

**Abstract**—The GABA<sub>A</sub> receptor is a ligand-operated chloride channel. It has a pentameric structure. In mammalian brain different subunits are recruited from four gene subfamilies. Using immunocytochemistry, we investigated the distribution of the 13 GABA<sub>A</sub> receptor subunits in the hippocampus of the rat. GABA<sub>A</sub> receptor subunits were heterogeneously distributed within different hippocampal subfields. High concentrations of α<sub>1</sub>-, α<sub>2</sub>-, α<sub>3</sub>-, β<sub>2</sub>-, γ<sub>2</sub>- and δ-immunoreactivities were observed within the molecular layer of the dentate gyrus, representing the dendritic area of the granule cells. In the hippocampus proper, the predominant GABA<sub>A</sub> receptor subunits were α<sub>2</sub>, α<sub>3</sub>, α<sub>4</sub>, β<sub>3</sub> and γ<sub>2</sub> that were located throughout the strata radiatum and oriens of CA1 to CA3. Immunocytochemical staining was there less prominent for α<sub>1</sub>-, β<sub>1</sub>-, β<sub>2</sub>-, γ<sub>2</sub>- and δ-subunits. In the hippocampus proper, the β<sub>1</sub>-subunit was preferentially located in CA2. The α<sub>x</sub>- and δ-subunits were somewhat more abundant in CA1 than in CA3. Numerous local circuit neurons in the hippocampus proper and the hilus of the dentate gyrus contained α<sub>1</sub>-, β<sub>2</sub>-, γ<sub>2</sub>- and/or δ-subunits. α<sub>3</sub> and γ<sub>2</sub> were present only in minute amounts and no α<sub>4</sub>-IR was detected in the hippocampal formation.

The distribution of the GABA<sub>A</sub> receptor subunits indicates the existence of heterogeneously constituted GABA<sub>A</sub> receptor complexes within various hippocampal subfields, which may exert different physiological or pharmacological properties upon stimulation by GABA or its agonists. © 1997 IBRO. Published by Elsevier Science Ltd.

Key words: basket cells, benzodiazepines, dentate gyrus, limbic system, local circuit neurons.

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**GABA<sub>A</sub> RECEPTOR SUBUNITS IN THE RAT HIPPOCAMPUS I: IMMUNOCYTOCHEMICAL DISTRIBUTION OF 13 SUBUNITS**

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Abbreviations: IR, immunoreactivity; KA, kainic acid; PBS, phosphate-buffered saline.

GABA is the principal inhibitory neurotransmitter in the vertebrate brain. It is acting through two major classes of receptors: (i) GABA<sub>A</sub> receptors, which are ligand-gated chloride channels, and (ii) GABA<sub>B</sub> receptors which are G-protein coupled. GABA<sub>A</sub> receptors are presumably assembled in a pentameric structure from a number of subunit classes. Four classes of subunits, with multiple isoforms, have been identified by their sequence homology so far. In rodents, they comprise six α-subunits (α<sub>1</sub>-α<sub>6</sub>), three β-subunits (β<sub>1</sub>-β<sub>3</sub>), three γ-subunits (γ<sub>1</sub>-γ<sub>3</sub>) and one δ-subunit. Proteins derived from a fifth gene subfamily encoding p-subunits (ρ<sub>1</sub>, ρ<sub>2</sub>) presumably constitute so-called GABA<sub>C</sub> receptors in the retina. Even assuming that a functional GABA<sub>A</sub> receptor is assembled in a pentameric structure and that it requires a combination of at least one α-, one β- and one γ- (or δ-) subunit, a rather high number of subunit combinations may be considered. Recent studies on the regional distribution of mRNA species encoding for the different subunits in the brain and first immunocytochemical investigations indeed suggest the existence of different subunit combinations which may constitute molecularly and functionally different GABA<sub>A</sub> receptor subtypes.11,30,45

The hippocampal formation is important for acquisition of short-term memory.3 Some of its structures are especially prone to neuronal damage in chronic epilepsy and ischemia.36 Benzodiazepines and barbiturates are the treatment of choice in severe status epilepticus, although they may induce mild retrograde amnesia.46 These actions, at least in part, may be mediated by GABA<sub>A</sub> receptors located within the hippocampal formation. Only limited information on the subunit composition of GABA<sub>A</sub> receptors in individual subfields of the hippocampus is available. Studies performed at the mRNA level suggest a widespread distribution of the 13 known GABA<sub>A</sub> receptor subunits within the hippocampus.30,45 On the protein level, the distribution of eight different subunits (α<sub>1</sub>, α<sub>2</sub>, α<sub>3</sub>, α<sub>5</sub>, β<sub>2</sub>-3, γ<sub>2</sub> and δ) has been described.10,14,25,28,50 The β<sub>2</sub>-3 antibodies used in these studies, however, did not distinguish between the β<sub>2</sub> and β<sub>3</sub> subunits.10,25 We now
investigated by immunocytochemistry the distribution of all 13 GABAA receptor subunits in the hippocampus including a differential analysis of the β2 and β3 subunits. In accompanying papers changes in immunoreactivities of these subunits and their mRNAs in an animal model of temporal lobe epilepsy are described.33,43

EXPERIMENTAL PROCEDURES

Animals and tissue preparation
Male Sprague-Dawley rats (250–350 g, Forschungsinstitut für Versuchstierzucht, Himberg, Austria) were injected with a lethal dose of thiopental (150 mg/kg, i.p., Sanabo, Austria) and perfused immediately through the ascending aorta with 50 ml ice-cold phosphate-buffered saline (PBS; 50 mM phosphate buffer, pH 7.4 in 0.9% NaCl) followed by 200 ml chilled 4% paraformaldehyde in PBS. The brains were removed from the skulls and divided by coronal cuts into three parts. They were postfixed in the same fixative for 90 min at 4°C, then transferred to 20% sucrose in PBS and kept there for 24 h at 4°C. Thereafter the brains were rapidly frozen by immersion in −70°C isopentane for 3 min and, after evaporating the isopentane, stored in tightly sealed vials at −70°C. Sections of the same animals at three different levels of the dorsal hippocampus were removed from the skulls and divided by coronal cuts (for details see references given in Table 1). Antisera against β1, β2 and δ were purified with affinity chromatography with the respective GST-subunit fusion proteins.26 All antibodies have been characterized extensively by various methods including immunoprecipitation, Western blotting and immunocytochemistry (for further details see references given in Table 1). Antibodies against α1, β3 and δ were recognized by the respective GABAA receptor subunits, coupled to keyhole-limpet haemocyanin (α1–α3, α5, α6 and γ3) or against fusion proteins derived from DNA constructs of the maltose-binding protein gene with specific sequences of the receptor subunit genes and transcribed in Escherichia coli (αs, β1–β3, γ2, γ2 and δ).26 All antibodies have been characterized extensively by various methods including immunoprecipitation, Western blotting and immunocytochemistry (for further details see references given in Table 1). Antibodies against β1, β2 and δ were purified with affinity chromatography with the respective GST-subunit fusion proteins.26 All antibodies were characterized by immunoprecipitation and Western blotting. The β1 and β2 antibodies did not immunoprecipitate the other β subunit proteins. The δ antibody recognized several isoforms of the δ subunit proteins with an apparent molecular weight of 53–57,000 and did not precipitate a 12β3/2 receptor complex.26

RESULTS

Antibodies
In the present experiments the antibodies appeared highly specific, revealing staining patterns characteristic for each subunit in different brain areas (data not shown). Notably, subunits which were less prominently expressed in the hippocampus were detected at considerable concentrations in other

<table>
<thead>
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<th>Subunit</th>
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<th>Amino acids</th>
<th>Final conc. (µg/ml)</th>
<th>References</th>
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<td>OPSQDELK D (KLH-coupled)</td>
<td>1–9</td>
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<td>12,48–50</td>
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<td>AIGMIRKQ (KLH-coupled)</td>
<td>459–467</td>
<td>3</td>
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<td>MBP–α4–7His-fusion protein</td>
<td>379–421</td>
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<td>29</td>
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<tr>
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<td>MPTSSVODE (KLH-coupled)</td>
<td>2–10</td>
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<td>α6</td>
<td>VSSTVF (KLH-coupled)</td>
<td>429–434</td>
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<td>1–44</td>
<td>5</td>
<td>(Pelz et al., unpublished observations)</td>
</tr>
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</table>

Table 1: Antibodies used for labelling different GABAA receptor subunits

peroxidase-antiperoxidase complex (1:300, Dako Z-113, Vienna, Austria) for 120 min at room temperature. These sections were reacted with 0.4 mM 3,3-diaminobenzidine (Sigma, Munich, Germany) and 0.01% H2O2 for 4–6 min. After each incubation step, except the precipitation, three 5-min washes with Tris-HCl-buffered saline were performed. All buffers and antibody dilutions, except those for washing and reacting with dianisobenzidine, contained 0.4% Triton X-100. Normal goat serum and egg albumin (4% each) were included at all antibody dilutions and during the incubation with the peroxidase-antiperoxidase complex. Sodium azide (0.1%) was added to the primary antibody solutions to avoid growth of microorganisms.

In each experiment controls were included using the primary antibodies preadsorbed with 10 µg/ml (24 h, 4°C) of the respective synthetic peptide or fusion protein (except for γ2, for which 50 µg/ml of the fusion protein were used). Furthermore, slices were incubated without the primary antibody.

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brain regions: e.g., \( \alpha_2 \) in the cortex, striatum and tuberculum olfactorium, \( \alpha_6 \) in granule cells of the cerebellum, \( \beta_1 \) in the cerebral cortex, tuberculum olfactorium and cerebellum, \( \gamma_1 \) in the ventral pallidum and \( \gamma_3 \) in the glomerular layer of the olfactory bulb and in Purkinje cells of the cerebellum (data not shown). The general distribution of immunostaining for the different subunits was in agreement with data published so far on the immunocytochemical distribution of \( \text{GABA}_A \) receptor subunits\(^{10,11,50} \) and with data on the distribution of mRNAs\(^{30,43,45} \). In all instances staining was blocked by pre-incubation of the antibodies with the respective antigens. Staining for the \( \alpha_3 \) subunit revealed a pattern slightly different from previously published data when paraformaldehyde-perfused brain sections were used (see below)\(^{11,48} \). Therefore, in accordance with a previous report on the characterization of this antibody, acetone-fixed sections were used for labelling (Fig. 1). The most prominent staining was observed for the \( \alpha_2 \)-, \( \alpha_3 \)-, and the \( \alpha_6 \)-subunits. Less immunoreactivity (IR) was detected for \( \alpha_4 \) and only minute amounts for \( \alpha_5 \). \( \alpha_6 \)-IR was not detected in the hippocampus although it was observed at high concentrations within the granular cell layer of the cerebellum (not shown).\(^{29} \)

**\( \alpha \)-Subunits**

Among the six \( \alpha \)-subunits investigated only five (\( \alpha_1-\alpha_5 \)) were present within the hippocampal formation (Fig. 1). The most prominent staining was observed for the \( \alpha_1 \)-, \( \alpha_2 \)-, and the \( \alpha_3 \)-subunits. Less immunoreactivity (IR) was detected for \( \alpha_4 \) and only minute amounts for \( \alpha_5 \). \( \alpha_6 \)-IR was not detected in the hippocampus although it was observed at high concentrations within the granular cell layer of the cerebellum (not shown).\(^{29} \)

- **\( \alpha_1 \)-subunit.** The \( \alpha_1 \)-subunit of the \( \text{GABA}_A \) receptor was found within fibres throughout the hippocampal formation (Fig. 2). It was almost evenly distributed in the dendritic areas of pyramidal cells, in strata oriens and radiatum CA1 to CA3. Within the molecular layer of the dentate gyrus and the stratum lacunosum moleculare, even somewhat higher concentrations of the protein were detected (Fig. 2a,c). There was no staining within the principal cell layers and in the stratum lucidum. Most of the \( \alpha_1 \)-IR appeared to be located in perikarya and fibres of interneurons at the hilar surface of the granule cell layer (pyramidal-shaped basket cells) and throughout the pyramidal cell layer (Fig. 2b,d,e).

- **\( \alpha_2 \)-subunit.** The \( \alpha_2 \)-subunit was found in all subfields of the hippocampus (Fig. 3a). The highest concentration of \( \alpha_2 \)-IR was present in the molecular layer of the dentate gyrus (Fig. 3a,c). Staining for \( \alpha_2 \) was more prominent in the strata oriens and radiatum of CA3 than of CA1. Noticeable \( \alpha_2 \)-IR was seen also in the stratum lacunosum moleculare, especially at the transition zone to CA3. Staining was restricted to fibres and was not observed within perikarya.

- **\( \alpha_3 \)-subunit.** As shown in Fig. 1, besides \( \alpha_6 \), the \( \alpha_3 \)-subunit was the least expressed \( \alpha \)-subunit. In acetone-fixed sections faint staining was detected in the inner molecular layer of the dentate gyrus, in CA3 and in the stratum lacunosum moleculare (Fig. 1). In sections obtained from paraformaldehyde-perfused brains, faint (but specific) staining was also observed within principal cells (not shown).

- **\( \alpha_4 \)-subunit.** Staining for the \( \alpha_4 \)-subunit was diffuse and weaker than for the \( \alpha_1 \)-, \( \alpha_2 \)- and \( \alpha_3 \)-subunits (Figs 1, 3b). It was quite strong in the molecular layer of the dentate gyrus. Staining was also observed in the stratum oriens and radiatum of CA1 and the stratum lacunosum moleculare (Fig. 3b,d). In other parts of the hippocampus only marginal staining was detected. \( \alpha_4 \)-IR appeared to be restricted to fibres and was not seen in perikarya. Its overall distribution paralleled that of the \( \delta \)-subunit (Figs 1, 7).

- **\( \alpha_6 \)-subunit.** The \( \alpha_6 \)-subunit was present in most parts of the hippocampus proper at high concentrations (Fig. 3f). In contrast to the other \( \alpha \)-subunits, \( \alpha_6 \) was considerably more concentrated in the dendritic areas (strata radiatum and oriens) of CA1 to CA3 and in the stratum lacunosum moleculare than in the molecular layer of the dentate gyrus (Fig. 3e-g). Staining appeared to be restricted to dendrites. \( \alpha_6 \)-IR was not observed within the hilus of the dentate gyrus and not within the principal cell layers.

**\( \beta \)-Subunits**

- **\( \beta_1 \)- and \( \beta_2 \)-subunits.** The \( \beta_1 \)-subunit was present throughout the dendritic areas of the hippocampus, the strata oriens and radiatum CA1 to CA3 and the molecular layer of the dentate gyrus (Fig. 4a). It was especially concentrated within the dendrites of the CA2 sector (Fig. 4a,c). No \( \beta_1 \)-IR was observed within the pyramidal cell layers and in the hilus of the dentate gyrus.

The distribution of the \( \beta_3 \)-subunit largely matched that of \( \beta_1 \), \( \beta_3 \)-IR, however, in general was found at higher concentrations than \( \beta_1 \)-IR (Fig. 4b,e). \( \beta_3 \)-IR was most prominent in the molecular layer of the dentate gyrus and intense in the strata oriens and radiatum of CA1 and CA3. In CA2 considerably less \( \beta_3 \)-IR was observed (Fig. 4a-c). The layers of principal neurons and the terminal field of mossy fibres were spared for \( \beta_1 \) and \( \beta_3 \) (Fig. 4a,b).

- **\( \beta_2 \)-subunit.** Only faint staining was detected when the \( \beta_2 \) antibody was used at a similar concentration as the other antibodies (2 µg/ml) although prominent...
staining was observed in brain areas outside the hippo-campus, e.g., in the thalamus (Fig. 1), globus pallidus and substantia nigra. Among the β-subunits, thus β2 was the least abundant in the hippocampus. In the course of this study we then used comparatively high concentrations of the β2 antibody (15µg/ml), obtaining still specific immunostaining. Marked labelling of a great number of interneurons throughout the hippocampal formation was observed (Fig. 5). Only weak staining was seen in dendrites of pyramidal neurons (preferentially in CA1) and of granule cells (molecular layer of the dentate gyrus). The β2 antibody intensely labelled the perikarya and the dendritic trees of pyramidal-shaped basket cells in

Fig. 1. Differential distribution of 13 GABA<sub>A</sub> receptor subunits in the dorsal hippocampus. Note the different subunit expression in the molecular layer of the dentate gyrus (containing preferentially α<sub>1</sub>-, α<sub>2</sub>-, α<sub>4</sub>, β<sub>2</sub>-, β<sub>3</sub>-, γ<sub>2</sub>-, δ-IR), in CA1 (containing especially α<sub>1</sub>, α<sub>5</sub>, β<sub>2</sub>- and some α<sub>2</sub>, α<sub>3</sub>, β<sub>3</sub>, δ-IR), in CA2 (containing α<sub>1</sub>, β<sub>2</sub> and γ<sub>2</sub>-IR but little β<sub>1</sub>-IR), in CA3 (being especially rich in α<sub>2</sub>, α<sub>5</sub>, α<sub>6</sub>, β<sub>1</sub> and γ<sub>2</sub>-IR). Note the rather similar distributions of the α<sub>4</sub>- and δ-subunits. For β<sub>2</sub> a similar concentration (2µg/ml) of the β<sub>2</sub> antibody was used as for the other antibodies, resulting in a faint staining. Compare staining for β<sub>2</sub> in Fig. 5, where a higher antibody concentration (15µg/ml) was applied. In the lower right corner a Nissl-stained section is shown. Scale bar=250µm.
the hilus of the dentate gyrus (Fig. 5b–d) and basket cells located in the CA1 pyramidal cell layer (Fig. 5e,g) and in CA3 (Fig. 5f). A band of neurons located within the middle and inner molecular layer of the dentate gyrus also exposed β2-IR (Fig. 5b,c).

Among the γ-subunits, only γ2-IR was found at high concentrations in neuronal structures of the hippocampus. It was concentrated in dendrites of the stratum radiatum of CA1 to CA3 (Fig. 6a,b,d,e), in the stratum lacunosum moleculare and in the molecular layer of the dentate gyrus (especially in its inner part; Fig. 6d). Staining in the stratum oriens was diffuse but strong. Prominent γ2-IR was present in perikarya located throughout the dentate hilus (Fig. 6c). Numerous presumable type II basket cells, located close to the inner surface of the granule cell layer, were strongly labelled (Fig. 6c). However, only a few faintly stained (type I) pyramidal-shaped basket cells were found. Occasionally staining of pyramidal neurons was seen in CA1 and CA3.

γ1- and γ3-subunits. For the γ1- and γ3-subunits only faint and diffuse immunoreactivity was detected in all parts of the hippocampus (Fig. 1). The faint γ1-immunostaining appeared to be associated with astrocyte-like cells and fibres (not shown). γ3-IR was observed in fibres throughout the hippocampus especially in the terminal field of mossy fibres and, to a lesser extent, in the molecular layer and the hilus of the dentate gyrus (Fig. 1).
The overall distribution of the α₂- and α₄-subunits largely matched that of α₅-IR in an aspect of CA2 is depicted. Note also the differential distribution of these three α-subunits in the molecular layer of the dentate gyrus (containing α₂- and α₄-, but almost no α₅-IR), the high concentration of α₅-IR, present throughout the dendritic fields of CA1 to CA3 (e-g), the distribution of α₂-IR (being enriched in CA2 and CA3) and the complementary expression of α₅-IR in CA1. Note also the differential expression of the subunits in the stratum lacunosum moleculare (slm in c-e). For abbreviations see Fig. 2. Scale bars: in d=50 µm (for c-d); in b and f=250 µm (for a, b, f); in g=10 µm.

α₅-Subunit
The overall distribution of the α₅-subunit largely matched that of α₅-IR was preferentially located in the molecular layer of the dentate gyrus (Fig. 7a-d). It was also observed within the granule cell layer (Fig. 7c,d). Faint staining was present in the strata radiatum and oriens of CA1 and in the pyramidal cell layer of CA1 to CA3 (Fig. 7a,e). Dense α₅-IR was also
observed in pyramidal-shaped basket cells in the hilus of the dentate gyrus and in numerous interneurons of CA3 (Fig. 7d,e). A plexus of immunoreactive fibres was observed in the stratum lacunosum moleculare (Fig. 7b).

DISCUSSION

The present data provide a detailed survey of the immunocytochemical distribution of 13 GABA<sub>A</sub> receptor subunits in the hippocampal formation in the rat.

Specificity of antibodies and efficacy of the immunocytochemical staining

The specificity of the subunit-directed antibodies is based on their extensive previous characterization (see references in Table 1). In the present study, the validity of the immunocytochemical staining was verified by competition of the primary antibodies with their corresponding peptide antigens and by replacing primary antibodies with a non-immune serum. The regional distribution of the individual subunit proteins within the hippocampal formation and in other brain areas (data not shown) generally is in agreement with the distribution of the respective mRNAs.30,43,45

Possible localization of GABA<sub>A</sub> receptor subunits in glia

By far the largest portion of immunoreactive GABA<sub>A</sub> receptor subunits appears to be located on neuronal structures. Faint β<sub>1</sub>-, γ<sub>2</sub>- and possibly α<sub>4</sub>-IR is found in astrocyte-like structures of the hippocampus (not shown). This staining is blocked by preincubation of the antibodies with the respective antigens. It was rather weak and not unequivocal. We therefore did not include these results in our present report. Following kainic acid-induced seizures, however, transient expression of some α<sub>3</sub>- (in
Fig. 5. The $\beta_2$-subunit of the GABA$_A$ receptor. In this experiment 15 µg/ml $\beta_2$ antibody were used (compare Fig. 1 in which 2 µg/ml $\beta_2$ antibody were used). An overview (a); aspect of the dentate gyrus (b); the area extending from the stratum granulosum to the stratum oriens CA1 (c); high magnification of pyramidal-shaped basket cells with their apical dendrites extending to the molecular layer (d); basket cell in CA1 with dendrites extending to the stratum oriens (up) and the stratum lacunosum moleculare (down; e); dense plexus of interneurons with their processes in CA2 (f); interneurons in CA1 with fibres extending into the stratum oriens (g). The $\beta_2$-subunit is preferentially located within interneurons of all hippocampal subfields. Note the faint $\beta_2$-IR neurons located in the middle molecular layer of the dentate gyrus (arrows in b and c) forming an immunoreactive band (a). Scale bars: in a = 250 µm; in b, c, f and g = 50 µm; in d and e = 10 µm.
paraformaldehyde-fixed sections), $\alpha_4$, $\alpha_5$, $\gamma_1$, $\gamma_3$ and $\delta$-IR is observed in astrocytes of the hippocampus, being most prominent two or 30 days ($\gamma_1$) after KA injection. Also in epileptic animals such a staining has to be judged with caution. Other proteins expressed at high concentrations in glia after KA-induced seizures easily could interfere.

Distribution of GABA<sub>A</sub> receptor subunits in the hippocampal formation

The distribution of GABA<sub>A</sub> receptor subunits in different parts of the hippocampal formation is summarized in Table 2 (see also Fig. 1). Within the hippocampal formation, immunoreactivity of the different subunits appears to be expressed at different levels. In general, extraordinarily strong staining was found for the $\alpha_1$, $\alpha_5$, $\beta_2$ and the $\gamma_2$-subunits. Within some hippocampal subfields (especially in the molecular layer of the dentate gyrus), rather strong staining is seen for $\alpha_4$, $\beta_1$ and for $\delta$. Weak immunoreactivity is observed for $\alpha_3$, $\gamma_1$ and for $\gamma_3$. This coincides with the comparatively low expression levels of mRNAs encoding the latter subunits. $\beta_1$ and $\beta_2$ mRNAs are expressed in all principal cell layers at a considerably lower level than $\beta_3$. This coincides with the moderate overall staining observed for these two subunits. The observed conspicuous labelling of interneurons for $\beta_3$-IR, as for $\alpha_1$ and $\gamma_2$, was in agreement with the mRNA expression.
The monoclonal antibody (62-361), recently developed by Moreno et al. and directed against an intracellular loop of the β₂-subunit as well as the one used by Fritschy and Möhler,11 are less specific and detect simultaneously the β₂- and β₃-subunits. Thus, staining obtained by these groups has to be compared with the almost complementary distributions of β₂ and β₃ as observed in the present study (see below). This fact, however, stresses the importance of using selective antibodies that differentiate between the two subunits. Our data also revealed that in the hippocampus the combination of α₁β₂γ₂, thought to be highly abundant in other brain areas,24,38 is present preferentially in interneurons.

Molecular layer of the dentate gyrus

The molecular layer of the dentate gyrus contains an extraordinary high variety of immunoreactive GABAₐ receptor proteins expressed at high concentrations, α₁, α₂, α₄, β₁, β₃, γ₂ and δ (and of β₂ in interneurons). In general these subunits are almost uniformly distributed within the different segments of the molecular layer. This implies that these GABAₐ receptor subunits are predominantly located upon dendrites of granule cells. A slight clustering of α₄ in the outer, of β₂-IR in the middle and inner molecular layer and of γ₂-IR in the inner molecular layer may be supportive of a partial direct association with synapses of GABAergic projections to these parts of

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Fig. 7. The δ-subunit of the GABAₐ receptor. An overview (a); the stratum lacunosum moleculare (b); the area between the stratum granulosum and the stratum oriens CA1 (c); pyramidal shaped basket cell at the hilary surface of the stratum granulosum (d); CA3a sector (e). Note the intense immunostaining within the molecular layer of the dentate gyrus. The δ-subunit is expressed at moderate concentration within interneurons of CA3 and in fibres of the stratum lacunosum moleculare (b). Moderate concentrations are found in dendrites of CA1 pyramidal neurons (a,c). For abbreviations see Fig. 2. Scale bars: in a=250 μm; in b, c, e=50 μm; d=10 μm.
the dentate gyrus. Granule cell dendrites are densely innervated by a great variety of GABAergic interneurons located in the hilus of the dentate gyrus and exerting an important role in gating the impulse flow transmitted through the granule cell axons, the mossy fibres, to the CA3 sector of the hippocampus. The co-expression of at least three different α-, two β- and one γ- and one δ-subunit suggests a striking heterogeneity of GABA<sub>A</sub> receptors within the same dendritic area. It has been demonstrated before that GABAergic interneurons synapse at different sites of the granule cells and their dendrites. The genes for these subunits are clustered on the human chromosome 5q34–35 and on the mouse chromosome 11. Thus, it may be imagined that such a diversity of GABAergic innervation in conjunction with the broad variety of functionally different GABA<sub>A</sub> receptors within granule cells may allow a more decisive differentiation of the information processed through these structures. Indeed, experimental evidence for a heterogenous localization of two important α-subunits of the GABA<sub>A</sub> receptor at different domains of the same hippocampal cell type has been provided recently by Somogyi’s group in an elegant study using immunoelectron microscopy. The authors demonstrated for the α2-subunit a preferential localization at the axon initial segments of pyramidal cells, whereas the α3-subunit was found at all postsynaptic domains. A differential distribution of GABA<sub>A</sub> receptor subunits within individual regions of the postsynaptic cell may be of high relevance because, e.g., GABA<sub>A</sub> receptors containing either α1- or α2-subunits exert different pharmacological properties and α4-subunit-containing receptors have a very low affinity for classical benzodiazepines such as diazepam. The subunit composition of the GABA<sub>A</sub> receptors might also influence the modulation by endogenous ligands (e.g., neurosteroids or Zn<sup>2+</sup>) and by second messenger systems (e.g., phosphorylation).

Interneurons of the hilus of the dentate gyrus and the hippocampus proper

Our data demonstrate that at least four subunits of the GABA<sub>A</sub> receptor, α4, β2, γ2 and δ are present in GABAergic interneurons of the hilus of the dentate gyrus and in interneurons located in the strata oriens and radiatum of CA1 to CA3. These data are in agreement with previous observations using immunocytochemistry and in situ hybridization. They suggest that interactions between GABAergic neurons (or possibly presynaptic GABA<sub>A</sub> receptor-mediated actions of GABA neurons) within the hippocampus may be preferentially mediated through the major class of type I GABA<sub>A</sub> receptors consisting of α1-, β2-, and γ2-subunits. The genes for these subunits are clustered on the human chromosome 5q34–35 and on the mouse chromosome 11.

It is interesting to note, that type I pyramidal-shaped basket cells in the dentate hilus express (at least in some instances) the δ-subunit but rarely γ2. The γ2-subunit was found at high concentrations in other interneurons of the dentate hilus. Thus, a heterogeneous distribution of the γ2- and the δ-subunits in various types of interneurons has to be considered. On the other hand, the α1- and the β2-subunits may be expressed by a great number of local circuit neurons of the hippocampus. Gao and Fritschy, however, have shown in an elegant study using double-labeling immunocytochemistry of
the α1 subunit with various neuropeptides and calcium-binding proteins, that α1 is not expressed in calbindin-D28k or cholecystokinin-containing neurons. And, whereas the α1 subunit is expressed in most somatostatin/GABA neurons of the hippocampus proper it is not contained in somatostatin neurons of the hilus of the dentate gyrus.7,13 Similar studies are required to establish the exact localization of the different GABA<sub>A</sub> receptor subunits on the neurochemically well-defined subpopulations of hippocampal interneurons.9

**Hippocampus proper**

There is also a striking heterogeneity of GABA<sub>A</sub> receptor subunits within the dendritic areas of the pyramidal neurons (Table 2). The α<sub>1</sub>, α<sub>2</sub>, α<sub>3</sub>, α<sub>5</sub>, β<sub>2</sub>, and the γ<sub>2</sub>-subunits are present at high concentrations throughout the strata oriens and radiatum CA1 to CA3. β<sub>1</sub>, β<sub>2</sub>, α<sub>5</sub> and δ- are expressed at somewhat lower concentrations. Only faint or no staining was detected for α<sub>2</sub>, α<sub>3</sub>, γ<sub>1</sub> and γ<sub>3</sub>. The α2-subunit is less, α<sub>4</sub> somewhat more extensively expressed in the CA1 sector than in other parts of the hippocampus proper. The dendritic areas of CA2 appear to contain more α<sub>5</sub>, and β<sub>1</sub>-protein, and in reverse somewhat less α<sub>2</sub>- and β<sub>3</sub>IR than the neighbouring areas. The δ-subunit is found preferentially in CA1, however, less concentrated than in the dentate molecular layer. Both, the immunocytochemical data and the cellular distribution of seven major subunits suggest a low level of expression for the γ<sub>2</sub>-subunit with different GABA neurons innervate pyramidal cells at different domains and impose

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**CONCLUSION**

A heterogeneous distribution of GABA<sub>A</sub> receptor subunits has been found within the hippocampus. The predominant subunits within the molecular layer of the dentate gyrus are α<sub>1</sub>, α<sub>2</sub>, α<sub>4</sub>, β<sub>3</sub>, γ<sub>2</sub> and δ. Within the dendritic areas of the hippocampus proper the α<sub>1</sub>, α<sub>2</sub>, α<sub>5</sub>, β<sub>2</sub> and the γ<sub>2</sub>-subunits predominate. GABA<sub>A</sub> receptors within local circuit neurons of the hippocampus consist mainly of α<sub>1</sub>, β<sub>2</sub>, and of either γ<sub>2</sub> or δ-subunits (or both γ<sub>2</sub> and δ). These data imply heterogeneously constituted GABA<sub>A</sub> receptors with different physiological and pharmacological properties within individual subfields of the hippocampus.

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