Subunit Composition, Distribution and Function of $GABA_A$ Receptor Subtypes

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Abstract: $GABA_A$ receptors are the major inhibitory neurotransmitter receptors in the brain and are the site of action of many clinically important drugs. These receptors are composed of five subunits that can belong to eight different subunit classes. Depending on their subunit composition, these receptors exhibit distinct pharmacological and electrophysiological properties. Recent studies on recombinant and native $GABA_A$ receptors suggest the existence of far more receptor subtypes than previously assumed. Thus, receptors composed of one, two, three, four, or five different subunits might exist in the brain. Studies on the regional, cellular and subcellular distribution of $GABA_A$ receptor subunits, and on the co-localization of these subunits at the light and electron microscopic level for the first time provide information on the distribution of $GABA_A$ receptor subtypes in the brain. These studies will have to be complemented by electrophysiological and pharmacological studies on the respective recombinant and native receptors to finally identify the receptor subtypes present in the brain. The distinct cellular and subcellular location of individual receptor subtypes suggests that they exhibit specific functions in the brain that can be selectively modulated by subtype specific drugs. This conclusion is supported by the recent demonstration that different $GABA_A$ receptor subtypes mediate different effects of benzodiazepines. Together, these results should cause a revival of $GABA_A$ receptor research and strongly stimulate the development of drugs with a higher selectivity for α2-, α3-, or α5-subunit-containing receptor subtypes. Such drugs might exhibit quite selective clinical effects.

Key words: $GABA_A$ receptors, subtypes, subunits, distribution, function, pharmacology

1. INTRODUCTION

γ-Aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter in the central nervous system. About 30% of all synapses use GABA as a transmitter [1,2]. Most of the physiological actions of GABA are generated via $GABA_A$ receptors. These receptors are chloride ion channels that can be opened by GABA and can be modulated by a variety of pharmacologically and clinically important drugs, such as benzodiazepines, barbiturates, steroids, anesthetics and convulsants [3,4]. These drugs produce at least part of their clinically relevant effects by interacting with distinct allosteric binding sites on $GABA_A$ receptors [5]. Based on their pharmacological action it was concluded that $GABA_A$ receptors are involved in controlling the excitability of the brain [6,7], in the modulation of anxiety [8,9], of feeding and drinking behaviour [10,11], circadian rhythms [12,13], and cognition, vigilance, memory, and learning [14-16].

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2. MOLECULAR STRUCTURE

The $GABA_A$ receptor is a member of a superfamily of ligand-gated ion channels that also includes the nicotinic acetylcholine receptor, the 5-hydroxytryptamine type 3 receptor and the glycine receptor [17]. Similar to the other members of this receptor superfamily, $GABA_A$ receptors seem to be composed of five subunits [18,19]. All $GABA_A$ receptor subunits consist of a large N-terminal extracellular domain, four transmembrane (TM) domains, and a large intracellular loop between TM3 and TM4 [20]. So far, a total of 6α, 4β, 3γ, one δ, one ε, one π, one θ and 3ρ subunits of $GABA_A$ receptors have been cloned and sequenced from the mammalian nervous system [21,22]. This heterogeneity is increased by alternative exon splicing of the pre-mRNA, which generates two forms of the γ2 subunit (γ2S and γ2L) from one gene [23,24] which can be distributed differently in the brain [25]. Splice variants have also been detected for other subunits [21]. In addition, subunit homologues have been identified in non-mammalian species [21,26,27].

Expression studies have indicated that depending on the subunits used for transfection of the cells, receptors with

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distinct pharmacological and electrophysiological properties do arise [4]. α, β, and γ subunits, however, have to combine to produce $GABA_A$ receptors with a pharmacology resembling that of most native receptors [4]. This indicates that the majority of native receptors is composed of αβγ subunits. The δ, ε, and π subunits seem to be able to replace the γ subunit in $GABA_A$ receptors [28-31], whereas the θ subunit might be able to replace a β subunit in these receptors [22]. Although the subunit stoichiometry has been contentious [32], evidence is now convincing that receptors composed of α, β, and γ subunits contain two α, two β, and one γ subunit [19,33-35], and that in these receptors a total of four alternating α and β subunits are connected by a γ subunit [19] (Fig. 1). Whether all receptors composed of αβγ subunits or those composed of αβδ, αβε, or αβπ subunits exhibit the same subunit stoichiometry and subunit arrangement, presently is not known.

ρ subunits are assumed not to coassemble with other classes of $GABA_A$ receptor subunits [36,37] and can form homo- as well as hetero-oligomeric channels with other ρ subunits that exhibit properties of the previously characterized $GABA_C$ receptors [38-40]. Since ρ subunits are structurally part of the family of $GABA_A$ receptor subunits, it was recommended that ρ-containing receptors should be classified as a specialized set of the $GABA_A$ receptors [21].

3. SUBUNIT COMPOSITION OF RECOMBINANT $GABA_A$ RECEPTORS

3.1. Homo-Oligomeric $GABA_A$ Receptors

Recombinant receptor studies have indicated that at least some of the $GABA_A$ receptor subunits can form homo-oligomers. The extent of formation of these homo-oligomers, however, varies dramatically. Whereas some are robustly formed in all recombinant expression systems, others seem to be formed with low efficiency only. For instance, although some electrophysiological studies could identify GABA-activated homo-oligomeric channels after expression of α1, β2, γ2, or δ subunits [28,41-43], in other studies such channels were not observed [44-46]. Similarly, ε or π subunits seem not to be able to form homo-oligomeric channels [29-31].

A robust expression of GABA-activated homo-oligomeric chloride channels, however, was observed with ρ subunits [36,37], or with β1 or β3 subunits [47,48]. Interestingly, channels formed by murine or rat β1 [49,50] or β3 subunits [51] were open in the absence of GABA. This effect seems to be species dependent, because human or bovine β1 subunits seem to be able to form homo-oligomeric channels that could be opened by GABA [42,50,52].

3.2. $GABA_A$ Receptors Composed of Two Different Subunits

In most cases, channels consisting of two different subunits formed more efficiently than homo-oligomeric channels and could be activated by lower GABA concentrations. In addition, the chloride ion flux induced in hetero-oligomeric channels was higher than that in homo-oligomeric channels [44,53]. The efficiency of receptor formation, however, seems to depend on the subunit combination. Whereas αβ subunit combinations were expressed efficiently and formed GABA-activated channels in all cell systems investigated, conflicting results were obtained with αγ or βγ subunit combinations [43-45,53]. The efficiency of formation of pentameric α1γ2 or β3γ2 receptors on heterologous expression in human embryonic kidney (HEK) cells seems to be low [19]. Thus, for cells coexpressing β3 and γ2L subunits, γ2L could be detected on the surface of only about 15% of cells, indicating that

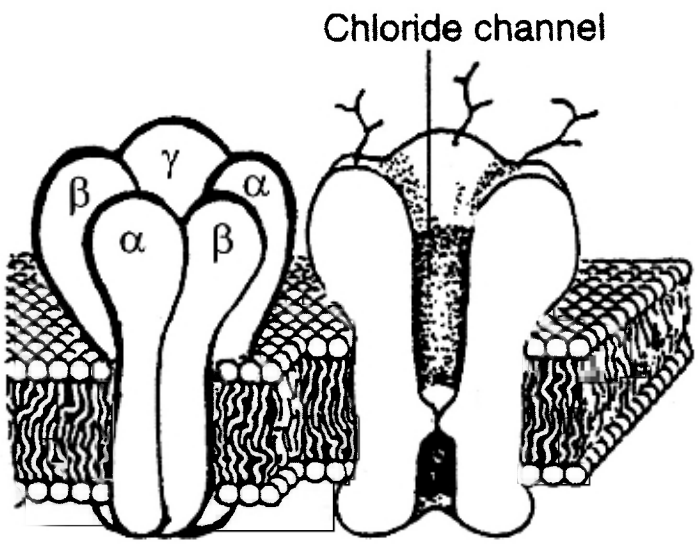

Fig. (1). Subunit stoichiometry and arrangement of recombinant α1β3γ2 receptors [19]. A mirror image arrangement of the subunits is equally possible.

most of the receptors formed in these cells were homo-oligomeric β3 receptors [54]. β3γ2S receptors also formed in HEK cells to a comparable extent and exhibited a pharmacology distinct from that of homooligomeric β3 receptor [54]. α1γ2 or β2γ2 subunit combinations, however, were retained within the endoplasmic reticulum [47,48]. It is thus possible that receptors composed of these subunit combinations can only be formed under certain experimental conditions, such as in the presence of suitable chaperones, at high subunit concentrations due to high synthesis rates (conditions that are present in some recombinant receptor systems), or in the absence of degrading enzymes.

No information is available on the possible formation of pentameric $GABA_A$ receptors composed of αδ, βδ, or γδ subunits. No functional channels, however, were formed on co-transfection of α1ε or β1ε [31] or of α1π or β1π [30] subunit combinations.

In contrast, different ρ subunits can combine with each other and might also co-assemble to functional receptors *in vivo* [37]. Although in one study it was demonstrated that ρ subunits are unable to assemble with α1, β1, or γ2 subunits [37], other studies indicated that ρ subunits can assemble with γ2 subunits and possibly also with glycine receptor subunits, and form functional receptors with properties found in certain cell types of the retina [55,56]. In addition, receptors similar to but not identical with those formed by ρ subunits have been identified in several brain regions [57-60].

3.3. $GABA_A$ Receptors Composed of Three and More Different Subunits

As mentioned above, the majority of $GABA_A$ receptor subtypes present in the brain seems to be composed of 2α, 2β, and 1γ subunit and recombinant αβγ receptor subtypes composed of one type of α, β, and γ subunit have been most thoroughly studied so far [4,61]. Recombinant receptor studies have also indicated that receptors containing two different α subunits as well as a β and a γ subunit can assemble and exhibit properties that are distinct from those of receptors containing only a single type of α subunit [43,44,62-64]. Similarly, it has been demonstrated that receptors containing two different types of β subunits together with one type of α and γ subunit are able to assemble and to exhibit properties different from receptors that contain only a single β subunit subtype [65]. Finally, it has been demonstrated that recombinant receptors composed of α1, β1, the long splice variant of γ2, and δ (α1β1γ2Lδ) subunits can also be formed and exhibit properties distinct from those of α1β1γ2L or α1β1δ receptors [66,67].

Although experiments investigating the coexpression of five different subunits have been performed in *Xenopus* oocytes, the results obtained were difficult to interpret [44]. This is not surprising because from the five different subunits simultaneously expressed in the oocytes a variety of different receptor subtypes composed of 3, 4, or 5 different subunits could have been formed, that all could have contributed to the chloride current measured in these cells.

4. SUBUNIT COMPOSITION OF $GABA_A$ RECEPTORS IN THE BRAIN

4.1. Extensive Heterogeneity of $GABA_A$ Receptors in the Brain

During the last 10 years a variety of $GABA_A$ receptor subunit selective antibodies have been generated and have been used for purifying $GABA_A$ receptor subtypes by immunoprecipitation or immunoaffinity chromatography. The analysis of the subunit composition of the purified receptors indicated an extreme promiscuity of the various subunits [68-75]. Although the antibodies used were highly specific for the respective subunits, most if not all of the other subunits investigated could be copurified with the respective subunit, suggesting that each subunit can combine with most of the other subunits to form a variety of different receptor subtypes.

These studies also indicated that two different α subunits [68-71,75] or two different β subunits [74,75] are present in at least some $GABA_A$ receptors. Discrepant results were obtained concerning a possible colocalization of different γ subunits, or of γ and δ subunits in the same $GABA_A$ receptor. Whereas in three studies [72,75,76] it was demonstrated that $GABA_A$ receptors seem to contain only a single type of γ subunit, other studies, in disagreement with the presumed subunit stoichiometry of 2α, 2β and 1γ subunit, suggested a significant colocalization of the alternatively spliced short and long form of the γ2 subunit [77] or of the γ2 and γ3 subunit [78] in the same receptor. Whereas one study concluded that γ and δ subunits might be present in the same $GABA_A$ receptor [71], in three other studies, γ and δ subunits could not be demonstrated in the same receptors [75,79,80].

4.2. Identification of $GABA_A$ Receptor Subtypes by Subtractive Purification

From these studies it was clear that it is impossible to purify a single $GABA_A$ receptor subtype by a one step immunoprecipitation. Recently, therefore, a generally applicable method for resolving the subunit composition of hetero-oligomeric receptors has been developed [75] that is based on a subtractive purification. In this strategy, immunoaffinity chromatography of receptors, elution of the retained receptors and Western blot analysis is used first to identify the subunits copurifying with a subunit to be investigated. This is then followed by the quantitative elimination of receptors containing the copurifying subunits one after the other until only one receptor subtype is left. Its subunit composition can then be determined by Western blot analysis. By measuring the total amount of receptors in the extract before and after chromatography on the individual immunoaffinity columns, this procedure can also be used to

determine the percentage of receptors removed by the column, and thus, to estimate the relative abundance of individual receptor subtypes [75,76,81,82].

Using this method, the subunit composition of α6 subunit-containing $GABA_A$ receptors from cerebellum was determined. Results obtained indicated that α6 receptors in rat cerebellum are composed predominantly of α6βxγ2 (32%), α1α6βxγ2 (37%), α6βxδ, (14%), or α1α6βxδ (15%) subunits [75]. In addition, these experiments indicated that 10%, 51%, or 21% of α6 receptors contained homogeneous β1, β2, or β3 subunits, respectively, whereas two different β subunits were present in 18% of all α6 receptors. The observation that β1 and β2 as well as β3 subunits are copurifying with α6 and γ2, or with α6 and δ subunits, additionally indicates that the α6βxγ2 or α6βxδ receptor subtypes might exist in up to six isoforms containing different β subunit combinations (homogeneous β1, β2, or β3 subunits, β1β2, β1β3, or β2β3). The same might be true for receptors consisting of α1α6βxγ2 or α1α6βxδ subunits. Whether all of the resulting 24 α6 receptors with different subunit composition actually do exist could not be clarified by this study [75].

These data are consistent with the results from recombinant receptor studies mentioned above and indicate that most of the receptors found in the brain consist of 2α, 2β and 1γ or 1δ subunits. In addition, a significant part of the receptors contains two different α and/or two different β subunits, and is thus composed of 4 or 5 different subunits. To finally prove the existence of all these receptors in the brain, however, these receptors have to be functionally identified in situ by electrophysiological and pharmacological techniques. This, of course, is the most difficult and tedious part of the identification of receptor subtypes because it requires a distinct pharmacological or electrophysiological fingerprint of the receptors to be identified. Such fingerprints only can be established by recombinant receptor studies and it can be only hoped that the properties of the native receptors are not significantly changed due to endogenous phosphorylation [4] or to interaction with other proteins at the synapse. Although some progress has been achieved recently in identifying $GABA_A$ receptors in the brain [83], in most cases an unequivocal identification of receptor subtypes in situ currently is not possible due to the lack of highly selective pharmacological tools.

4.3. Relative Abundance of $GABA_A$ Receptors Containing a Certain Subunit

To estimate the relative abundance of $GABA_A$ receptors containing a certain subunit, in most cases immunoprecipitation with subunit specific antibodies followed by [^{3}H]muscimol binding studies was used. [^{3}H]muscimol is a ligand binding to the GABA-binding site of $GABA_A$ receptors that is located between an α and a β subunit of these receptors [5] and it is assumed that [^{3}H]muscimol binds to all receptors containing α and β subunits [84]. This ligand, however, is not suitable for the detection of receptors containing ρ subunits.

Results of these studies are shown in Table **1**. Overall, there is agreement that α1, β2, and γ2 subunits are the most abundant $GABA_A$ receptor subunits in the brain. Antibodies directed against these subunits precipitate 70-90%, 55-90% and 50-70% of [^{3}H]muscimol binding sites from rat or mouse brain membrane extracts, respectively (Table **1**). Subunits α2 or α3 seem to be present in 35% or >14% of all $GABA_A$ receptors of the brain, respectively. Whereas on average only 6% of $GABA_A$ receptors in the brain seem to contain α4 subunits, in the hippocampus about 13% and in the thalamus about 20% of $GABA_A$ receptors might contain this subunit (Table **1**). α5 subunits are present in only 7-8% of all $GABA_A$ receptors in the brain, but this subunit is especially enriched in hippocampus, where it might be present in up to 31% of all receptors. As mentioned above, α6 subunits are present only in cerebellar granule cells and in the cochlea. In the cerebellum about 58% of all $GABA_A$ receptors contain α6 subunits (Table **1**).

As discussed above for recombinant receptors, alpha subunits in most cases cannot readily form functional $GABA_A$ receptors. It is therefore assumed that α subunits have to co-assemble with β subunits and in most cases also with a γ, a δ, or an ε subunit to form completely assembled pentameric receptors that are incorporated into the neuronal membrane. Using antibodies specifically directed against individual β-subunits for purification of $GABA_A$ receptors from the brain, all the other subunits could be co-precipitated. Whereas the β2 subunit is the most abundant β subunit, β1 and β3 subunits are also quite abundant compared with some α subunits (Table **1**). And whereas a large part of the $GABA_A$ receptors in the brain seems to contain a γ2 subunit, receptors containing a γ1, a γ3, or a δ subunit on the average are not very abundant in the brain (Table 1). These receptors, however, again are enriched in certain brain regions (Table 1, and see below). So far, the relative abundance of receptors containing the ε, π, θ, or ρ subunits in the brain has not been determined.

4.4. Evidence for the Existence of αβ Receptors in the Brain

Four lines of evidence support the existence of receptors composed of αβ subunits in the brain. Firstly, it has been demonstrated that about 50% of α4 subunit containing $GABA_A$ receptors in forebrain did not contain γ1, γ2, γ3, or δ subunits, although 98% of α1 receptors in this tissue contained either γ1, γ2, γ3 or δ subunits [76]. From these and other data it was concluded that a significant part of α4 receptors in the brain is composed of α4 and β1–3 subunits, only. Further experiments, however, have to clarify whether α4 receptors lacking γ1, γ2, γ3, or δ subunits might contain ε [29], π [30], or so far unidentified subunits.

Secondly, it was demonstrated that in δ subunit knockout mice a significant percentage of $GABA_A$ receptors in the cerebellum did not contain γ1, γ2, or γ3 subunits.

Table 1. Percentage of [^{3}H]Muscimol Binding Sites Immunoprecipitated from Rat Brain by Subunit-Specific Antibodies

Antibody	%[^{3}H]muscimol binding	References
α1	70-90	Sieghart *et al.*, unpublished
	90 ± 9	85,86
	75 ± 6 (in cerebellum)	81
α2	35	Sieghart *et al.*, unpublished
α3	>14	Sieghart *et al.*, unpublished
α4	6 ± 1	76
	6 ± 2	87
	>5	88
	20 ± 3 (in thalamus)	89
	13 ± 3 (in hippocampus)	89
α5	7-8	Sieghart *et al.*, unpublished
	31 (in hippocampus)	90
α6	58 ± 8 (in cerebellum)	91
	56 ± 1 (in cerebellum)	81
β1	18 ± 3(in purified receptors)	92
	32 ± 1 (in cerebral cortex)	74
	>20-30	Sieghart *et al.*, unpublished
β2	55 ± 3 (in purified receptors)	92
	64 ± 4(in cerebral cortex)	74
	>65	Sieghart *et al.*, unpublished
	90 ± 4 (in cerebellum)	81
β3	19 ± 7 (in purified receptors)	92
	48 ± 3 (in cerebral cortex)	74
	>55	Sieghart *et al.*, unpublished
	36 ± 2 (in cerebellum)	81
γ1	11 ± 1	78
	3-7	Sieghart *et al.*, unpublished
γ2	59 ± 3	78
	60-70	Sieghart *et al.*, unpublished
	50 ± 6	85,86
	68 ± 6 (in cerebellum)	81
	59 (in hippocampus)	90
γ3	14 ± 2	78
	3-4	Sieghart *et al.*, unpublished
	8 (in hippocampus)	90
δ	11 ± 2	91
	8 ± 4 (in cortex)	80
	23 ± 2 (in cerebellum)	91
	27 ± 1.5 (in cerebellum)	81
	20 ± 2 (in cerebellum)	80
	16 ± 3 (in thalamus)	89
	13 ± 2 (in hippocampus)	89
	11 ± 3 (in hippocampus)	80
	19 ± 1 (in olfactory bulb)	80

Data were derived from membranes of whole brain if not indicated otherwise. Data from Sieghart *et al.*, unpublished, were obtained by immunoprecipitation with a pool of subunit-specific antibodies that was characterized for absence of crossreactivity with other subunits. In addition, the data were corrected for the precipitation efficiency (determined by using recombinant receptors) of the antibodies used.

These receptors, thus, either are composed of α and β subunits only, or additionally contained so far unidentified $GABA_A$ receptor subunits [82].

Thirdly, similar results were obtained in γ2 subunit knockout mice [93]. In the brain of these mice 94% of the benzodiazepine binding sites were absent, while the number of GABA sites was only slightly reduced. In addition, except for the γ2 subunit the level of expression and the regional and cellular distribution of the major $GABA_A$ receptor subunits were unaltered. The single channel main conductance levels, however, were reduced to values consistent with recombinant $GABA_A$ receptors composed of α and β subunits only [93]. The absence of γ2 subunits (and thus, the formation of αβ receptors) obviously does not disturb embryonic development, as suggested by the normal body weight and histology of newborn γ2-knockout mice. Postnatally, the reduced function in most of the $GABA_A$ receptors, however, is associated with retarded growth, sensorimotor dysfunction and drastically reduced life-span. The majority of these mice die within a few days after birth [93].

Finally, there is some evidence that not only during embryonic development of γ2-knockout mice [93], but also during the postnatal development of cerebellar granule cells in wild-type mice, αβ receptors might be formed, they can be distinguished by their lower channel conductance and are probably located extrasynaptically [94]. Taken together, these results indicate that receptors composed only of α and β subunits can be present in the brain.

So far, homo-oligomeric $GABA_A$ receptors composed of α, β, γ, δ, ε, π, or θ subunits have not been identified in the brain. This is not surprising considering the weak (or absent) formation of recombinant homo-oligomeric receptors containing these subunits. In addition, due to the promiscuity of these subunits, such homo-oligomeric receptors are difficult to detect amongst the large variety of other receptor subtypes formed. As mentioned above, there is some evidence, however, that homo-oligomeric receptors are formed from different ρ subunits *in vivo* [37,39].

5. REGIONAL, CELLULAR AND SUBCELLULAR DISTRIBUTION OF $GABA_A$ RECEPTOR SUBUNITS

5.1. Regional Distribution of α, β, γ, and δ Subunits in the Brain

"In situ" hybridization [25,95-97] and immunohistochemical studies [98-104] have indicated that the individual subunits exhibit a distinct but overlapping regional and cellular distribution. Subunits α1, β1, β2, β3, and γ2 are found throughout the brain, although differences in their distribution were observed. Subunits α2, α3, α4, α5, α6, γ1, and δ are more confined to certain brain areas (Table **2**). Thus, the α1 subunit is the most abundant subunit and is ubiquitously distributed throughout the brain. The α2 subunits are less abundant than α1 subunits and are preferentially located in forebrain areas. The highest concentrations were found in olfactory bulb, striatum, nucleus accumbens, septum, dentate gyrus, amygdala and hypothalamus. But α2 subunits were less abundant in thalamus (except reticular nucleus) midbrain and brainstem areas (Table **2**) [103]. α3 subunits were strongly present in the glomerular and external plexiform layers of the olfactory bulb, in the inner layers of the cerebral cortex, the reticular thalamic nucleus, the zonal and superficial layers of the superior colliculus, the amygdala and cranial nerve nuclei. Subunit α4 was strongly detected in the thalamus, dentate gyrus, olfactory tubercle and basal ganglia. The α5 subunit immunoreactivity was strongest in Ammon's horn, the olfactory bulb and hypothalamus, whereas the α6 subunit was only present in granule cells of the cerebellum and the cochlear nucleus [103]. The β subunits are widely distributed. The β2 subunit is one of the most widely distributed subunits in the brain. It preferentially seems to be co-assembled with α1 and γ2 subunits. β2 and β3 subunits are less abundant. The subunit γ1 is a minor subunit and exhibits a quite specific distribution in the brain. It is preferentially located in the central and medial amygdaloid nuclei, in pallidal areas, the substantia nigra pars reticulata and the inferior olive. In contrast, the γ3 subunit is expressed in most brain areas but with low abundancy. The δ subunit is frequently co-distributed with the α4 subunit , e.g. in the thalamus, striatum, outer layers of the cortex and in the dentate molecular layer. In the cerebellum, however, it is co-distributed with the α6 subunit [103].

Striking examples of complementary distribution of certain subunits were also observed. Thus, subunits α2, α4, β1, β3, and δ were considerably more concentrated in the neostriatum than in the pallidum and entopeduncular nucleus. In contrast, α1, β2, γ1, and γ2 subunits prevailed in the pallidum compared to the striatum [103] (Table **2**). The striatum, the nucleus accumbens, and the olfactory tubercle displayed strong, diffuse staining for the subunits α2, α4, β3, and δ, presumably located on dendrites of the principal medium spiny neurons. Subunits α1, β2, and γ2 were apparently mostly resticted to interneurons of these areas [104]. In contrast, the globus pallidus, the entopeduncular nucleus, the ventral pallidum, the subthalamic nucleus, and the substantia nigra pars reticulata revealed dense networks of presumable dendrites of resident projection neurons, which were darkly labeled for subunits α1, β2 and γ2. The globus pallidus, ventral pallidum, entopeduncular nucleus, and substantia nigra pars reticulata, all areas receiving innervations from the striatum, displayed strong subunit γ1-immunoreactivity compared to other brain areas. In the substantia nigra pars compacta and in the ventral tegmental area numerous presumptive dopaminergic neurons were labeled for subunits α3, γ3, and/or δ [104,105].

With the exception of the reticular thalamic nucleus, which was prominently stained for subunits α3, β3, and γ2, most thalamic nuclei were rich in α1, α4, β2, and δ subunits (Table **2**). Whereas the dorsal lateral geniculate nucleus was strongly immunoreactive for subunits α4, β2, and δ, the ventral lateral geniculate nucleus contained sub-

Table 2. Regional Distribution of $GABA_A$ Receptor Subunits in the Brain

Region	α1	α2	α3	α4	α5	α6	β1	β2	β3	γ1	γ2	γ3	δ	ε	θ
Olfactory bulb															
glomerular layer	xx	x	xx	o	x	-	-	xx	xx	-	xx	o	x	-	
ext. plexiform layer	xxx	x	xx	o	x	-	xx	xxx	xxx	-	xxx	o	o	-	
granular layer	xx	xx	o	x	xx	-	-	x	xx	-	xx	-	o	-	
mitral cell layer	xx	-	o	-	xx	-	x	xx	-	-	xx	o	x	-	
Olfactory tubercle	x	x	-	xx	x	-	x	x	xx	-	x	x	x	-	
Cerebral cortex															
all layers	xx	x	x	xx	x	-	xx	xx	xx	-	xx	o	x		
outer layers	xx	x	x	x	x	-	xx	xx	xx	-	xx	o	x	-	
inner layers	xx	x	xx	x	x	-	xx	xx	xx	-	xx	o	x	-	
Hippocampus															
molecular layer	x	xx	-	xx	x	-	xx	x	xx	-	xx	-	x	-	
hilar neurons	xx	-	x	-	-	-	o	xx	-	-	xx	-	x	-	
strat. oriens/radiatum	xx	xx	-	x	xx	-	xx	x	xx	-	xx	o	-	-	
Septum															
medial	xx	x	x	-	o	-	o	xx	x	-	xx	o	-	x	x
lateral	xx	xx	x	x	o	-	xx	x	x	x	xx	o	-		
Basal ganglia															
Striatum/n. accumbens	x	xxx	x	xx	xx	-	x	x	xxx	x	x	o	x	x	x
Globus pallidus	xx	o	o	x	o	-	o	xx	o	xx	xx	o	o		
Subst. nigra	x	x	x	o	x	-	x	x	-	x	x	x	o	xx	x
Thalamus															
reticular nucleus	x	-	xx	x	x	-	xx	-	xx	-	xx	o	o		
ventr. lat. geniculate	xxx	x	x	x	o	-	x	xx	x	-	x	o	o	xx	x
dors. lat. geniculate	xxx	-	-	xxx	o	-	x	xxx	x	-	x	x	xxx		
medial and central	x	xx	x	o	o	-	xx	xx	xx	xx	x	x	x	xx	x
Hypothalamus															
ventromedial	x	xx	x	o	xx	-	xx	x	xx	x	xx	x	x	xx	xx
supraopticus	xxx	xxx	x	x	o	-	xxx	xx	x	-	x	x	x		
paraventricular	xx	xxx	-	-	x	-	xx	x	xx	-	x	x	x	xx	x
arcuate	x	x	x	x	x	-	x	x	x	-	-	x	x	xx	x
med. preoptic area	xx	xx	x	-	x	-	x	x	x	-	xx	x	x	xx	x

(Table 2). contd....

Region	α1	α2	α3	α4	α5	α6	β1	β2	β3	γ1	γ2	γ3	δ	ε	θ
Amygdala															
lateral	xx	xx	xx	x	o	-	xx	xx	xx	-	xx	x	o	x	x
basolateral	xx	xx	xx	x	o	-	xx	xx	xx	-	xx	x	o		
medial and central	x	xx	x	o	o	-	xx	xx	xx	xx	xx	x	x	x	x
Cerebellum															
granule cell layer	xxx	x	o	o	x	xxx	x	xxx	xxx	x	xx	-	xxx		
molecular layer	xx	xx	-	-	xx	-	x	x	-	-	x	o	-		
Midbrain/Pons															
Ventral tegmental area	xx	x	o	-	o	-	xx	x	xx	x	xx	xx	o	-	-
Raphe nuclei	xx	xx	x	-	o	-	xx	xx	x	-	xx	xx	xx	x	x
Inferior colliculus	xx	-	-	-	o	-	x	xx	o	-	o	o	o		
Olive superior	o	-	x	-	o	-	xx	-	x	-	x	o	x		
Medulla															
Trigeminal sensory complex	xx	-	xx	o	o	-	x	x	x	x	xx	x	x		
Dorsal cochlear nucleus	xx	x	xx	o	x	xx	o	x	o	o	x	x	xx		
Solitary tract nucleus	xx	o	xx	-	xx	-	x	o	x	-	xx	x	xx		

xxx extremely high
xx high
x low
o very low

Data are from Refs. 102-104, 108,109

units α2, α3, β1, β2, β3, and γ2. Subunits α1 and α5 were about equally distributed in both areas.

In most hypothalamic areas, immunoreactivities for subunits α1, α2, β1, β2, and β3 were observed, raising the possibility of a preferential formation of αβ receptors. In the supraoptic nucleus, staining of conspicuous dendritic networks with subunit α1, α2, β2, and γ2 antibodies was contrasted by perikarya labeled for α5, β1, and δ subunits. In most pontine and cranial nerve nuclei and in the medulla, only subunits α1, β2, and γ2 were strongly expressed, whereas the inferior olive was significantly labeled only for subunits β1, γ1, and γ2 [103]. The composition of $GABA_A$ receptors in this tissue thus might be unique. In interpreting these data, however, it has to be considered that individual antibodies differ in their ability to detect their respective subunit. It is thus possible that strongly reactive antibodies are able to detect subunits present in receptors with a weak abundance whereas other subunits present in the same receptors are not detected because the respective antibodies are only weakly reactive. In addition, it is possible that subunit epitopes might be differentially accessible by the antibodies depending on the tissue investigated.

5.2. Regional Distribution of ε, π, θ, and ρ Subunits in the Brain

The ε subunit originally has been cloned from human tissues [29,31,106]. An unusually high level of divergence from their human homologs was detected in the rat tissue in one report [107] that could not be confirmed in another study [108]. Northern blots and in situ histochemistry originally were contradictory in the human brain [29,31] and also in the rat brain [107,108]. Subsequent extensive in situ hybridization studies in the rat have indicated that this subunit is expressed by neurons located in septal and preoptic areas, as well as in various hypothalamic nuclei, amygdala and thalamus [108,109]. The mRNA was also detected in major neuronal groups with broad-range influence, such as the cholinergic (basal nucleus), dopaminergic (substantia nigra compacta), serotonergic (raphe nuclei) and noradrenergic (locus coeruleus) systems [108,109]. Immunohistochemistry revealed the presence of ε-subunit immunoreactivity over the somatodendritic domain of neurons with a distribution closely matching that of the respective mRNA-expressing cells [108,109].

The π subunit was detected in several peripheral human tissues as well as in the brain (hippocampus and temporal cortex) and was particularly abundant in the uterus [30]. So far, no study investigating the detailed regional distribution of the π subunit in the brain has been published. The π subunit protein can co-assemble with α, β, and γ subunits and confer unique ligand binding and electrophysiological properties to the recombinant receptors in which it combines [30,110].

The θ subunit was the last so far identified [22] and seems to be expressed in various brain regions, including the hypothalamus, amygdala, hippocampus, substantia nigra, dorsal raphe and locus coeruleus [109]. θ subunits showed strikingly overlapping expression patterns with ε subunits throughout the brain, especially in the septum, preoptic areas, various hypothalamic nuclei, amygdala, and thalamus, as well as in monoaminergic cell groups [109]. Surprisingly, θ produced a functional receptor only when coexpressed (and probably coassembled) with an α, a β and a γ subunit [22]. As with the ε subunit, there were some discrepancies in the cDNA sequence obtained by different groups [22,107].

The ρ subunits seem to be preferentially expressed in the retina. Immunohistochemistry in the retina using an antibody recognizing all 3 ρ subunits revealed staining restricted to the terminals of bipolar cells in the inner plexiform layer which did not overlap with GABA$_A$ α or β subunits [111,112]. mRNA encoding ρ subunits, however, is present also in the superior colliculus, dorsal lateral geniculate nucleus and cerebellar Purkinje cells [113,114]. In addition, bicuculline-resistant and baclofen-independent GABA effects have been reported in the cerebellum [57,115], superior colliculus [58,116], amygdala [60], hippocampus [117,118], dorsal geniculate nucleus [119] and spinal cord [120]. This indicates that ρ subunits may be present in many CNS regions and are more prevalent than previously suspected.

5.3. Cellular Distribution of GABA$_A$ Receptor Subunits

To understand the functional and pharmacological significance of GABA$_A$ receptor heterogeneity, the allocation of receptor subtypes to identified GABA-receptive neurons *in situ* is essential. Using electrophysiological recordings followed by single-cell polymerase chain reaction, the GABA$_A$ receptor subunit mRNA content was analyzed in granule and Purkinje neurons from rat cerebellar slices [121]. It was demonstrated that in all Purkinje neurons assayed, α1 subunit mRNA but not α6 mRNA was detected. In contrast, among individual granule neurons a heterogeneous distribution of the mRNA for the α1 and α6 GABA$_A$ receptor subunits was found: 8 out of 16 granule cells investigated contained both α1 and α6 subunits, whereas 4 out of 16 cells contained α1 subunits only and 4 out of 16 cells contained α6 subunits only [121].

Immunocytochemical studies indicated that a large part of α1β2γ2 receptors is located on GABAergic interneurons in hippocampus and other brain regions [102-104], because for no other α or β subunits was such an intense labeling of cell bodies and dendrites of interneurons obtained. Using confocal laser microscopy, various neurons in distinct brain regions that receive specific GABAergic inputs were investigated for the expression of GABA$_A$ receptor subunits. In support of the above conclusion it was demonstrated that in the hippocampus in both Ammon's horn and the dentate gyrus, all parvalbumin-positive interneurons and 50% of the calretinin-positive neurons contained the α1 subunits, whereas interneurons containing calbindin-D$_{28k}$ were devoid of α1 subunit staining [122]. Similarly, most neurons positive for neuropeptide Y and a subset of somatostatin-positive cells contained α1 subunits, in contrast to cholecystokinin- and vasoactive intestinal peptide-containing cells, which lacked the α1 subunit staining. These results demonstrate cell-specific expression of GABA$_A$ receptors containing the α1 subunit among subsets of hippocampal interneurons, pointing to a pronounced functional specialization of these cells and supporting the conclusion that disinhibition may be of major functional relevance in regulating the balance between excitation and inhibition in hippocampal circuits [122].

In the raphe nuclei the vast majority of serotonergic neurons express strong α3-subunit-immunoreactivity but are devoid of α1 subunit staining. Only a small population of serotonergic neurons co-express these two subunits. In contrast, both the α1- and α3-subunit-immunoreactivities are present in glutamate decarboxylase-positive neurons [123]. These data indicated that serotonergic and GABAergic neurons selectively express distinct patterns of α subunits, suggesting that they possess distinct subtypes of GABA$_A$-receptors.

In other studies the medial septum-diagonal band of Broca complex was investigated, in which cholinergic and GABAergic neurons represent the two main types of neurons [105]. In this brain region α1, α3, β2,3 and γ2 subunits of GABA$_A$ receptors were ubiquitously expressed. By using double- and triple-immunofluorescence staining it was demonstrated that 84-95% of the choline acetyltransferase containing neurons in the various cholinergic subgroups of the basal forebrain expressed the α3 subunit but not the α1 subunit. In contrast, parvalbumin-positive GABAergic neurons in these brain regions were frequently co-stained with the α1-subunit antiserum, and to a lesser extent with the α3-subunit antibody [105]. Triple immunofluorescence staining revealed that 45-60% of parvalbumin-immunoreactive neurons expressed both the α1- and the α3-subunit in the various subnuclei of the medial septum-diagonal band of Broca complex, whereas most of the remaining parvalbumin-positive neurons were labeled with only the α1-subunit antiserum [105]. The α3 subunit, however, not only is associated with serotonergic or cholinergic neurons, but also with noradrenergic and dopaminergic neurons in the brainstem [105].

In addition, using in situ hybridization and dual labeling immunohistochemistry, α3, θ, and ε GABA$_A$ subunit mRNAs were all detected with an overlapping distribution in neurons of the dorsal raphe and the locus coeruleus.