this observation: the genetic programs governing the expression of zebrins (Hawkes *et al*., 1992; Hawkes & Herrup, 1995) may also entrain the expression of $GABA_B$-receptors. Alternatively, since zebrin II is identical to the respiratory enzyme aldolase C (Ahn *et al*., 1994), zebrin-positive compartments may reflect a higher level of metabolic activity, suggesting that the expression of $GABA_B$-receptors is dynamically regulated depending on the requirements of Purkinje cells. Finally, $GABA_B$-receptors may be involved in the regulation of zebrins or other enzymes, e.g. acetylcholine esterase, that are also enriched in zebrin-positive stripes (Hawkes *et al*., 1992).

3 The selective enrichment of GB1b in dendritic spines is unrelated to GABAergic input, and is therefore difficult to reconcile with the proposed role of $GABA_B$-receptors as mediating part of the synaptic inhibitory action of GABA in Purkinje cells (Vigot & Batini, 1997). Furthermore, the extrasynaptic localization of GB1b suggests a neuromodulatory role for $GABA_B$-receptors. In particular, $GABA_B$-receptors could be activated by spillover of GABA released from adjacent synapses. However, since the distribution of GB1b in Purkinje cell dendrites and spines closely matches that of taurine (Magnusson *et al*., 1988; Ottersen, 1988), it is tempting to speculate on an interaction between taurine and $GABA_B$-receptors in Purkinje cells. Taurine is a weak agonist of $GABA_B$-receptors (Kontro & Oja, 1990) and has been shown to inhibit Purkinje cells, in part by blocking Ca^{2+} entry into their dendrites (Okamoto *et al*., 1983a, 1983b). $GABA_B$-receptors might thus mediate an autoregulatory action of taurine on Purkinje cells. In any case, the results show that the functional role of $GABA_B$-receptors *in vivo* extends beyond that of mediating synaptic GABAergic neurotransmission, and may include taurine as a physiological ligand.

Acknowledgements

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Abbreviations

CNS, central nervous system; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; GB1a, $GABA_B$-R1a isoform; GB1b, $GABA_B$-R1b isoform; IR, immunoreactivity.

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