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$GABA_A$-RECEPTOR ASSEMBLY IN VIVO: LESSONS FROM SUBUNIT MUTANT MICE

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Summary

The rules governing the assembly of $GABA_A$ receptors in vivo were assessed in subunit mutant mice. The transcription of individual subunit genes was regulated independently. The lack of a particular subunit did not result in a molecular rescue by an enhanced transcription of other subunits. In addition, the availability of an α- and β-subunit was essential for receptor formation. Finally, highly selective recognition processes directed the subcellular targeting of receptors. The loss of a particular receptor subtype (α_5) did not lead to a subcellular redistribution of the remaining subtype ($\alpha 2$) present in the same cell.

Key Words: $GABA_A$-receptor subtype, gene transcription, subcellular targeting

Brain function is based on an overall balance of exitatory and inhibitory neuronal transmission. GABA is the major inhibitory neurotransmitter and its main actions are mediated via $GABA_A$-receptors. By gating the flow of chloride ions, these heteropentameric protein complexes determine the level of excitability of the vast majority of neurons in the brain. In addition, $GABA_A$-receptors are of major clinical significance as molecular targets for anxiolytic, anticonvulsant, muscle relaxant and hypnotic drugs (1,2,3).

$GABA_A$-receptors are formed by the assembly of various subunits which, in mammalian brain, are encoded by a total of 14 different genes (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ϵ). In heterologous expression systems, no major restrictions for the combinatorial assembly of the subunits were apparent. However, in vivo, the expression of $GABA_A$-receptor subunits is regulated in an ontogenetic and cell-specific manner providing particular populations of neurons with distinct sets of $GABA_A$-receptor subtypes (4). In addition, the process of receptor assembly is expected to include specific interactions among its constituent subunits. These interactions may govern not only the subunit stoichiometry but also the formation and membrane targeting of multiple $GABA_A$-receptors in a single cell. Thus, in vivo, the assembly and targeting of $GABA_A$-receptors appears to be determined by rules which may not be apparent in in vitro expression systems.

Recently, mutant mouse lines were generated in which the genes for the $GABA_A$-receptor subunits $\gamma 2$, $\beta 3$ and $\alpha 6$ were inactivated (5,6,7,8). In addition, mouse strains with radiation-induced chromosomal deletions which include the $\alpha 5$, $\beta 3$ and $\gamma 3$ subunit genes are available (9,10,12,13). These animal model provided insight into the physiological function of particular $GABA_A$-receptors and their contribution to behavioral phenotypes. On the structural level, the mutant mouse lines helped to uncover at least some of the constraints which govern subunit expressions, assembly and receptor targeting. The following report is restricted to

an analysis of the mutant mice with regard to the assembly and targeting of $GABA_A$-receptors subunits in vivo.

Independent regulation of subunit gene transcription

The inactivation of a particular $GABA_A$-receptor subunit gene does not appear to induce a compensatory up- or down-regulation of the transcription of other $GABA_A$-receptor subunit genes in mutant mouse brain. For instance, in the $\gamma 2$-subunit null mutants the protein level of the subunits $\alpha 1$, $\alpha 2$, $\alpha 3$, $\beta 2,3$, $\gamma 1$, $\gamma 3$ remained unaltered as shown by Western blotting (5). Similarly, in the $\beta 3$ subunit mutant, the $\beta 2$-subunit was unable to substitute, as deduced from radioligand binding experiments performed in newborn and adult animals (6). In addition, the inactivation of the $\alpha 6$ subunit gene, which selectively affected $GABA_A$-receptors in cerebellar granule cells, left the receptors containing the $\alpha 1$, $\beta 2,3$ and $\gamma 2$ subunits in this brain region unaltered as shown by radioligand binding and $\alpha 1$ subunit immunohistochemsitry, although a slight and variable downward trend in $\alpha 1$ protein levels was apparent in Western blotting (7). Similarly, in another $\alpha 6$ mutant, diazepam-insensitive 3H-Ro 15-4513 binding in cerebellar granule cells, corresponding to $\alpha 6$ receptors, was reduced while the mRNA for $\alpha 1$, $\alpha 3$, $\beta 2$, $\gamma 2$ and δ remained unchanged (8). These results clearly demonstrate that the lack of a particular subunit of $GABA_A$ receptors does not result in a molecular rescue by an enhancement of the transcription of a functionally related subunit.

Post-translational modifications

Im mice containing a targeted mutation of the $\alpha 6$-subunit gene, a post-translational influence on a related subunit was discovered (7). In addition to the lack of the $\alpha 6$ subunit protein a concommitant and selective loss of the δ subunit was found as demonstrated by immunoprecipitation, immunocytochemistry and immunoblot analysis with δ subunit-specific antibodies (7). The δ-subunit mRNA was present at wild type levels in the mutant granule cells, indicating a post-translational loss of the δ subunit. These results provide genetic evidence for a specific association between the $\alpha 6$ and δ subunits (7). However, in the absence of the $\alpha 6$-subunit the δ subunit appears to be degraded. Thus, in contrast to the transcription of the subunit genes, the protein level of a related subunit might be affected in mutant animals. A posttranslational effect has so far been observed only for the δ-subunit.

Identification of $GABA_A$-receptor subtypes in vivo.

The loss of a population of $GABA_A$-receptors by the inactivation of a subunit gene can facilitate the analysis of the remaining $GABA_A$-receptors which may otherwise be difficult to distinguish. For instance, in cerebellar granule cells several distinct $GABA_A$ receptor subtypes are expected to be expressed based on the presence of six subunits ($\alpha 1$, $\alpha 6$, $\beta 2$, $\beta 3$, $\gamma 2$ and δ). Current views accommodate the subunit combinations $\alpha 1\beta 2,3\gamma 2$, $\alpha 6\beta 2,3\gamma 2$, $\alpha 1\alpha 6\beta 2,3\gamma 2$, $\alpha 1\alpha 6\beta 2,3\gamma 2\delta$, $\alpha 1\beta 2,3\gamma 2\delta$ and $\alpha 6\beta 2,3\delta$. The lack of the $\alpha 6$-subunit was accompanied by a complete posttranslational loss of the δ subunit. However, the subunits $\alpha 1$, $\beta 2,3$ and $\gamma 2$ remained essentially unchanged in the δ-deficient/$\alpha 6$ null mutants as shown by radioligand binding and immunobiochemistry (7). Thus, the delta-subunit is unlikely to be associated with the $\alpha 1$ subunit indicating that the subunit combinations $\alpha 1\beta x\delta$ and $\alpha 1\beta x\gamma 2\delta$ are unlikely to be formed to large extent in vivo (7). Similarly, in dorsal root ganglion cells, new information on the subunit composition of the receptor subtypes was obtained. As shown by the electrophysiologic comparison between wildtype and the $\beta 3$ null mutant, the majority of

$GABA_A$-receptors in these neurons in neonatal brain contain the β3-subunit. The remaining $GABA_A$-receptors on these sensory neurons contain the β2-subunit rather than the β1 subunit since the remaining small GABA-evoked currents in neurons from the β3 mutant mice were sensitive to potentiation by the anticonvulsant loreclezole (6).

Subunits required for receptor assembly

Most $GABA_A$-receptors contain an α-subunit variant, a β-subunit variant and the γ2-subunit. It is however unknown, whether all three types of subunits are necessarily required for the formation of a receptor or whether, in mutant animals, the remaining subunits are sufficient to form functional receptors. When the β3-subunit gene was inactivated, about half of all $GABA_A$-receptors in the brain were lost (6). This result indicated, that the β3 subunit was essential for the assembly of the corresponding receptors and could not be substituted by the corresponding α- and γ2- subunits (6). Corresponding results were found in those cases in which an α-subunit-gene was deleted or inactivated. In mice with a deletion in chromosome 7 which includes the α5-subunit gene, the corresponding receptor subtype was not formed (11). Furthermore, the lack of the α6-subunit resulted in the loss of the corresponding receptor subtype in cerebellar granule cells (7,8). Thus, it appears that both the α and the β-subunit are essential components for the assembly and membrane targeting of $GABA_A$-receptors.

In contrast, in mice devoid of the γ2-subunit, $GABA_A$-receptors were formed in practically unaltered numbers from the remaining α- and β-subunits. This was evident from radioligand binding studies in newborn mice, in which the maximum number of GABA binding sites (^{3}H-99531 binding) was unaltered (5). Only the number of benzodiazepine binding sites (^{3}H-flumazenil binding) was reduced by 90 % concomitant with the absence of the γ2-subunit (5). The remaining α- and β-subunits were targeted to the cell surface as shown immunohistochemically in dorsal root ganglion cells. The receptors displayed a normal dose response curve for GABA, were potentiated by a barbiturate, but lacked the benzodiazepine response. In addition, the single channel conductance was reduced (5). Nevertheless, these experiments in newborn mice show that the γ2-subunit is not a necessary prerequisite to permit the assembly of $GABA_A$-receptors from the remaining subunits. It therefore appears that the assembly of α- and β-subunits is the main step in determining receptor formation and targeting.

Independent subcellular targeting of receptor subtypes

Various neurons express more than a single type of $GABA_A$-receptor. This can be most clearly visualised in hippocampal pyramidal cells, where receptors containing the α2-subunit are largely contentrated in the axon-initial segment, while receptors containing the α5-subunit are distributed on the cell soma and dendrites (14,15). The mechanisms governing the differential assembly and subcellular targeting of receptors subtypes are unknown. In mice lacking the α5-subunit due to a chromosomal deletion, the α5-receptors are absent in hippocampal pyramidal cells as shown by a reduction in radioligand binding and immunhistochemical staining of the α5-subunit. The α2-receptors however remained targeted to the axon-initial segment and did not display a compensatory distribution on the soma and dendrites (11). These results show that each receptor subtype has its own target identity presumably determined by domains of the distinctive α- and β-subunit variants. Highly selective recognition processes appear to be operative to ensure differential assembly and subcellular distribution of the receptor subtypes within a single cell.

Degradation of subunit fragments

Targeted disruption of a subunit gene with a replacement vector may not necessarily be sufficient to prevent the formation of a truncated subunit transcript to be formed. It therefore has to be tested whether a subunit fragment is generated which might possibly interfere with the process of receptor assembly and thereby affect the null mutation phenotype. In case of the α6- and γ2-subunit deficient mice, gene targeting was directed to exon 8 (5,7,8). Although a truncated mRNA of low abundance was described for the α6-mutant, a subunit fragment was not detectable on the protein level in either the α6- or the γ2-subunit mutants (5,7). In case of the β3-subunit mutant, part of the promotor and exon 1-3 of the β3-subunit gene were replaced. The non-targeted part of the gene did not result in a truncated transcript as tested by Northern blotting (6). Thus, these control experiments demonstrate that the gene targeting technology is suitable to analyse the process of subunit assembly of $GABA_A$-receptors in vivo.

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