Published in final edited form as:

Neurosci Lett. 1998 January 9; 240(2): 81–84.

A deficit of functional GABAA receptors in neurons of β_3 subunit knockout mice

Matthew D. Krasowski^a, Caroline E. Rick^b, Neil L. Harrison^{a,b,c,*}, Leonard L. Firestone^d, and Gregg E. Homanics^d

^a Committee on Neurobiology, University of Chicago, 915 East 57th Street, Chicago, IL 60637, USA

^b Department of Anesthesia/Critical Care, University of Chicago, 915 East 57th Street, Chicago, IL 60637, USA

^c Department of Pharmacological and Physiological Sciences, University of Chicago, 915 East 57th Street, Chicago, IL 60637, USA

^d Department of Anesthesiology/Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh PA 15261, USA

Abstract

Mice whose γ -aminobutyric acid type A (GABA_A) β_3 subunit gene is inactivated (' β_3 knockout mice') have been previously shown to have epilepsy, hypersensitive behavior, cleft palate, and a high incidence of neonatal mortality. In this study, we analyze whole-cell responses to GABA in neurons from $\beta_3^{+/+}$, $\beta_3^{+/-}$ and $\beta_3^{-/-}$ mice. We demonstrate markedly decreased responses to GABA in both hippocampal and dorsal root ganglion neurons isolated from $\beta_3^{-/-}$ mice without major differences in the GABA concentration-response curves. We also utilize the subunit selective pharmacology of Zn²⁺ and the anticonvulsant drug loreclezole to help infer the presence of β_2 and γ subunits in the GABA_A receptors remaining in neurons from $\beta_3^{-/-}$ mice.

γ -Aminobutyric acid type A (GABA_A) receptors mediate most of the rapid inhibitory synaptic transmission in the mammalian brain. GABA_A receptors are pentameric complexes consisting of subunits which assemble to form a ligand-gated chloride channel. To date, 16 GABA_A subunits have been identified and cloned (α_{1-6} , β_{1-4} , γ_{1-4} , δ , ϵ) [1,12,14]. GABA_A receptors are modulated by clinically important drugs such as the benzodiazepines, general anesthetics, and barbiturates [5,11].

The β_3 subunit is a major constituent of neuronal GABA_A receptors and is the predominant β subunit isoform mRNA in a number of brain and spinal cord regions [9,10,20]. β_3 subunit mRNA is particularly abundant in the prenatal and neonatal brain, which suggests a possible role in development [10]. Recently, mice in which the GABA_A β_3 subunit gene was inactivated (' β_3 knockout mice') were demonstrated to have epilepsy, hypersensitive behavior, cleft palate, and a high incidence of neonatal mortality [6]. The phenotype of the β_3 knockout mice strengthens the hypothesis that deficits in GABA_A receptor-mediated transmission may underlie certain forms of epilepsy [13] and indicates the importance of GABA_A receptor function in development and behavior. In the present study, we studied the function of GABA_A receptors in the brain and spinal cord of these animals.

*Corresponding author: University of Chicago, Whitman Laboratory, 915 East 57th Street, Room 202, Chicago, IL 60637, USA, Tel: +1-773-702-4054; fax: +1-773-702-4791; nh17@harper.uchicago.edu.

The production and phenotypic characterization of β_3 knockout mice has been previously described in detail [6]. For the experiments involving cultured hippocampal neurons, the hippocampus was microdissected, and neurons were dissociated from brains of 1-day-old mouse pups by methods described by Jones and Harrison [7], placed onto a layer of confluent mouse cortical astrocytes on a glass cover slip and maintained in tissue culture for 4–5 days prior to recording. For the experiments involving acutely dissociated dorsal root ganglion neurons (DRGs), DRGs were dissociated from postnatal day 1 mouse pups [18], plated onto poly-D-lysine coated coverslips and used for recordings within 12 h.

For all electrophysiological experiments, coverslips containing neurons were transferred to a chamber which was continuously perfused with extracellular medium containing (in mM): 145 NaCl, 3 KCl, 1.5 CaCl₂, 1 MgCl₂, 5.5 D-glucose, and 10 HEPES/NaOH (pH 7.4, osmolarity 320–330 mosmol). Recordings were made using the whole-cell patch-clamp technique [6,8]. The intracellular solution contained (in mM): 145 *N*-methyl-D-glucamine hydrochloride, 5 K₂ATP, 5 HEPES/KOH, 2 MgCl₂, 0.1 CaCl₂, and 1.1 EGTA/KOH (pH 7.2, osmolarity 315 mosmol). Neurons were voltage-clamped at –60 mV. Since the intracellular and extracellular solutions contained approximately equal concentrations of chloride ions, the chloride equilibrium potential was approximately 0 mV.

GABA and other drugs were rapidly applied (>1 ml/min) to neurons by local perfusion using a motor-driven solution exchange device (Bio Logic Rapid Solution Changer RSC-100; Molecular Kinetics, Pullman, WA, USA) as previously described [8]. Responses were low-pass-filtered at 5 kHz and digitized (TL-1–125 interface; Axon Instruments) using pCLAMP5 and stored for off-line analysis. Drugs used in this study were methohexital sodium (Brevital® sodium; Eli Lilly, Indianapolis, IN, USA) and zinc chloride (Sigma, St. Louis, MO, USA). Loreclezole was a kind gift from Janssen Pharmaceutica (Beerse, Belgium).

A marked reduction in the maximal amplitude of GABA-evoked whole cell currents was observed in hippocampal neurons from $\beta_3^{-/-}$ mice. Mean maximal GABA responses recorded in hippocampal neurons from β_3 mice were nearly 50% lower than those from neurons of either $\beta_3^{+/+}$ and $\beta_3^{+/-}$ mice (Fig. 1). This is very similar to the reported 50% decrease in the $B_{\max}$ for [³H]muscimol binding in whole brain homogenates from $\beta_3^{-/-}$ versus $\beta_3^{+/+}$ mice [6]. This suggests that there are fewer GABA_A receptors in neurons from $\beta_3^{-/-}$ mice. The amplitudes of GABA-evoked currents in hippocampal neurons from $\beta_3^{+/+}$ or $\beta_3^{+/-}$ mice do not differ significantly (Fig. 1C). The GABA concentration-response relationships for neurons from $\beta_3^{+/+}$, $\beta_3^{+/-}$, and $\beta_3^{-/-}$ mice are very similar, although the Hill slopes for neurons from $\beta_3^{+/-}$ and $\beta_3^{-/-}$ mice differ significantly from those from $\beta_3^{+/+}$ mice ($P < 0.001$ for each of the Hill slopes of $\beta_3^{+/-}$ and $\beta_3^{-/-}$ mice versus $\beta_3^{+/+}$ mice). Similar small differences in GABA concentration-response relationships have also been noted in recombinant studies of GABA_A receptor subunit combinations containing either the β_2 or β_3 subunit isoform [3].

In marked contrast to the situation in hippocampal neurons, where substantial GABA-evoked currents are observed in neurons from $\beta_3^{-/-}$ mice, maximal GABA responses are drastically reduced in DRGs from $\beta_3^{-/-}$ mice [6]. In particular, a majority (>80%) of DRGs from $\beta_3^{-/-}$ mice (27/36 cells) respond to a maximal GABA concentration (1 mM) with less than 100 pA of current.

Since *in situ* hybridization studies have shown that the β_3 subunit is the predominant GABA_A receptor β subunit isoform mRNA in neonatal rat DRGs [10], GABA currents in DRGs can reveal whether there is any replacement of the lost β_3 subunit by another β subunit isoform. A useful pharmacological tool to distinguish between GABA_A β subunit isoforms is the anticonvulsant drug loreclezole, which has been demonstrated to have a much higher apparent affinity at GABA_A receptors containing β_2 or β_3 subunits, relative to those containing

β_1 subunits [17,19]. Only a very small minority of DRG neurons from $\beta_3^{-/-}$ mice have whole-cell GABA currents large enough to perform potentiation experiments (approximately 1 in every 15 cells tested), but in these cells we determined that submaximal (EC_{20}) GABA currents from $\beta_3^{+/+}$, $\beta_3^{+/-}$ and $\beta_3^{-/-}$ mice were all potentiated equally by a low concentration (5 μ M) of loreclezole, consistent with most or all GABA_A receptors in DRGs from all three genotypes of mice containing β_2 and/or β_3 subunits (Fig. 2). Mean data for potentiation by 5 μ M loreclezole were: $\beta_3^{+/+}$ ($52.3 \pm 15.1\%$ potentiation, $n = 8$), $\beta_3^{+/-}$ (34.1 ± 8.3 , $n = 8$), and $\beta_3^{-/-}$ (56.1 ± 27 , $n = 3$). The barbiturate methohexital also potentiated submaximal GABA currents in neurons from all three mouse genotypes (Fig. 2). Mean data for potentiation by 5 μ M methohexital were: $\beta_3^{+/+}$ ($82.7 \pm 24.7\%$ potentiation, $n = 5$), $\beta_3^{+/-}$ (100.1 ± 31.5 , $n = 4$), and $\beta_3^{-/-}$ (134.0 ± 22.2 , $n = 3$). We also studied the effect of Zn^{2+} , which has a much higher apparent affinity for GABA_A receptors lacking a γ subunit; in particular, currents at GABA_A $\alpha\beta$ receptors can be inhibited completely by low micromolar concentrations of Zn^{2+} [2,16]. There was very little inhibition by a low concentration of zinc chloride (10 μ M) for GABA currents from DRGs from either $\beta_3^{+/+}$ ($9.7 \pm 3.1\%$ inhibition of EC_{50} GABA current, $n = 4$) or $\beta_3^{-/-}$ mice ($3.4 \pm 13.4\%$ inhibition, $n = 4$).

In the present report, we have extended previous observations [6] to show a clear reduction in GABA-evoked currents in hippocampal neurons of $\beta_3^{-/-}$ mice. The approximately 50% reduction in functional GABA_A receptors in hippocampal neurons from $\beta_3^{-/-}$ mice is consistent with evidence from in situ hybridization experiments demonstrating substantial expression of β_3 mRNA in the CA1 neurons of the hippocampus [9]. The GABA currents in the hippocampal neurons from β_3 mice presumably reflect β_2 subunit-containing GABA_A receptors since β_2 mRNA is also detected in appreciable amounts in the hippocampus [9].

Pharmacological experiments in sensory neurons support the idea that there is little or no replacement of the product of the inactivated β_3 subunit gene by another β subunit isoform. Potentiation experiments using loreclezole show that the small GABA currents in a minority of DRG cells from $\beta_3^{-/-}$ mice probably result from β_2 -containing GABA_A receptors, which are also likely to be present even in $\beta_3^{+/+}$ mice, since β_2 mRNA is detectable at low levels in neonatal animals [10]. This is consistent with the β_1 isoform being predominantly expressed in glial cells [4,15]. In addition, most or all of the GABA_A receptors in DRG cells from $\beta_3^{-/-}$ mice still contain the γ subunit (i.e. they are probably $\alpha\beta_2\gamma$ receptors) since there is little or no inhibition of GABA currents by micromolar concentrations of Zn^{2+} .

The β_3 knockout mouse appears to be a good model to examine the physiological and behavioral consequences of the lack of a significant fraction of the total GABA_A receptor population. The reduced pre- and post-synaptic inhibition resulting from the absence of many functional GABA_A receptors may well result in the hypersensitive behavior and epilepsy seen in $\beta_3^{-/-}$ mice and may also contribute to the developmental problems (e.g. cleft palate and early mortality) evident in some of these mice.

Acknowledgments

The authors thank Dr. Shubha Tole for her help with hippocampal dissections. MDK is supported by National Institute of Mental Health training fellowship MH11504.

References

1. Davies PA, Hanna MC, Hales TG, Kirkness EF. Insensitivity to anaesthetic agents conferred by a class of GABA_A receptor subunit. *Nature (London)* 1997;385:820–823. [PubMed: 9039914]
2. Draguhn A, Verdorn TA, Ewert M, Seeburg PH, Sakmann B. Functional and molecular distinction between recombinant rat GABA_A receptor subtypes by Zn^{2+} . *Neuron* 1990;5:781–788. [PubMed: 1702644]

3. Ducic I, Caruncho HJ, Zhu WJ, Vicini S, Costa E. γ -Aminobutyric acid gating of Cl^- channels in recombinant GABA_A receptors. *J Pharmacol Exp Ther* 1995;272:438–445. [PubMed: 7815361]
4. Gu Q, Perez-Velazquez JL, Angelides KJ, Cynader MS. Immunocytochemical study of GABA_A receptors in the cat visual cortex. *J Comp Neurol* 1993;333:94–108. [PubMed: 8393462]
5. Harris RA, Mihic SJ, Dildy-Mayfield JE, Machu TK. Actions of anesthetics on ligand-gated ion channels: role of receptor subunit composition. *FASEB J* 1995;9:1454–1462. [PubMed: 7589987]
6. Homanics GE, DeLorey TM, Firestone LL, Quinlan JJ, Handforth A, Harrison NL, Krasowski MD, Rick CEM, Korpi ER, Makela R, Brilliant MH, Hagiwara N, Ferguson C, Snyder K, Olsen RW. Mice devoid of γ -aminobutyric type A receptor β_3 subunit have epilepsy, cleft palate, and hypersensitive behavior. *Proc Natl Acad Sci USA* 1997;94:4143–4148. [PubMed: 9108119]
7. Jones MV, Harrison NL. Effects of volatile anesthetics on the kinetics of inhibitory postsynaptic currents in cultured rat hippocampal neurons. *J Neurophysiol* 1993;70:1339–1349. [PubMed: 7506753]
8. Krasowski MD, O'Shea SM, Rick CEM, Whiting PJ, Hadingham KL, Czajkowski C, Harrison NL. α subunit isoform influences GABA_A receptor modulation by propofol. *Neuropharmacology* 1997;36:941–949. [PubMed: 9257938]
9. Laurie DJ, Wisden W, Seeburg PH. The distribution of thirteen GABA_A receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. *J Neurosci* 1992;12:4151–4172. [PubMed: 1331359]
10. Ma W, Saunders PA, Somogyi R, Poulter MO, Barker JL. Ontogeny of GABA_A receptor subunit mRNAs in rat spinal cord and dorsal root ganglia. *J Comp Neurol* 1993;338:337–359. [PubMed: 7509352]
11. Macdonald RL, Olsen RW. GABA_A receptor channels. *Ann Rev Neurosci* 1994;17:569–602. [PubMed: 7516126]
12. McKernan RM, Whiting PJ. Which GABA_A -receptor subtypes really occur in the brain? *Trends Neurosci* 1996;19:139–143. [PubMed: 8658597]
13. Olsen RW, Avoli M. GABA and epileptogenesis. *Epilepsia* 1997;38:399–407. [PubMed: 9118844]
14. Rabow LE, Russek SJ, Farb DH. From ion currents to genomic analysis: recent advances in GABA_A receptor research. *Synapse* 1995;21:189–274. [PubMed: 8578436]
15. Rosier A, Arckens L, Orban GA, Vandesande F. Immunocytochemical detection of astrocyte GABA_A receptors in cat visual cortex. *J Histochem Cytochem* 1993;41:685–692. [PubMed: 8385682]
16. Smart TG, Moss SJ, Xie X, Haganir RL. GABA_A receptors are differentially sensitive to zinc: dependence on subunit composition. *Br J Pharmacol* 1991;103:1837–1839. [PubMed: 1655141]
17. Wafford KA, Bain CJ, Quirk K, McKernan RM, Wingrove PB, Whiting PJ, Kemp JA. A novel allosteric modulatory site on the GABA_A receptor β subunit. *Neuron* 1994;12:775–782. [PubMed: 8161449]
18. White G. Heterogeneity in EC_{50} and n_H of GABA_A receptors on dorsal root ganglion neurons freshly isolated from adult rats. *Brain Res* 1992;585:56–62. [PubMed: 1324774]
19. Wingrove PB, Wafford KA, Bain C, Whiting PJ. The modulatory action of loreclezole at the γ -aminobutyric acid type A receptor is determined by a single amino acid in the β_2 and β_3 subunit. *Proc Natl Acad Sci USA* 1994;91:4569–4573. [PubMed: 8183949]
20. Wisden W, Laurie DJ, Monyer H, Seeburg PH. The distribution of 13 GABA_A receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *J Neurosci* 1992;12:1040–1062. [PubMed: 1312131]

Fig. 1. GABA-evoked whole cell currents from cultured hippocampal neurons isolated from newborn $\beta_3^{+/+}$, $\beta_3^{+/-}$, and $\beta_3^{-/-}$ mice. (A) Representative currents from individual neurons from each group in response to GABA (10 μ M, 30 μ M, 100 μ M and 1 mM). The bar above the trace represents the duration of GABA application. (B) Pooled GABA concentration-response curves ($5 \leq n \leq 10$) yielding EC_{50} values of 15.9 ± 0.7 μ M, 27.4 ± 0.9 μ M, 26.8 ± 3.5 μ M for $\beta_3^{+/+}$, $\beta_3^{+/-}$ and $\beta_3^{-/-}$ mice, respectively. The Hill slopes were 1.7 ± 0.1 , 1.1 ± 0.03 and 1.0 ± 0.1 . (C) Pooled data for maximal GABA current amplitudes (mean $\pm$ SEM) for neurons tested. Numbers above bars indicate number of neurons contributing to mean value. The maximal response to 1 mM GABA for hippocampal neurons from $\beta_3^{-/-}$ mice differs significantly from

that for both $\beta_3^{+/+}$ ($P < 0.002$) and $\beta_3^{+/-}$ ($P < 0.0001$) mice (Student's unpaired t-test). Maximal GABA current amplitudes for hippocampal neurons from $\beta_3^{+/+}$ and $\beta_3^{+/-}$ mice do not differ significantly from each other.

Fig. 2.

Low concentrations of loreclezole (LCZ) and the barbiturate methohexital (MTX) enhance submaximal GABA currents in DRGs from both $\beta_3^{+/+}$ and $\beta_3^{-/-}$ mice. Potentiation of approximately EC₂₀ (25 μ M) GABA responses by LCZ and MTX at DRGs from both $\beta_3^{+/+}$ mice (A) and $\beta_3^{-/-}$ mice (B). Note the much lower amplitude of currents in response to the maximal concentration (1 mM) of GABA in the neuron from the $\beta_3^{-/-}$ mouse, which accounts for the noisier appearance of the traces from $\beta_3^{-/-}$ cells.