Changes in the expression of GABA<sub>A</sub> receptor subunit mRNAs in parahippocampal areas after kainic acid-induced seizures

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INTRODUCTION
Temporal lobe epilepsy (TLE) is the most common and difficult to treat form of focal epilepsies. It comprises about 30% of all epilepsies (Fisher et al., 1998). The most common pathology underlying TLE is hippocampal damage, termed Ammon’s horn sclerosis, and primarily affects the hilus of the dentate gyrus (DG) and hippocampal sectors CA1 and CA3, and subunit α<sub>1</sub> also in the EC layer II (30 and 90 days after KA). We also observed sustained overexpression of subunits α<sub>4</sub> and α<sub>2</sub> in the subiculum and in the Ammon’s horn. Subunit γ<sub>2</sub> mRNA was also increased in sector CA1 at the late intervals after KA. Taken together, our results suggest distinct regulation of mRNA expression for individual GABA<sub>A</sub> receptor subunits. Especially striking was the wide-spread down-regulation of the often peri- or extrasynaptically located subunits γ<sub>2</sub> and δ. These subunits are often associated with tonic inhibition. Their decrease could be related to decreased tonic inhibition or may merely reflect compensatory changes. In contrast, expression of subunit α<sub>4</sub> that may also mediate tonic inhibition when associated with the δ-subunit was significantly upregulated in the DG and in the proximal subiculum at late intervals. Thus, concomitant up-regulation of subunit γ<sub>2</sub>, α<sub>1</sub> and α<sub>4</sub> mRNAs (and loss in δ-subunits) ultimately indicates significant rearrangement of GABA<sub>A</sub> receptor composition after KA-induced seizures.

Keywords: epilepsy, tonic inhibition, GABA<sub>A</sub>-receptor, temporal lobe epilepsy, subiculum, entorhinal cortex, epileptogenesis

The parahippocampal areas including the subiculum, pre- and parasubiculum, and notably the entorhinal cortex (EC) are intimately involved in the generation of limbic seizures in temporal lobe epilepsy. We investigated changes in the expression of 10 major GABA<sub>A</sub> receptor subunit mRNAs in subfields of the ventral hippocampus, ventral subiculum, EC, and perirhinal cortex (PRC) at different intervals (1, 8, 30, and 90 days) after kainic acid (KA)-induced status epilepticus priming epileptogenesis in the rat. The most pronounced and ubiquitous changes were a transient (24 h after KA only) down-regulation of γ<sub>2</sub> mRNA and lasting decreases in subunit α<sub>5</sub>, β<sub>2</sub>, and γ<sub>2</sub> mRNAs that were prominent in all hippocampal and parahippocampal areas. In the subiculum similarly as in sectors CA1 and CA3, levels of subunit α<sub>1</sub>, α<sub>2</sub>, α<sub>4</sub>, and γ<sub>2</sub> mRNAs decreased transiently 11 days after KA-induced status epilepticus. They were followed by increased expression of subunit α<sub>1</sub> and α<sub>4</sub> mRNAs in the dentate gyrus (DG) and sectors CA1 and CA3, and subunit α<sub>1</sub> also in the EC layer II (30 and 90 days after KA). We also observed sustained overexpression of subunits α<sub>4</sub> and γ<sub>2</sub> in the subiculum and in the Ammon’s horn. Subunit γ<sub>2</sub> mRNA was also increased in sector CA1 at the late intervals after KA. Taken together, our results suggest distinct regulation of mRNA expression for individual GABA<sub>A</sub> receptor subunits. Especially striking was the wide-spread down-regulation of the often peri- or extrasynaptically located subunits γ<sub>2</sub> and δ. These subunits are often associated with tonic inhibition. Their decrease could be related to decreased tonic inhibition or may merely reflect compensatory changes. In contrast, expression of subunit α<sub>4</sub> that may also mediate tonic inhibition when associated with the δ-subunit was significantly upregulated in the DG and in the proximal subiculum at late intervals. Thus, concomitant up-regulation of subunit γ<sub>2</sub>, α<sub>1</sub> and α<sub>4</sub> mRNAs (and loss in δ-subunits) ultimately indicates significant rearrangement of GABA<sub>A</sub> receptor composition after KA-induced seizures.

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INTRODUCTION
Temporal lobe epilepsy (TLE) is the most common and difficult to treat form of focal epilepsies. It comprises about 30% of all epilepsies (Fisher et al., 1998). The most common pathology underlying TLE is hippocampal damage, termed Ammon’s horn sclerosis, and primarily affects the hilus of the dentate gyrus (DG) and hippocampal sectors CA1 and CA3, and while other brain areas are considerably less affected (Babb et al., 1984). In recent years, however, neurodegeneration and epilepsy-induced neurochemical changes were found also in areas closely associated with the hippocampus, such as the subiculum (Andròli et al., 2007) and the entorhinal cortex (EC; Du et al., 1995; Bartolomei et al., 2003). These brain regions may also be intimately involved in seizure propagation in human TLE (Cohen et al., 2002; Wozny et al., 2005; Fabo et al., 2008; Huberfeld et al., 2011) and in animal models of TLE (Knopp et al., 2005; de Guzman et al., 2006; Kumar and Buckmaster, 2006). Malfunctioning of GABA<sub>A</sub>-receptor transmission is one of the major hypotheses for generation of epilepsy. Thus, preferential losses of GABAergic neurons have been proposed to be responsible for impaired inhibition (Houser et al., 1986; Sloviter, 1987; Andre et al., 2001; Dinocourt et al., 2003). Reports showing overexpression of neurochemical markers, such as glutamate decarboxylases or neuropeptides, indicating enhanced GABA<sub>A</sub>-receptor transmission in surviving GABA neurons, however challenged this view (Marksteiner and Sperk, 1988; Esclapez and Houser, 1999; Sperk et al., 2003). Several groups, however, demonstrated a selective loss of parvalbumin-containing interneurons (Best et al., 1993; DeFelipe et al., 1995; Knopp et al., 2008; Drexel et al., 2011) or down-regulation of parvalbumin (Wittner et al., 2001; Magloczky and Freund, 2005) in these neurons in the sector CA1 and the subiculum of epileptic rats and in TLE patients. Also a role of possibly impaired GABA<sub>A</sub>-receptor transmission through altered GABA<sub>A</sub> or GABA<sub>B</sub> receptors has been extensively investigated (Campbell et al., 1995; Sperk et al., 1998; Furtinger et al., 2003). One of the initially unexpected findings was that GABA<sub>A</sub> receptor binding is increased, not decreased in kindled rats (Shin et al., 1985). Later...
an altered subunit constitution of GABAA receptors and conse-
sequently altered GABAergic transmission was proposed as a cause
of epileptogenesis (Sperk et al., 1998, 2004). Several groups per-
formed neurochemical and electrophysiological experiments in rat
models and in hippocampal tissue removed from patients suf-
f ering from TLE (Loep et al., 2000; Pirker et al., 2003; Peng et al.,
2004; Nishimura et al., 2005). These studies mainly focused on the
hippocampal formation and epilepsy-induced changes included
increased expression of α-4, -γ2, and β-subunits along with
decreased expression of β-subunit in the DG or down-regulation
of α5-subunits in CA1 pyramidal cells (Schwarzer et al., 1997; 
Tsunashima et al., 1997; Loep et al., 2000; Houser and Easlepe,
2003; Peng et al., 2004). The changes observed indicate distinct
effects of epilepsy on subunits implicated in phasic or tonic
GABAergic neurotransmission, respectively.

Most studies in animal models of TLE so far focused on changes
in GABAA receptor subunit expression in the dorsal hippocampus
including the DG and the Ammon’s horn (Sperk et al., 1998-2004).
We recently became aware of a crucial role of parahippocampal
areas, notably of the subiculum and the EC in epileptogenesis.
Recent key findings were increased excitability of the subiculum
in rodent models of TLE (Knopf et al., 2005; de Guzman et al.,
2006) and in tissue obtained from TLE surgery (Cohen et al.,
2002; Wonyy et al., 2003; Hubertfeld et al., 2011), massive losses
of parvalbumin-expressing interneurons in the subiculum and in
deep layers of the EC (Andrioli et al., 2007; Knopf et al., 2008;
Dreuel et al., 2011), and a correlation of the loss of parvalbumin-
expressing interneurons in the subiculum with the numbers of
spontaneous seizures in the rat kainic acid (KA)-model of TLE
(Dreuel et al., 2011).

To elucidate possible changes in GABAergic transmission in
parahippocampal areas we investigated changes in GABAA recep-
tor subunit expression in the ventral hippocampus including the
subiculum and the entorhinal and perirhinal cortices.

MATERIALS AND METHODS

ANIMALS

Adult male Sprague-Dawley rats (220–250 g; Institut für Versuch-
ierzucht, Hämberg, Austria) were used in the study. The rats were
housed in single-ventilated cages at a temperature of 22–24 ◦C, a
relative humidity of 50–60%, and a 12 h light/dark cycle. They had
access to food and water ad libitum. All animal experiments were
conducted according to national guidelines and European Com-
munity laws and were approved by the Committee for Animal
Protection of the Austrian Ministry of Science.

KAINIC ACID INJECTION

Twenty-nine rats were injected i.p. with 10 mg/kg KA (5 mg/ml in
saline, pH 7.0, Ascent Scientific, Bristol, UK) and 13 control rats
with saline. Two hours after the first generalized seizure the rats
were treated with diazepam (10 mg/kg i.p., Gwäcalm, Nycomed
Austria GmbH, Linz, Austria) to reduce mortality and severity of
the neuropathological outcome. Their seizure behavior was inves-
tigated for at least 3 h and rated according to a five-stage rating
scale described previously (Sperk et al., 1993). Rats without obvi-
ous behavioral changes were rated as stage 0, rats showing wet
dog shakes only as stage 1, rats with chewing, head bobbing and
forelimb cloni as stage 2, rats with generalized seizures and rear-
ing as stage 3, rats with generalized seizures, rearing and loss of
postural tone (falling over) as stage 4, and rats that died dur-
ing status epilepticus were rated as stage 5. Only rats exhibiting
rating 3 or 4 were used. In brains of 19 rats (+9 controls) in
site hybridization and in four rats (+4 controls) neuron specific
nucl ear protein (NeuN) immunohistochemistry was performed
30 days after injection of KA.

TISSUE PREPARATION

For in situ hybridization, rats were killed by exposure to CO2-
gas either 1 day (n = 5), 8 days (n = 6), 30 days (n = 5), or
90 days (n = 3) after KA-induced status epilepticus. Controls
were killed 1 day (n = 3), 30 days (n = 3), or 90 days (n = 3)
after saline injection. These intervals were chosen for assessing
changes directly related to consequences of the status epilepti-
cus (1 day), to changes in the presumed silent phase (8 days),
and changes due to the chronic epilepsy syndrome (30 and 90
days). Brains were quickly removed and snap-frozen in isopentane
(−70 ◦C). Horizontal 20 μm sections were cut using a cryostat-
microtome (Microm HM 560 M, Carl Zeiss AG, Vienna, Austria),
thaw-mounted on silane-coated slides and stored at −70 ◦C. Every
11th section was stained with cresyl violet, dehydrated, cleared in
butyl acetate, and coverslipped using Eukitt mounting medium (O.
Kindler GmbH, Freiburg, Germany). These sections were used for
matching the individual brains at the same anatomical level along
the dorso-ventral axis for later histochemistry.

IN SITU HYBRIDIZATION

In situ hybridization was performed as described previously
in detail (Tsunashima et al., 1997). The sequences of custom-
synthesized oligonucleotides (Microsynth AG, Balgach, Switzer-
land) complementary to the respective mRNAs for GABAA
receptor subunits that were used as probes are listed in Table 1.
Briefly, the oligonucleotides (2.5 pmol) were labeled at the 3’-end
with [35S] a-thio-dATP (1,300 Ci/mmol, New England
Nuclear, Boston, MA, USA) by reaction with terminal deoxynu-
clearotidetransferase (Roche Austria GmbH, Vienna, Austria)
and precipitated with 75% ethanol and 0.4% NaCl. Frozen sections
(20 μm) were immersed in ice-cold paraformaldehyde (2%) in
phosphate-buffered saline (PBS), pH 7.2 for 10 min, rinsed in PBS,
immersed in acetic anhydride (0.25% in 0.1 mol/l triethylamine
hydrochloride) at room temperature for 10 min, dehydrated by
ethanol series, and delipidated with chloroform. The sections
were then hybridized in 50 μl hybridization buffer containing
about 50 fmol (0.8 to 1 × 108 cpm) labeled oligonucleotide
probe for 18 h at 42 ◦C. The hybridization buffer consisted of 50% 
formamide (Merck, Darmstadt, Germany), 2× SSC (1× SSC con-
sisting of 150 mmol/l NaCl and 15 mmol/l sodium citrate, pH
7.2). The sections were then washed twice in 30% formamide in
1× SSC (42 ◦C, 4 × 15 min), briefly rinsed in 1× SSC, rinsed
in water, dipped in 70% ethanol, dried, and then exposed to
BioMax MR films (Sigma-Aldrich, Vienna, Austria) together with
[14C]-microscales for 7–42 days. After exposure to BioMax MR
films, the sections were dipped at 42 ◦C in photosensitive emul-
sion (NTB-2; Kodak, Rochester, NY, USA) diluted 1:1 with distilled
water, air dried, and exposed for 14–40 days. Dipped sections and

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BioMax films were developed using Kodak D19 developer (Sigma-Aldrich, Vienna, Austria). After counterstaining with cresyl violet, photoemulsion-dipped sections were dehydrated, cleared in butyl acetate, and coverslipped with Eukitt.

**DENSIOMETRICAL ANALYSIS OF mRNA EXPRESSION**

Autoradiographic films were digitized and opened in NIH ImageJ (version 1.46; U.S. National Institutes of Health, Bethesda, MD, USA; http://imagej.nih.gov/ij/). The following regions were investigated for epilepsy-induced changes in mRNA expression of individual GABA<sub>A</sub> receptor subunits: the granule cell layer of DG, pyramidal cell layers of hippocampal sectors CA3, CA1, and the proximal and distal parts of the subiculum, layers II and V/VI of the medial and lateral EC and layers II/III of the PRC. As the values obtained in the medial and lateral EC were not significantly different from each other, they were pooled. Briefly, a line selection (20 pixels width) was drawn perpendicular to the layer of interest and a density profile plot (gray values) was created using the function “analyze – plot profile.” Values for relative optical densities (RODs) were calculated from gray values according to the following formula: ROD = log[256/(255−gray value)]. ROD values obtained from left and right hemispheres were averaged and film background ROD was subtracted.

**HYBRIDIZATION DATA IN SITU**

Apart from neuronal losses in the hilus of the DG and degeneration of CA3- and CA1-pyramidal neurons the rats displayed widespread losses of principal neurons and GABAergic interneurons in the subiculum and in subareas of the parahippocampal region (Figure 1). As shown previously, cell losses occurred already 1 day after KA-induced status epilepticus and were most intense in layer III of the medial EC (about −30%; Figure 1C, arrowhead; and in the proximal subiculum (about −40%; Figure 1C, arrowshead; Drexel et al., 2012).

**DISTRIBUTION OF GABA<sub>A</sub> RECEPTOR SUBUNIT mRNAs IN CONTROLS**

For the hippocampus proper and the DG the subunit distribution was rather similar as that described for the dorsal and ventral hippocampus of the rat (Widgren et al., 1992; Tsunashima et al., 1997) and for the ventral hippocampus of the mouse (Hornung et al., 2013). To our knowledge no comprehensive study on the expression of all GABA<sub>A</sub> receptor subunits in horizontal sections of parahippocampal areas of the rat is yet available. As shown in...
As shown in Figure 3, mRNAs for the β-subunits were abundantly expressed in all principal cell layers of the ventral hippocampal formation (DG, hippocampus proper, and subiculum) and parahippocampal region (presubiculum, parasubiculum, EC, and PRC) of saline-injected rats.

Transcripts for a2 and a3 were especially abundant. Strongest expression of a2 mRNA was present in the granule cell layer of the DG, in the pyramidal cell layer of the hippocampus proper, and in the subiculum as well as in layer II of the EC and throughout the PRC (Figure 2). Expression of subunit γ2 was strongest in sectors CA1–CA3 and in the EC and somewhat less prominent in the stratum granulosum. In the PRC, it was predominantly expressed in the deepest layers. It was weaker in the proximal subiculum and in the presubiculum.

Subunit a1 was almost equally distributed throughout the granule cell layer and the stratum pyramidale CA1–CA3. It was even more prominent in the subiculum, pre-, and parasubiculum and in the EC notably in layers II/III and in deep layers. Its presence in the hilar of the DG indicates expression in hilar interneurons. Subunit a3 expression appeared to be weaker (Figure 2).

It was strongest in the deep layers of the EC and PRC and more prominent in sector CA3 than in sector CA1 and in the stratum granulosum. Interestingly, clear labeling of the hilus of the DG was observed presumably reflecting the location of the a3-subunit on hilar interneurons. Subunit a4 mRNA was concentrated in the granule cell layer of the DG while the remaining subregions revealed only modest expression levels.

Distribution of subunit β1-β3 mRNAs

As shown in Figure 3, mRNAs for all three β-subunits were distributed throughout principal cell layers of all hippocampal and parahippocampal areas including the PRC. For β1 and β3 it was somewhat more prominent in the granule and pyramidal cell layers than in parahippocampal areas. Subunit β2 mRNA appeared to be slightly more concentrated in the EC and in the DG than in hippocampal pyramidal cells and showed a somewhat weaker expression in the subiculum and PRC (Figure 3). All three subunits were also expressed in interneurons of the dentate hilus (Figure 3).

Changes in the Expression of GABAA Receptor Subunits After KA-Induced Status Epilepticus

Film autoradiographs after in situ hybridization are shown in Figures 2 and 3. ROD values obtained by densitometrical analysis of transcript levels are depicted in Figures 4-7. In addition to the brain areas depicted we also examined separately layers III and V/VI of the medial and lateral EC. There was no significant difference in the expression level of GABAA receptor subunits between the medial and lateral parts of the EC. We therefore pooled the data obtained in the medial and lateral EC and depict them as “EC layer II” and “EC layers V/VI.”
FIGURE 2 | Expression of α-subunit mRNAs after KA-induced seizures

 Autoradiographs of horizontal sections of the hippocampus and para-hippocampal areas after in situ hybridization for GABAA receptor subunits α1–α5 in untreated controls (Co) and at different intervals after KA-induced status epilepticus are depicted. Subunit α1–α5 mRNAs were expressed in all principal cell layers of the hippocampal/parahippocampal formation of controls. Note the sustained down-regulation of α5 mRNA throughout the hippocampal formation. On the other hand, subunits α1 (CA3, subiculum, EC, PRC) and α2 (in most regions) were only transiently reduced 1 day after KA-induced seizures. Subunit α3 mRNA is only moderately altered, whereas subunit α4 mRNA is upregulated in the dentate gyrus at all intervals.

Changes in α1 and α5 mRNAs after KA-induced seizures

Expression of α1 mRNA was significantly decreased in sector CA3, proximal and distal subiculum, and EC (deep layers) 1 day after status epilepticus (Figures 2, 4, and 5). At later intervals, however, α1 mRNA concentrations increased again in these regions and were similar to or exceeded expression levels in controls. Significantly increased levels of α1 mRNA were present 30 and 90 days after KA injection in the DG, sectors CA3 and CA1, and in the EC (layer II) and PRC (Figures 4 and 5).

Messenger RNA encoding for the α2-subunit was significantly reduced in the DG, hippocampus proper, subiculum, and PRC 1 day after KA injection. While α2-subunit mRNA concentrations in the DG, hippocampus proper, and PRC increased at later intervals (30 and 90 days) to levels observed in controls, α2 mRNA levels in the subiculum were still reduced by about 45–60% after 90 days (Figures 4 and 5). As shown in Figure 5, expression of α3 mRNA did not change in the granule cell layer of the DG over the course of the experiment. The other areas investigated revealed transiently decreased expression of α3 mRNA 1 and 8 days after KA injection. These decreases were compensated by approaching control levels in most parts of the hippocampal formation, however were markedly increased (220% of controls) in the sector CA1 after 90 days (Figure 4).

Subunit α4 mRNA concentration was reduced by 35–45% in the hippocampus proper 1 and 8 days after KA injection (Figure 4) but reached approximately control levels at later time intervals (30 and 90 days). Also in the proximal subiculum, in the EC (layer II), and in the PRC, α4 mRNA levels were decreased by about 30, 25, and 35%, respectively, after 24 h. At later intervals (30 and 90 days) α4 mRNA levels, however, increased in the DG (about 178% of controls) and in the proximal subiculum (about 130–170% of controls).

Subunit α5 mRNA showed the most drastic and widespread changes in its expression. Considerably decreased concentrations of α5 mRNA were already evident 1 day after KA injection in all investigated subregions and ranged from about −60% in the proximal subiculum to about −75% in the DG and hippocampal sector CA1 (Figures 4 and 5). After a transient increase in expression after 8 or 30 days, α5 mRNA was again decreased after 90 days (from −50 to −80%) in all investigated regions.

Changes in β1–β3 mRNAs after KA-induced seizures

Figures 3, 6, and 7 show changes in the expression of β-subunit mRNAs. In the hippocampus proper, mRNA expression for the β1-subunit was significantly reduced from 1 to 30 days after KA injection, but almost reached control levels after 90 days.
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FIGURE 3 | Expression of subunit β1, β2, β3, γ2, and δ mRNAs after KA-induced seizures. Autoradiographs of horizontal sections of the hippocampus and parahippocampal areas after in situ hybridization for GABAA receptor subunits β1, β2, β3, γ2, and δ in saline-injected controls (Co) and at different intervals after KA-induced status epilepticus are shown. Messenger RNAs encoding for β-subunits and for δ appear to be widely reduced, those for γ2 appear to increase after an initial (1 day) reduction.

Changes in β1 and δ mRNAs after KA-induced seizures

Expression of β1 mRNA was transiently decreased by 40–60% in sectors CA3 and CA1 of the hippocampus, in the proximal subiculum, and in deep layers of the EC layer V/VI 24 h after KA injection (Figures 6 and 7). At later time intervals, we observed significantly increased expression of β1 mRNA in the proximal subiculum (up to 180% of controls), in layer II of the EC (up to 165%), and in the PRC (up to 169%). In the remaining regions, there was a (statistically not significant) trend for increased γ2 mRNA levels at the 30 and 90 days intervals. Expression of mRNA encoding the δ-subunit was lastingly decreased in all regions except sector CA3 and at all time points investigated (Figures 6 and 7).

DISCUSSION

We now report changes in the mRNA expression of 10 GABAA receptor subunits in the hippocampal formation and in parahippocampal regions between one and 90 days after KA-induced status epilepticus. The main findings are (1) transient decreases in mRNA levels of all α-subunits, in subunits β2 and β3 and of subunit γ2 mRNA in the proximal subiculum and in the EC layer V/VI 24 h after KA injection, (2) lastingly decreased expression of subunits α5 and δ (with an onset at day 1 after KA injection) virtually in all hippocampal and parahippocampal areas (for subunit δ most prominently seen in the DG and the PRC, and for subunit α5 in sectors CA1 to CA3, the subiculum and the ento- and perirhinal cortices where these subunits are most prominently expressed in controls), (3) increased expression of α4-subunit mRNA in the DG and in the proximal subiculum (30 and 90 days after KA),
FIGURE 4 | Semi-quantitative assessment of mRNA levels for GABA\textsubscript{A} receptor subunits \(\alpha_{1}-\alpha_{5}\) in the hippocampus proper and subiculum after KA-induced seizures. The autoradiographic films were digitized and analyzed using the open source NIH ImageJ software. Note the lasting down-regulation of subunit \(\alpha_{5}\) mRNA levels in the hippocampus proper and subiculum, whereas mRNAs of the other \(\alpha\) subunits are only transiently down-regulated. \(\alpha_{2}\) mRNA levels are still reduced in the subiculum after 90 days. Data are expressed as mean relative optical densities (RODs) ± SEM. Numbers of animals are given in the upper left graph. Statistical analysis was done by ANOVA and Dunnett’s multiple comparison post hoc test (\(*p < 0.05; **p < 0.01; ***p < 0.001\)). In cases where, due to the low number of animals, no significant difference was found for the 30 and 90 days intervals, data for this time points were pooled and re-analyzed: \(a_{1}, p < 0.05\); \(a_{2}, p < 0.01\).
FIGURE 5 | Semi-quantitative assessment of mRNA levels for GABAA receptor subunits α1–α5 in the dentate gyrus (DG), entorhinal cortex (EC), and perirhinal cortex (PRC) after KA-induced seizures. Density profile plots were performed in layers of the hippocampal/parahippocampal region on autoradiograms after radioactive in situ hybridization and relative optical densities (RODs) were calculated. Note the lasting down-regulation of subunit α5 mRNA in the DG, EC, and PRC and concomitant up-regulation of subunit α1 mRNA (DG, EC layer II, PRC) and α4 mRNA (only DG) at late intervals after status epilepticus. Data are given as mean ROD values ± SEM. Animal numbers are given in the upper left graph. Statistical analysis was done by ANOVA and Dunnett’s multiple comparison post hoc test (*p < 0.05; **p < 0.01; ***p < 0.001; 30 and 90 days pooled: δp < 0.01).
FIGURE 6 | Semi-quantitative assessment of mRNA levels for GABAA receptor subunits β1–β3, γ2, and δ in the hippocampus proper and subiculum at different time intervals after KA-induced seizures. Note reduced β1–β3 mRNA levels in the hippocampus proper and subiculum 24 h after KA increasing again in the hippocampus proper at later intervals. In the subiculum (β1–β3) mRNA levels remain reduced 90 days after KA. Subunit γ2 mRNA levels are transiently decreased in the hippocampus proper and proximal subiculum 1 day after status epilepticus, however, increasing at later intervals. Levels of the δ-subunit are permanently reduced in sector CA1 and in the distal subiculum. Numbers of rats per group are given in the upper left graph. Data are expressed as mean ROD ± SEM; statistical analysis was done by ANOVA and Dunnett’s multiple comparison post hoc test (*p < 0.05; **p < 0.01; ***p < 0.001). In cases where, due to the low number of animals, no significant difference was found for the 30 and 90 days intervals, data for this time points were pooled and re-analyzed: a, p < 0.05; b, p < 0.01.
FIGURE 7 | Semi-quantitative assessment of mRNA levels for GABA<sub>A</sub> receptor subunits β<sub>1</sub>–β<sub>3</sub>, γ<sub>2</sub>, and δ in the dentate gyrus (DG), entorhinal cortex (EC), and perirhinal cortex (PRC) after KA-induced seizures. Note the reduced β<sub>3</sub> subunit mRNA levels in the superficial (β<sub>3</sub>) and deep entorhinal cortex (EC; β<sub>1</sub>–β<sub>3</sub>) and in the PRC (β<sub>1</sub>–β<sub>3</sub>) at late intervals after KA. While subunit δ mRNA levels were decreased in the DG, entorhinal, and PRC at all intervals after KA, γ<sub>2</sub> mRNA levels were increased in the DG, superficial EC, and PRC at late intervals. Numbers of rats per group are given in the upper left graph. Data are shown as mean ROD values ± SEM, statistical analysis was done by ANOVA and Dunnett’s multiple comparison post-hoc test (*p < 0.05; **p < 0.01; ***p < 0.001). In cases where, due to the low number of animals, no significant difference was found for the 30 and 90 days intervals, data for this time points were pooled and re-analyzed: a, p < 0.05; b, p < 0.01.
increased expression of γ2-subunit mRNA in the DG, sector CA1, layer II of the EC, and PRC at late intervals after KA injection (30–90 days after KA). (3) In contrast, we observed lasting decreased levels of α2- and all β-subunit mRNAs in the subiculum and of β2- and β3-subunit mRNAs in the perihinal and deep entorhinal cortices, (4) and increased expression of subunit α1 mRNA in the DG, hippocampus proper, superficial EC, and PRC 30 and 90 days after KA.

Our data reflect semi-quantitatively assessed mRNA levels. They likely reflect respective changes in the mRNA expression, which are mostly translated into protein (Schwarzer et al., 1997; Tsunashima et al., 1997; Nishimura et al., 2005). It has also always to be considered that neurodegeneration may obscure the results of mRNA expression. Neurodegeneration was most severe in the CA1 and CA3 sectors of the hippocampus, in parts of the subiculum and in layers II/III of the EC. And, neurodegeneration was almost maximal already after 24 h (Drexel et al., 2012), the earliest time interval reported here. In brain areas undergoing significant neurodegeneration, decreased mRNA levels may be due to this pathological change and increased mRNA concentrations could be apparently reduced by the underlying cell losses. Therefore it is always advisable to view the time course of changes and to compare changes in different subunits in the same brain area and of one subunit in different brain areas.

Decreases in subunit α1- and γ2-immunoreactivities due to rapid internalization were reported during the status epilepticus (induced by KA, pilocarpine or electroconvulsively. Brooks-Kayal et al., 1998; Nafyori and Wasterlain, 2005; Nishimura et al., 2005). Since the α-subunits are crucial for the binding of benzodiazepines, it was suggested that this event may be causatively related to the partial resistance to benzodiazepine treatment during status epilepticus (Brooks-Kayal et al., 1998; Nafyori and Wasterlain, 2005). Also our present study demonstrates an initial decrease in mRNA expression of these subunits in several hippocampal areas. This indicates that the reported internalization of α1- and γ2-subunits is accompanied by decreased expression of these subunits. These initial decreases in mRNA level, however, were followed by rapid overexpression of subunit γ2 mRNA and protein in all subfields of the hippocampus and may compensate for the initial losses (Schwarzer et al., 1997; Nishimura et al., 2005). Interestingly, our present study also revealed that mRNA levels of almost all other subunits transiently decreased in their expression. Thus, the transient down-regulation of the GABA A receptor subunits may be more general and may affect a great number of differently assembled receptors.

**Changes in Subunits Mediating Tonic Inhibition**

Inhibition via GABA A receptors comprises phasic inhibition by activating GABA A receptors at the synapse and tonic inhibition by stimulating high affinity GABA A receptors located at peri- and extrasynaptic sites (Mohler et al., 1996; Semyanov et al., 2004; Farquhar and Nusser, 2005). Tonic inhibition is responsible for about 75% of the total inhibitory charge received by hippocampal principal neurons (Mody and Pearce, 2004). Receptors containing the γ2-subunit are mainly located within the synaptic cleft and thus primarily are involved in generation of phasic inhibition. Key components of GABA A receptors implicated in tonic inhibition in the DG and hippocampus proper are subunits a5, α4, and β2 (Nusser et al., 1998; Carascosti et al., 2004). Additionally, subunit α4 is considered to be the main partner of the β3-subunit in the thalamus and forebrain (Sur et al., 1999). Epilepsy-induced decreased expression of GABA A receptor subunits α5 and α3 notably in the DG and CA1, layer II of the EC, and PRC at late intervals after KA injection (30–90 days after KA), (5) in contrast, we observed lastingly decreased expression (30–90 days after KA), (5) in contrast, we observed lastingly decreased expression of almost all other subunits transiently decreased in their expression. Thus, the transient down-regulation of the GABA A receptor subunits may be more general and may affect a great number of differently assembled receptors.

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