

GABA_A receptor β_{1-3} subunit gene expression in the hippocampus of kindled rats

W. Kamphuis*, T.C. De Rijk, F.H. Lopes da Silva

Graduate School for the Neurosciences, University of Amsterdam, Institute of Neurobiology, Kruislaan 320, 1098 SM Amsterdam, The Netherlands

Received 15 February 1994; Revised version received 13 April 1994; Accepted 13 April 1994

Abstract

The effect of Schaffer collateral/commissural fiber kindling on the expression levels of GABA_A receptor β_1 , β_2 , and β_3 subunit mRNA in the pyramidal and granular neurons of the rat dorsal hippocampus was studied, using semi-quantitative in situ hybridization. In pyramidal neurons of CA1 and CA3, only small changes (10–15%) were found. In dentate granule neurons, the expression level of GABA_AR- β_3 mRNA was significantly, enhanced, bilaterally, in animals that were partial or fully kindled. At long-term, 4 weeks after the last convulsion no significant changes were found in pyramidal or granular neurons.

Key words: Kindling; GABA_A receptor β subunit; γ -Aminobutyric acid; mRNA; Hybridization, in situ; Hippocampus; Fascia dentata; Epileptogenesis

The repeated application of a high-frequency electrical stimulation to discrete regions of the brain results in the occurrence of epileptiform afterdischarges of increasing duration and intensity and in the gradual appearance of behavioural epileptiform seizure activity [4]. This process of epileptogenesis, called 'kindling', leads to a long-lasting increased seizure susceptibility and has been extensively studied as an experimental model of focal epilepsy [8,12].

In previous work we have reported several changes that take place in the GABAergic inhibitory system in the hippocampus as a result of kindling stimulations. In CA1 area, a reduction of the inhibition takes place as evidenced by a reduced paired-pulse inhibition of local evoked field potentials [7,9,27], a decreased sensitivity of the pyramidal neurons for iontophoretically applied GABA [6], and a reduced binding of the GABA_A receptor agonist [³H]muscimol [22]. In contrast to the decreased inhibition in CA1, in the fascia dentata paired-pulse inhibition is strengthened [7,23,28], together with a robust increase in [³H]muscimol binding in this area [14,18,22].

Structurally, the GABA_A-receptor is a hetero-oligomeric complex composed of several subunits (α , β , γ , δ , ρ), each of which exists in the brain in different variants [11,16]. In recombinant expression studies, the association of different cloned subunit variants leads to a functional diversity of the GABA_A receptor complexes with regard to GABA-activated chloride currents, benzodiazepine pharmacology and sensitivity for agonists [11,16,20]. In purified preparations of GABA_A receptors of the brain, the binding of [³H]muscimol shows a preference for bands that co-migrate with those that stain with a β subunit-specific antibody [1,2,3,15]. Therefore, we have investigated whether the observed alterations in [³H]muscimol binding in the two hippocampal areas may be related to a modified expression of the genes that encode for the three identified variants of the β -class [10,24,26]. To allow a differential measurement of the GABA_AR- β_1 , β_2 , and β_3 subunit mRNA levels in the hippocampal subregions, we used the technique of in situ hybridization.

Male Wistar rats ($n = 54$) were used in this study. Stainless-steel electrodes were implanted in the CA1 area of the left dorsal hippocampus of rats under pentobarbital anaesthesia. The stimulation bundle was placed in the Schaffer-collateral/commissural fiber pathway and the

*Corresponding author. Fax: (31) (20) 525-7709.

recording bundle was placed in stratum radiatum of CA1 as described previously [5,9]. After two weeks of recovery, 29 rats received kindling stimulations (200–300 μ A, 50 Hz, 1–2 s, twice daily) which evoke afterdischarges (ADs) with increasing duration, while the other 25 implanted animals were handled only and served as controls. The expression of GABA_AR- β_1 , β_2 , and β_3 subunit mRNA was studied in following kindled groups: (1) a 6-AD group, studied after the 6th tetanic stimulation; (2) a 14-AD group, sacrificed after the 14th afterdischarge; (3) a Fully Kindled (FK) group sacrificed after 22–33 afterdischarges when rats had experienced at least 6 generalized tonic-clonic convulsions; (4) a Long-Term Kindled (LK) group. Animals of the 6-AD, 14-AD and FK group were sacrificed 24 h after the last seizure, while the LK group was studied 28 days after the last generalized seizure. Along with each group of kindled animals, control animals were sacrificed. GABA_A- β_1 , β_2 , and β_3 mRNA levels were detected by [³⁵S]dATP 3'-end tailed oligonucleotide probes [24]. The sequences and the specificity of oligonucleotides used here were described by Wisden et al. [24], together with a detailed account of the distribution of the GABA_A- β subunit mRNAs in the rat brain. In situ hybridization and the subsequent densitometric analysis was carried out as described elsewhere [5,24,25].

All three β subunit mRNAs were present in the pyramidal and granular cell layers of the hippocampus (Fig. 1), what is in agreement with other reports [10,17,24]. The densitometric analysis of the autoradiograms revealed no statistical differences in expression levels between ipsi- and contralateral hemisphere in any of the groups. Therefore, further analysis was carried out using the mean extinction value of the left and right hemisphere. The mean extinction values of kindled groups were compared with the control animals fixed at the same time

using the Student's *t*-test. To facilitate the comparison of the changes found in the different kindled groups, we determined the percentual change of the extinction values in the kindled groups in comparison to the matching control groups by dividing the mean extinction of the kindled group by that of the control group and multiplying the outcome by 100. The difference from the 100% (= control) value is presented in Table 1 for the CA1, CA3 and fascia dentata.

Most changes of the different subunits mRNAs were in the same direction for the kindled animals sacrificed 24 h after 6- and 14-AD and for the FK group. Therefore, we pooled the three groups (Kindle-pooled, $n = 21$) and compared the results with the controls ($n = 17$). The numerical results are shown in Table 1. The following significant ($P < 0.05$, Student's *t*-test) changes were encountered in the three areas studied.

(1) In CA1, there was an increase of β_1 and a decrease of β_2 , while β_3 did not show a consistent change.

(2) In CA3, there was a similar increase of β_1 and decrease of β_2 , but here β_3 increased also.

(3) In FD, β_1 increased but β_2 did not show a consistent change, while there was a conspicuous increase of β_3 .

In summary, the pooled kindled groups showed an increase of β_1 in all areas, a decrease of β_2 in CA1 and CA3, and an increase of β_3 in CA3 and FD.

We examined next, whether there were significant changes of individual subunits for the distinct groups. Although these groups were rather small, a few significant changes were found, as indicated in Table 1. The most pronounced was the increase of β_3 in FD, that was significant at all kindling stages (6-, 14-AD and -FK). It is also worthwhile mentioning that β_3 of the CA3 area increased significantly at the FK stage, although this subunit did not change consistently for the other two kindled groups in the same area.

Table 1

Relative changes in mean extinction values of the in situ hybridization autoradiograms of GABA_A receptor β_1 , β_2 , and β_3 in the different kindled groups (% \pm S.E.M.) and the pooled animals of 6-AD, 14-AD and FK group (kindle-pooled)

	Kindle-pooled ($n = 21$)	6-AD ($n = 7$)	14-AD ($n = 6$)	FK ($n = 8$)	LK ($n = 8$)
CA1					
GABA _A - β_1	9.2 \pm 2.1****	4.3 \pm 2.8	9.6 \pm 4.5	9.4 \pm 3.0**	2.6 \pm 2.6
GABA _A - β_2	-7.5 \pm 2.1*	-8.8 \pm 3.4	-9.8 \pm 2.8	-2.5 \pm 2.9	-9.9 \pm 3.9
GABA _A - β_3	3.4 \pm 2.5	4.1 \pm 5.7	-1.2 \pm 4.0	7.2 \pm 2.4*	0.4 \pm 4.8
CA3					
GABA _A - β_1	5.6 \pm 1.5*	0.5 \pm 2.2	6.2 \pm 1.8	7.1 \pm 3.4	-2.3 \pm 1.8
GABA _A - β_2	-11.4 \pm 2.6***	-12.6 \pm 2.7	-14.3 \pm 3.1	-6.4 \pm 5.3	6.6 \pm 4.6
GABA _A - β_3	7.4 \pm 2.1**	5.9 \pm 3.7	2.3 \pm 2.4	14.5 \pm 2.9***	-2.2 \pm 4.2
Fascia dentata					
GABA _A - β_1	11.6 \pm 3.5**	3.2 \pm 4.4	9.1 \pm 6.0	6.6 \pm 5.6	9.7 \pm 5.2
GABA _A - β_2	6.3 \pm 3.2	-4.2 \pm 2.0	0.8 \pm 7.7	21.1 \pm 6.6***	-0.2 \pm 4.8
GABA _A - β_3	29.9 \pm 3.0****	26.7 \pm 6.1****	31.0 \pm 5.7****	29.9 \pm 4.2****	4.5 \pm 6.0

The value obtained in the control group was used as reference and set at 100%. Statistical comparisons with respect to controls were carried out on the determined extinction values using the Student's *t*-test. * $P < 0.05$; ** $P < 0.03$; *** $P < 0.01$; **** $P < 0.002$ (two tailed).

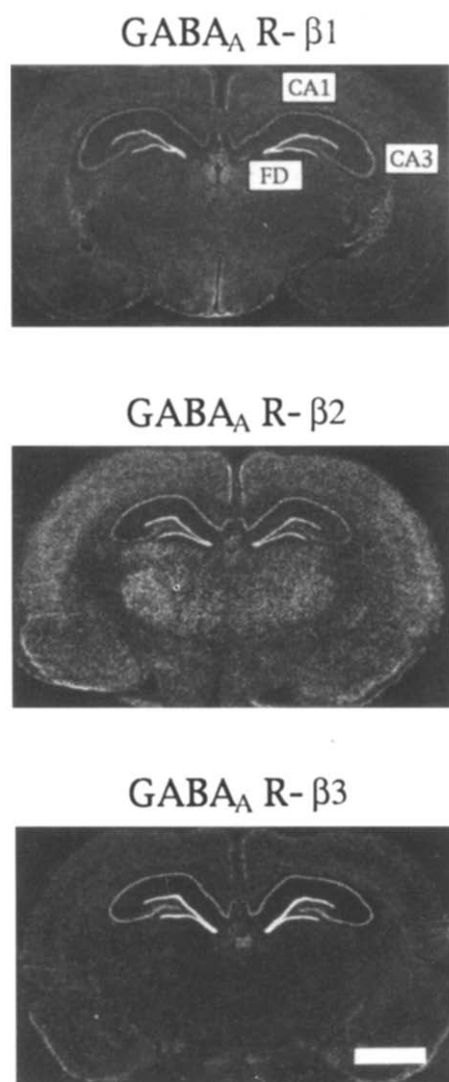


Fig. 1. GABA_A receptor β_1 , β_2 , and β_3 mRNA distribution in sections of control rats. Illustrations were obtained by direct enlargement of the autoradiograms on photographic paper. The ipsilateral electrode implanted hemisphere is on the left side. Bar = 2.5 mm.

In the LK group no significant changes were detected.

In order to confirm the increased expression of GABA_AR- β_3 in all principal neurons of the hippocampus in fully kindled animals, we studied the mRNA levels in a second series of fully kindled animals. Animals were kindled in a comparable way as described above, and sacrificed 24 h ($n = 6$) after the last generalized seizure. A significantly enhanced expression level of GABA_AR- β_3 mRNA in all hippocampal areas was found in comparison with controls ($n = 6$). The relative increase was: in CA1: $+17.7 \pm 5.2\%$ ($P \leq 0.05$), in CA3: $+23.5 \pm 4.7\%$ ($P \leq 0.02$), and in fascia dentata: $+31.9 \pm 5.3\%$ ($P \leq 0.004$). In an additional group of kindled animals, sacrificed 28 days ($n = 6$) after the last class 5 seizure, again no changes in GABA_AR- β_3 mRNA levels were found that persisted as long as 4 weeks.

Kindling of the Schaffer collateral/commissural path-

way leads to a gradual increase of the GABAergic inhibition in the fascia dentata [7,23,28] whereas in CA1 inhibition decreases [6,7,9,27]. These opposite changes in GABAergic inhibition are accompanied by a bilateral increased binding of GABA_A-receptor agonist [³H]muscimol in the fascia dentata [14,18,22] and a decreased binding in CA1 [22]. The absence of changes restricted to the stimulated hemisphere is probably due to the rapid spread of the afterdischarge activity from the site of stimulation to the contralateral hippocampus already occurring with the first kindling session.

Based on the observations described here, we conclude that kindling stimulations result only in small (10–15%) changes of GABA_AR- β subunit expression in CA1 and CA3 areas, but in opposite directions; a decrease of GABA_AR- β_2 and an increase of GABA_AR- β_1 mRNA levels. The decreased [³H]muscimol binding in CA1 in fully kindled animals cannot be related in a straightforward way to an altered expression of the GABA_AR- β subunit encoding genes. It may be possible that changes in the phosphorylation level of the GABA_AR-subunits rather than changes in receptor density or composition are primarily responsible for the reduction in GABAergic inhibition in this brain area [13,19,21]. In the fascia dentata the GABA_AR- β mRNA levels were clearly increased bilaterally in the course of kindling, most prominently the β_3 levels. We hypothesize, that the enhanced expression of the GABA_AR- β genes in this area underlies the robust increase of [³H]muscimol binding sites in the fascia dentata in fully kindled animals which most likely subserves the observed increase of recurrent inhibition [7,23,28]. Such an alteration would counterbalance changes in the same area that result in enhanced glutamatergic excitatory synaptic transmission [5]. Obviously, further quantification of the receptor complex proteins, using subunit specific antibodies, will be needed to substantiate this conclusion and to establish the precise time course and persistence of the long-term changes.

These investigations were supported by the Royal Netherlands Academy of Arts and Sciences (KNAW) and the Committee on Epilepsy of the Division for Health Research of the National Epilepsy Fund (CLEO, project number A-77). We would like to thank Dr. H. Monyer for kindly providing us with the DNA probes for the GABARs and Dr. P.C. Diegenbach for his assistance on the densitometric analysis of the autoradiograms.

- [1] Bureau, M. and Olsen, R.W., Multiple distinct subunits of the γ -aminobutyric acid_A receptor protein show different ligand binding affinities, *Mol. Pharmacol.*, 37 (1990) 497–502.
- [2] Casalotti, S.O., Stephenson, F.A. and Barnard, E.A., Separate subunits for agonist and benzodiazepine binding in the γ -aminobutyric acid_A oligomer, *J. Biol. Chem.*, 32 (1986) 15013–15016.
- [3] Deng, L., Ransom, R.W. and Olsen, R.W., [³H]Muscimol labels the γ -aminobutyric acid receptor binding site on a peptide subunit

- distinct from that labeled with benzodiazepines, *Biochem. Biophys. Res. Commun.*, 138 (1986) 1308–1314.
- [4] Goddard, G.V., McIntyre, D.C. and Leech, C.K., A permanent change in brain function resulting from daily electrical stimulation, *Exp. Neurol.*, 25 (1969) 295–330.
 - [5] Kamphuis, W., De Rijk, T.C., Talamini, L.M. and Lopes da Silva, F.H., Hippocampal kindling induces changes in the glutamate receptor mRNA expression patterns in dentate granule neurons, *Eur. J. Neurosci.*, in press.
 - [6] Kamphuis, W., Gorter, J.A. and Lopes da Silva, F.H., A long-lasting decrease in the inhibitory effect of GABA on glutamate responses of hippocampal pyramidal neurons induced by kindling epileptogenesis, *Neuroscience*, 41 (1991) 425–431.
 - [7] Kamphuis, W., Gorter, J.A., Wadman, W.J. and Lopes da Silva, F.H., Hippocampal kindling leads to different changes in paired-pulse depression of local evoked potentials in CA1 area and in fascia dentata, *Neurosci. Lett.*, 141 (1992) 101–105.
 - [8] Kamphuis, W. and Lopes da Silva, F.H., The kindling model of epilepsy: The role of GABAergic inhibition, *Neurosci. Res. Commun.*, 6 (1990) 1–10.
 - [9] Kamphuis, W., Wadman, W.J. and Lopes da Silva, F.H., Changes in local evoked potentials in the rat hippocampus (CA1) during kindling epileptogenesis, *Brain Res.*, 440 (1988) 205–215.
 - [10] Lolait, S.J., O'Carroll, A.M., Kusano, K. and Mahan, L.C., Pharmacological characterization and region-specific expression in brain of the β_2 - and β_3 -subunits of the rat GABA_A receptor, *FEBS Lett.*, 258 (1989) 17–21.
 - [11] Lüddens, H. and Wisden, W., Function and pharmacology of multiple GABA_A receptor subunits, *Trends Pharmacol. Sci.*, 12 (1991) 49–51.
 - [12] McNamara, J.O., Bonhaus, D.W., Shin, C., Crain, B.J., Gellman, R.L. and Giacchino, J.L., The kindling model of epilepsy: a critical review, *CRC Crit. Rev. Clin. Neurobiol.*, 1 (1985) 341–391.
 - [13] Moss, S.J., Smart, T.G., Blackstone, C.D. and Huganir, R.L., Functional modulation of GABA_A receptors by cAMP-dependent protein phosphorylation, *Science*, 257 (1992) 661–665.
 - [14] Nobrega, J.N., Kish, S.J. and McIntyre Burnham, W., Regional brain [³H]muscimol binding in kindled rat brain: a quantitative autoradiographic examination, *Epilepsy Res.*, 6 (1990) 102–109.
 - [15] Olsen, R.W., Bureau, M.H., Endo, S. and Smith, G., The GABA_A receptor family in the mammalian brain, *Neurochem. Res.*, 16 (1991) 317–325.
 - [16] Olsen, R.W. and Tobin, A.J., Molecular biology of GABA_A receptors, *FASEB J.*, 4 (1990) 1469–1480.
 - [17] Pehrsohn, E., Malherbe, P. and Richards, J.G., Comparative molecular neuroanatomy of cloned GABA_A receptor subunits in the rat CNS, *J. Comp. Neurol.*, 326 (1992) 193–216.
 - [18] Shin, C., Pedersen, H.B. and McNamara, J.O., γ -Aminobutyric acid and benzodiazepine receptors in the kindling model of epilepsy: A quantitative radiohistochemical study, *J. Neurosci.*, 5 (1985) 2696–2701.
 - [19] Sigel, E., Baur, R. and Malherbe, P., Activation of protein kinase C results in down-modulation of different recombinant GABA_A-channels, *FEBS Lett.*, 291 (1991) 150–152.
 - [20] Sigel, E., Baur, R., Trube, G., Möhler, H. and Malherbe, P., The effect of subunit composition of rat brain GABA_A receptors on channel function, *Neuron*, 5 (1990) 703–711.
 - [21] Stelzer, A., Intracellular regulation of GABA_A-receptor function, *Ion Channels*, 3 (1992) 83–136.
 - [22] Titulaer, M.N.G., Kamphuis, W., Pool, C.W., Van Heerikhuizen, J.J. and Lopes da Silva, F.H., Kindling induces time-dependent and regional specific changes in the [³H]muscimol binding in the rat hippocampus: a quantitative autoradiographic study, *Neuroscience*, (1994) 817–826.
 - [23] Tuff, L.P., Racine, R.J. and Adamec, R., The effects of kindling on GABA-mediated inhibition in the dentate gyrus of the rat. I. Paired-pulse depression, *Brain Res.*, 277 (1983) 79–90.
 - [24] Wisden, W., Laurie, D.J., Monyer, H. and Seeburg, P.H., The distribution of 13 GABA_A receptor subunit mRNA's in the rat brain. I. Telencephalon, diencephalon, mesencephalon, *J. Neurosci.*, 12 (1992) 1040–1062.
 - [25] Wisden, W., Morris, B.J. and Hunt, S.P., In situ hybridization with synthetic DNA probes. In J. Chad and H. Wheal (Eds.), *Molecular Neurobiology – a Practical Approach*, Oxford, 1991, pp. 205–225.
 - [26] Ymer, S., Schofield, P.R., Draguhn, A., Werner, P., Kohler, M. and Seeburg, P.H., GABA_A receptor β subunit heterogeneity: functional expression of cloned cDNAs, *EMBO J.*, 8 (1989) 1665–1670.
 - [27] Zhao, D. and Leung, L.S., Effects of hippocampal kindling on paired-pulse response in CA1 in vitro, *Brain Res.*, 564 (1991) 220–229.
 - [28] Zhao, D. and Leung, S.L., Hippocampal kindling induced paired-pulse depression in the dentate gyrus and paired-pulse facilitation in CA3, *Brain Res.*, 582 (1992) 163–167.