Distribution of GABA$_{\alpha}$ Receptor mRNA in the Motor Cortex of ALS Patients

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Abstract. The pathomechanism of amyotrophic lateral sclerosis (ALS) remains unclear. There is some evidence that excitotoxic cell death is involved in the degeneration of the motor nervous system, and that ligand-gated receptor channels play a role in the pathogenesis of the disease. Several electrophysiological and anatomical studies support the pathophysiological concept of an impaired inhibitory, namely GABAergic, control of the motoroneurons in the cerebral cortex of ALS patients. The aim of our study was to investigate the expression of GABA$_{\alpha}$-receptor subunit mRNAs and the GABA synthesizing enzyme glutamic acid decarboxylase (GAD) in the motor cortex of ALS patients compared to tissue of control persons. We performed in situ hybridization histochemistry (ISH) on human postmortem motor cortex sections of ALS patients (n = 5) and age matched controls with no history of neurological disease (n = 5). The most intriguing finding was a significantly reduced mRNA expression of the $\alpha$1-subunit in ALS patients while the level of $\beta$1-subunit mRNA was elevated in the patients group. This may indicate specific alterations of the GABA$_{\alpha}$ receptor subunit composition and result in distinct physiological and pharmacological properties of these receptors in ALS patients.

Key Words: Amyotrophic lateral sclerosis; Excitotoxicity; GABA$_{\alpha}$ receptor subunits; Inhibitory neurotransmission; In situ hybridization histochemistry.

INTRODUCTION

The clinical phenomenology of amyotrophic lateral sclerosis (ALS) that was first described by Charcot in 1874 is the consequence of a selective degeneration of motoneurons in the cerebral cortex, brainstem, and the anterior horn cells of the spinal cord (1), and, as shown recently, of other neuronal systems such as the frontal lobe (2–4). The etiology of ALS remains unclear to date. Around 10% of cases are genetically determined. A defined genetic defect (mutations in the superoxide dismutase (SOD) I gene) is detected in about 20 percent of familial cases (5–8); however, most ALS cases occur sporadically. Many different pathomechanisms, such as SOD-induced toxicity, mitochondrial dysfunction, dysregulation of neurotrophic factors, autoimmunity, and oxidative stress are discussed in sporadic ALS (7, 9–12). There is a lot of evidence that all these factors lead to a chronic imbalance of the intracellular calcium homeostasis and that the resulting chronic calcium overload causes damage to motor neurons. Excessive entry of calcium into the cell is mediated by ionotropic glutamate receptors, and especially, AMPA-type glutamate receptors seem to play a major role in acute and chronic excitotoxic cell death (13, 14). There are several in situ hybridization histochemistry (ISH) and RT-PCR studies evaluating the distribution of the excitatory AMPA-type glutamate receptors in normal human brain and spinal cord (15–18) as well as in spinal cord tissue of ALS patients (19, 20). These data provide some evidence that motor neurons in the spinal cord in non-ALS affected, but even more so in ALS postmortem spinal cord tissue, are more vulnerable to glutamate mediated excitotoxicity due to distinct AMPA receptor subunit compositions. However, a detailed study of the expression of inhibitory, namely GABA$_{\alpha}$ receptors, in the motor cortex of ALS patients has not yet been done. A PET study showed that binding of the benzodiazepine antagonist flumazenil was reduced in the motor cortex and several extramotor brain regions of ALS patients (4). A significant loss of cortical inhibition or corticomotor hyperexcitability in ALS patients was demonstrated by transcranial magnetic stimulation (TMS) studies (21–25). Whether loss of inhibitory interneurons is the reason for disturbed cortical inhibition is controversial (26, 27). Reduced intracortical inhibition might also be due to a different expression pattern of postsynaptic inhibitory receptor channels in the motor cortex of ALS patients. GABA$_{\alpha}$ is the most widely distributed inhibitory neurotransmitter in the mammalian central nervous system (CNS) (28). GABA$_{\alpha}$ receptors are responsible for the fast inhibitory synaptic transmission in the CNS. The pentameric receptor is formed by members of at least 3 distinct subunit families detected by molecular cloning and grouped according to their degrees of identity: $\alpha_{1-6}$, $\beta_{1-3}$, $\gamma_{1-3}$, $\delta$, $\epsilon$, $\rho_{1-3}$ (29, 30). The most abundant subunit stoichiometry of GABA$_{\alpha}$ receptors seems to be 2 $\alpha$-subunits, 2 $\beta$-subunits, and 1 $\gamma$-subunit (31, 32). The $\gamma$-subunits can alternatively be replaced by $\delta$- or $\epsilon$-subunits (33, 34). The large number of different subunits and splice variants gives rise to a broad variety of functionally different GABA$_{\alpha}$ receptor
subtypes (35, 36) that depend on the subunit composition of the GABA<sub>A</sub> receptor (37–41).

The primary goal of the present ISH study was to investigate the distribution of the mRNA of the most abundant GABA<sub>A</sub> receptor subunits (α1–6, β1–3, γ1–3, δ, and ε) and of the GABA synthesizing enzyme glutamic acid decarboxylase (GAD) in the primary motor cortex (area 4 according to the nomenclature of Brodmann) of ALS patients. Additionally, we wanted to determine if there are characteristic changes in the expression patterns in ALS patients compared to normal controls.

**MATERIALS AND METHODS**

Postmortem brain specimens of 5 patients aged between 61 and 82 years (mean age 67 years) with a definite diagnosis of ALS according to the El Escorial criteria (42) and of 5 control patients aged between 65 and 84 years (mean age 65 years) with no evidence of neurological or psychiatric disease were available from autopsies. The postmortem delay ranged from 8 to 32 hours. Brains were cut into coronal slices of 1-cm thickness and rapidly frozen on dry ice. Prior to the in situ hybridization experiments, cryostat sections of the assayed and shock-frozen tissue blocks from ALS patients and controls were Nissl-stained, and their cytoarchitecture was studied histologically. Dipped sections were counterstained with 0.05% thionin, dehydrated, and coverslipped. For negative control, a 100-fold excess of nonlabeled oligonucleotides was added to the radioactive probe and applied to the adjacent section, leading to a complete suppression of the signal (not shown).

Semi-quantitative analysis of film autoradiograms was performed according to the method of Akbarian et al (44). Optical density readings were taken in vertical strips 0.5 mm wide across the full thickness of the cortex of area 4, and optical densities were determined for the thickness of each cortical layer separately, by matching the autoradiogram readings to digitized images of the adjacent Nissl-stained section. Optical density over white matter more than 3 mm deep to overlaying structures was counted for the background substraction. Absolute values of radioactivity were determined from 14C plastic standards (Amersham, Freiburg, Germany) on the same sheet of film (NIH image software). Quantified hybridization signals for the patient and the control group were compared using the Student t-test.

Cellular expression patterns of the mRNA transcripts were determined microscopically throughout the cortical layers by classifying the amount of silver grains over individual cells at the Nissl counterstained sections as strong, moderate, or weak according to a visual analogue scale (Table 2). To further investigate the differences in the optical density readings concerning the α1-subunit, we classified the labeling intensity of 100 pyramidal cells and 100 small and medium sized neurons, respectively, in five randomly chosen areas of layers III and V in each section. The percentage of cells that were labeled strongly, moderately, or weakly was counted for the α1-subunit (that had different amounts of mRNA in the semiquantitative

<table>
<thead>
<tr>
<th>Gene Sequences of the Oligonucleotide Probes Designed Complementary to the GABA&lt;sub&gt;A&lt;/sub&gt; Receptor Subunit mRNAs</th>
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<tbody>
<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;α1  5'-ggg tcg ccc ctc ggc aaa tta ggg tgt tag ctc tgt gtt ggt-3'</td>
</tr>
<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;α2  5'-ggc aac agc cac tgc ata agc gtt gtt ctc tat cat aac gga agc-3'</td>
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<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;α3  5'-ata ggt gtt gcc cac gat gtt gaa ggt agt gct gtt ttt ctt gc-3'</td>
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<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;α4  5'-acc ctc gac caa act gtc cag gat ggc gtt gaa att ttc ttc gtc ca-3'</td>
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<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;α5  5'-ttg atc ttc gct gct tcc aag gct tgt ttt tgg cca tcc cag gcc cag-3'</td>
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<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;α6  5'-ggc ctc gga aga tga aac tat ggc gaa aga agt gat gac-3'</td>
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<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;β1  5'-ctt ggc gta ctc gat gct ggc gct tgt ctc atga gta cat gat ggc-3'</td>
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<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;β2  5'-tcg tcc aga gca ttt cgg cca aaa tca tgc gtc ggc aac cca cc-3'</td>
</tr>
<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;β3  5'-ctc gtc ccc cag gaa tcc ctc att ccc ttc tgc agg cat gct ctg-3'</td>
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<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;γ1  5'-ctg cag tct tca aag cca cag aag aag agt ctc gca caa tct tgt cc-3'</td>
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<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;δ2  5'-cat tgt ttc gat gtc tca tct tgg ggc gat atc aat ggt agg ggc-3'</td>
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<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;γ3  5'-gga agg ggc tgt gat gct tat ctc ctc ggc tca gat cca tct cc-3'</td>
</tr>
<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;δ  5'-gtg gtc ggc tgt gaa atg agc aaa ggc gta ctc cac cag gcc ggc-3'</td>
</tr>
<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;ε  5'-acg ggc atg ggc acg gct att gat acg agg atg gca ggc gct ttt agg-3'</td>
</tr>
<tr>
<td>GAD  5'-ctc tgt ttt taa tct tgg cat aga gtt att cag cca gtt cc-3'</td>
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The specificity of the probes was counterchecked using the GeneBank BLAST software.
analysis of film autoradiograms of patients and controls), as well as for the δ-subunit, which had an equal mRNA expression in both groups.

For immunohistochemistry, additional fresh frozen sections were identically prepared, fixed in ice-cold acetone for 15 min, and rinsed in 1× phosphate buffered saline (PBS). Endogenous peroxidase was blocked with 3% hydrogen peroxide and 10% methanol in 1× PBS. After preincubation with 1% bovine serum albumin (BSA) in 1× PBS/0.3% Triton X-100 for 30 min at room temperature, sections were incubated with a monoclonal mouse antibody directed against the α1-subunit of the GABA_A receptor (bd24) (Chemicon, Hofheim, Germany) overnight at 4°C, followed the next day by an incubation with biotinylated rabbit-anti-mouse IgG (1:100 in PBS) for 30 min each at room temperature. The final enzymatic reaction was performed for 5 min using 0.01% H_2O_2 as substrate and 0.1% diaminobenzidine as chromogen in 0.05 M PBS.

**RESULTS**

We investigated the distribution of GABA_A receptor subunits in the motor cortex of ALS patients and controls by in situ hybridization techniques using film autoradiograms and for cellular localization of the hybridization signal by emulsion-dipped tissue sections. For semiquantitative analysis of film autoradiograms, optical density readings were converted to measures of radioactivity (nCi/g). Figure 1 shows the histological differentiation of the cortical layers of the primary motor cortex (Brodmann area 4) in a film autoradiogram obtained with a radiolabeled probe of the δ-subunit (Fig. 1a), with the adjacent section shown with Nissl staining on the right side (Fig. 1b). By overlay of the digitized Nissl sections and the adjacent film autoradiograms, the cortical layers could be identified optically by their content of different cell types. For the semiquantitative analysis, the mean values of radioactivity were attributed to the cortical layers that were identified microscopically. The identification of layer IV was difficult as the primary motor cortex is “agranular,” which means that layer IV is small and often hardly discernible from the adjacent layers (45, 46). The values of radioactivity obtained for this layer by analysis of the film autoradiograms may in part represent labeling of pyramidal cells in layers III and V. The mRNA expression of 14 GABA_A receptor subunits and of the GABA synthesizing enzyme GAD was analyzed in the primary motor cortex (Brodman area 4) in postmortem tissue from ALS and control patients. Instructive examples of the most important results are shown in Figure 2. The hybridization signal of subtype-specific oligonucleotide probes shows the expression of the GABA_A receptor subunits at ALS patients and controls. The results of the semiquantitative analysis of the film autoradiograms are shown in Figure 3. We found a distinct expression pattern of GABA_A subunit RNA in the cortical layers of the primary motor cortex of ALS patients and controls. The α6-, γ1- and ε-subunits displayed no signal above the background level throughout all cortical layers (Fig. 2). The α3-, α5-, and γ3-subunits were weakly expressed at all cortical layers (<100 nCi/g) (Figs. 2, 3). The other subunits investigated (α1-, α2-, α4-, β1-, β3-, γ2- and δ-subunits) showed a weak signal (<100 nCi/g) in the cortical layer I of patients and controls. The signal intensity was also <100 nCi/g for the α1-subunit in layer II, and between 100 and 200 nCi/g for the α2-, α4-, β2-, β3-, δ- and γ2-probes at both groups (Fig. 3). A slight but significant difference was found for the β1-subunit, where tissue from ALS patients was more intensively labeled (>100 nCi/g) compared to controls (<75 nCi/g) (p < 0.02). In cortical layers III to VI, the signal intensity was between 50 and 200 nCi/g for α2-, α4-, β3-, and γ2-subunits and >300 nCi/g for the δ- and β2-probes, but we found no difference between ALS patients and controls (Fig. 3). The mRNA of the β1-subunit was again more intensively expressed in ALS-Patients (100 to 150 nCi/g) compared to control tissue (<100 nCi/g) in the cortical layers III to VI (p < 0.002) (Fig. 3). However, the most important finding of the study was the significant reduction of the mRNA expression of the GABA_A receptor α1-subunit throughout cortical layers II to VI (p < 0.05), IV, V, and VI (p < 0.02) in brain tissue from ALS (<200 nCi/g) compared to control patients (200–350 nCi/g) (Fig. 3). The GAD mRNA expression level was <50 nCi/g in layer I and between 100 and 200 nCi/g in the cortical layers II to VI, with a maximum intensity in layer III. The slightly higher intensity in ALS patients compared to controls did not reach statistical significance (Fig. 3).

To get information about the mRNA expression of GABA_A receptors at the cellular level, emulsion-dipped slides were Nissl-counterstained and analyzed (Fig. 4; Table 2). This allowed more detailed study of the cortical architecture, the attribution of distinct expression patterns to the cortical layers, and a differentiation between pyramidal cells and other cellular elements of the pyramidal, ganglionar, and multiform layers. Labeling of adjacent sections with the GAD-specific probe identified the GABAergic interneurons. We found strong correlation with the data from film autoradiograms (Figs. 4, 5). As shown in Table 2 and in accordance with Figures 2 and 3, only weak GABA_A receptor expression was detected in molecular layer I. Most cells in layer II were labeled moderately by the α1-, α2-, β2-, γ2-, and δ-specific probes. In the external pyramidal layer III, a strong cellular hybridization signal of the mRNA of the α2-, β2-, and δ-subunits was seen in controls, with most intense labeling of large pyramidal neurons. In the patient group, however, the majority of pyramidal cells was only moderately labeled by the α1-subunit specific probe (Fig. 5a), while small and medium sized cells showed no distinct

labeled cells for the β in adjacent sections were strongly positive for the some presumed interneurons identified via GAD labeling granular layer IV were labeled moderately to weakly, mainly over the pyramidal cells. The smaller cells of the a subunits and a weak signal for the a1-subunits and a weak signal for the a2-, a5-, and γ3-subunits in tissue from ALS patients and controls. Particularly the a1-, β2-, γ3-, and δ-subunits were detected mainly over the pyramidal cells. The smaller cells of the granular layer IV were labeled moderately to weakly, some presumed interneurons identified via GAD labeling in adjacent sections were strongly positive for the a1-, β2-, and δ-subunits. This difference from the semiquantitative analysis of the autoradiograms in layer IV (where very similar densitometric values as in layers III and V were measured) results probably from the fact that layer IV in our sections was not sufficiently discernible in the autoradiograms, as it is very small in the primary motor cortex. The inner pyramidal layer V is characterized by its content of large pyramidal neurons and a lower cell density. The expression pattern of these cells was almost identical with those of layer III in ALS patients as well as in the control sections. Again, distinct proportions of strongly and moderately labeled pyramidal cells for the a1-subunit (Fig. 5a, b) and of moderately and weakly labeled cells for the β1-subunit were observed in layer V in the patient and control groups corresponding to the different mRNA levels seen in the semiquantitative analysis of the film autoradiograms (Figs. 2, 3). Cells in layer VI showed similar signal intensities for the studied mRNA transcripts to morphologically similar cells in the other layers of the motor cortex in patients and in controls. The mRNA of the GABA synthesizing enzyme GAD was strongly expressed by approximately 30% of the interneurons in both groups and several large pyramidal neurons in layer III and V were labeled weakly (Fig. 4; Table 2).

Immunohistochemical staining with an antibody specific for the a1-subunit showed a predominant localization of the receptor protein at the neuronal membranes and dendrites of pyramidal cells as well as smaller neurons in patient and control tissues (Fig. 6). The presence of positive neurons was highest in cortical layers III and IV, followed by layers V, II, and I in both patients and controls (not shown).

**DISCUSSION**

In the present study we investigated mRNA expression of the most abundant 14 GABA<sub>a</sub> receptor subunits in the primary motor cortex of ALS patients and normal controls. To our knowledge, this is the first study dealing with the distribution of GABA<sub>a</sub> receptor mRNA in the human motor cortex. So far, only the human prefrontal cortex (area 46 according to Brodmann) has been investigated by ISH for the expression of the GABA<sub>a</sub> receptor subunits a1, a2, a5, β1, β2, and γ2 in sections of schizophrenics compared to control tissue (44). Concerning the values of mRNA levels expressed in nCi/g, as well as the relative abundance of the different receptor subunit mRNAs, the results of our study are quite similar (44).

In the rodent brain there are some differences in the expression pattern of GABA<sub>a</sub> receptors compared to our findings in human brain tissue. In the rat neocortex, the expression of the a5-subunit was limited to a weak signal in layer V and IV and of the δ-subunit to a moderate signal in layer I and II (47, 48). In contrast, we found a weak to moderate mRNA expression of the a5- and, as the most obvious difference between human and rodent brain, a strong expression of the δ-subunit in the layers II, III, IV, V and VI in brain tissue sections of the precentral cortex of human Brodmann area 4 in patients as well as in controls (Figs. 2, 3). The mRNA of the GABA synthesizing enzyme GAD was detected in 20% to 30% of the cortical neurons in the patient as well as in the control group, with a predominance in interneurons in layers II to V (Fig. 4). This is consistent with previous immunohistochemical data of human and monkey cortices (49, 50).
The most abundant GABA<sub>α</sub> receptor of the vertebrate brain contains 2 α<sup>1</sup>, 2 β<sup>2</sup>, and 1 γ<sup>3</sup>, or δ-subunit (31, 32). The high mRNA levels of the α<sup>1</sup>-, β<sup>2</sup>-, and γ<sup>3</sup>-subunits in the human motor cortex of ALS patients and controls (Figs. 2–4) indicate a preferred expression of BDZ-sensitive GABA<sub>α</sub> receptors (29, 35, 36). The moderate mRNA expression of the α<sup>2</sup>-<sup>1</sup>, α<sup>3</sup>-, and α<sup>4</sup>-subunits in the human motor cortex of ALS patients and controls (Figs. 2, 3) also points to the presence of receptor subtypes with lower (α<sup>2</sup>, α<sup>3</sup>) or without (α<sup>4</sup>) BDZ binding affinity (30, 51, 52). Our finding of very high mRNA levels of the δ-subunit in layers II to VI (Figs. 2, 3) (in contrast to its weaker and more restricted expression described in rodents [47, 48]) indicate that the δ-subunit may be coexpressed with different α- and β-subunits even more frequently to functional GABA<sub>α</sub> receptors than the γ<sub>2</sub>-subunit in the human motor cortex. A coassembly of the δ-subunit instead of the γ<sub>2</sub>-subunit with different α- and β-subunits was shown to influence the receptor kinetics and pharmacological properties (39, 53).

It was presumed that the reduced cortical inhibition and the reduced flumazenil binding in ALS patients might be due to a loss of GABAergic interneurons (4, 23). This hypothesis was not confirmed by our data because the level of GAD mRNA as a marker of GABAergic interneurons was similar in tissue from control and ALS (Figs. 2, 3). Other studies of ALS tissue (26, 54), as well as in the SODI animal model (55, 56), showed interneuron loss during the time course of the disease. However, an immunohistochemical study investigating the calcium binding proteins parvalbumin and calbindin (which are associated with GABAergic interneurons) in the motor system of patients with motor neuron disease (27) also showed no alteration of these proteins in ALS, and is therefore consistent with our finding of unchanged GAD expression at the transcriptional level.

Comparing the results of normal controls with ALS patients, we found a significant reduction of the mRNA level of the α<sup>1</sup>-subunit (Figs. 3, 5) without a difference in the cellular distribution of the receptor protein (Fig. 6). At the cellular level this decreased α<sup>1</sup>-subunit mRNA expression was most impressive in pyramidal neurons of the patient group is functionally relevant cannot be decided so far. According to several electrophysiological studies, the functional and pharmacological meaning of the β1-subunit is not clearly defined (29, 57–59).

Our histological findings in postmortem tissue correlate with a PET study that shows a decrease in [11C]flumazenil binding in the motor and prefrontal cortex of ALS patients (4). With [11C]flumazenil PET, the presence of BDZ-sensitive GABA<sub>α</sub> receptors can be demonstrated in vivo. The BDZ sensitivity of GABA<sub>α</sub> receptors, however, depends mainly on the α<sup>1</sup>-subunit, and this subunit was markedly reduced in the motor cortices of ALS patients, as shown in our ISH study. Analysis of film autoradiograms revealed that the α<sup>1</sup>-subunit is reduced in cortical layers III to VI. At the cellular level
Fig. 2. X-ray autoradiographs of GABA\_ receptor subunit mRNA expression in the primary motor cortex (area 4 according to the nomenclature of Brodmann) of ALS patients (left columns) and controls (right columns). a: GABA\_ receptor \(\alpha 1\) - to \(\alpha 6\)-subunits. b: GABA\_ receptor \(\beta 1\) - to \(\beta 3\)- and \(\gamma 1\)- to \(\gamma 3\)-subunits. c: GABA\_ receptor \(\delta\)- and \(\epsilon\)-subunits, glutamic acid decarboxylase (GAD).

this reduced \(\alpha 1\)-subunit mRNA level is due to a decreased mRNA expression of large pyramidal neurons (Fig. 5a, b). As the semiquantitative evaluation of the distinct GABA\_ receptor subunit mRNA levels showed a reduction of the \(\alpha 1\)-subunit while the mRNA levels of other subunits were not altered (Fig. 3) compared to normal controls, the significant loss of \(\alpha 1\)-subunit mRNA expression can not be explained simply by a general cell loss. Several histopathological studies of the primary motor cortex of ALS patients did not find marked loss of large pyramidal cells in area 4 (60, 61) corresponding to our observations. Taken together, the PET data showing reduced flumazenil binding sites and our data demonstrating a reduced mRNA level of the \(\alpha 1\)-subunit of the

Fig. 3. Levels of subunit-specific mRNA expression in nCi/g (abscissae) across the 6 cortical layers (ordinates). GABA\_ receptor mRNA expression in the motor cortex of ALS patients and controls was assessed semiquantitatively by densitometry of the film autoradiograms. Abscissae indicate density readings converted to measures of radioactivity (nCi/g) by reference to [\(^{14}\)C] standards exposed on the same sheet of film. Layers have been added by matching to digitized images of adjacent Nissl-stained sections. Data are shown as mean values ± SD. *p < 0.05; **p < 0.02. White bars, controls; black bars, patients.
Fig. 4. Photomicrographs of emulsion-dipped sections exemplarily depicting the mRNA expression of the GABA_\textsubscript{A} receptor subunits in cellular resolution in pyramidal cells (arrows) and GABAergic interneurons identified by glutamate acid decarboxylase (GAD) labeling (arrowheads) in layer V of the motor cortex of patients and controls. ×400.

GABA_\textsubscript{A} receptor suggest that alterations of the subunit assembly of inhibitory neurotransmitter receptors on cortical motor neurons in area 4 might be important in the pathogenesis of motor neuron disease. Until now it has not been clear if the observed altered expression patterns of inhibitory and excitatory receptor channels are one of the factors that directly lead to an imbalance of inhibitory and excitatory stimulation of motor neurons in ALS, or if they only reflect an adaptive process in form of a rearrangement of receptors during the disease.

Studies in cell culture models showed the neuroprotective capacities of GABAergic inhibition (62, 63). Our finding of an alteration in the GABA_\textsubscript{A} receptor mRNA expression pattern in pyramidal cells in the primary
Fig. 5. Cellular analysis of the α1-subunit that had shown different mRNA levels in patients and controls in the film autoradiograms and of one of the equally expressed subunits (δ) by liquid emulsion autoradiography. In the emulsion-dipped Nissl-counterstained slides in randomly chosen areas of layer III and V of each section, the mRNA expression of 100 pyramidal cells (a, c) and small and medium sized cells (b, d), respectively, was classified as strong, moderate, or weak according to a visual analogue scale. It can be seen that in the patients group (grey bars) in layer III and V, the majority of pyramidal neurons was labeled only moderately by the δ1-subunit specific probe, while in the control group (white bars) most cells displayed intensive labeling (a). The labeling intensities of small and medium sized cells were similar in both groups (b). The δ-subunit labeling of pyramidal and nonpyramidal cells was similar in patients and controls (c, d). White bars, controls; grey bars, patients.

Fig. 6. Examples of immunohistochemical staining of large pyramidal neurons in patients (a) and controls (b) with an antibody specific for the α1-subunit. ×630.
motor cortex of ALS patients may provide a basis for the
development of a more disease specific pharmacothera-
peutic modulation.

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