GABA$_A$ Receptors in Human Temporal Lobe Epilepsy

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Summary

Temporal lobe epilepsy (TLE) associated with hippocampal sclerosis (HS) is a form of focal epilepsy, which is particularly resistant to medical treatment. It is, however, established that patients with TLE and HS respond favorably to surgery. Accumulating evidence indicates that TLE is associated with impairment in synaptic signaling via GABA (γ-aminobutyric acid), the main inhibitory neurotransmitter in the brain. GABA$_A$ receptors, which mediate the fast synaptic inhibitory action of GABA, are also targets for several medications used in treating seizures, including benzodiazepines or barbiturates. Nineteen genes have been identified coding for distinct subunits, which assemble into a functional GABA$_A$ receptor composed of a combination of five of these subunits. This heterogeneity is reflected in the multitude of structurally distinct GABA$_A$ receptor subtypes expressed in various brain regions.

A comparative analysis based on immunohistochemical techniques revealed significant changes in the distribution and expression of three major GABA$_A$ receptor subtypes in hippocampal tissue removed during surgery from TLE patients with HS versus tissue from autopsy controls. In TLE with HS, which is characterized by neuronal loss and gliosis in the hippocampus, we found first that, although GABA$_A$ receptor subunit staining was decreased in areas of extensive cell death, surviving neurons of the dentate gyrus had more GABA$_A$ receptors, in particular the α2-subtype. Second, we observed changes in numbers and morphology of interneurons expressing the α1-subtype. These results, demonstrating marked reorganization of specific GABA$_A$ receptor subtypes in surviving hippocampal neurons, provide new insights into the role of inhibitory mechanisms in the pathophysiology of focal epilepsy.

Key words: human, temporal lobe epilepsy, GABA, hippocampus, dentate gyrus, interneurons

GABA$_A$-Rezeptoren bei Patienten mit Temporallappenepilepsie

Temporallappenepilepsie (TLE), die mit einer Hippokampussklerose (HS) einhergeht, ist eine besonders pharmakotherapieresistente Art der fokalen Epilepsie, in der auch pharmakologisch ausgefeilte Kombinationstherapien bei vielen Patienten keine befriedigende Anfallskontrolle ermöglichen. Erfreulicherweise sprechen Patienten mit TLE und HS in der Regel jedoch sehr gut auf epilepsiechirurgische Eingriffe an. TLE scheint mit einer Störung der synaptischen Übertragung mittels GABA (γ-Aminobuttersäure), dem wichtigsten inhibitorischen Neurotransmitter im Gehirn, im Zusammenhang zu stehen. Die GABA$_A$-Rezeptoren vermitteln die schnelle synaptische inhibitorische Wirkung von GABA und sind zugleich Zielstrukturen von verschiedenen Pharmaka, die zur Therapie epileptischer Anfälle eingesetzt werden, wie unter anderem Benzodiazepine und Barbiturate. GABA$_A$-Rezeptoren werden aus einer Kombination von fünf Untereinheiten aufgebaut, kodiert von neunzehn Genen. Diese Heterogenität erklärt die Vielfalt von GABA$_A$-Rezeptor-Subtypen mit unterschiedlichsten Strukturen und Funktionen in den verschiedenen Hirnarealen.

Wir konnten durch eine vergleichende immunhistochemische Analyse im resezierten Hippokampus von Patienten mit TLE und HS signifikante Veränderungen der Verteilung und Expression von drei wichtigen GABA$_A$-Rezeptor-Subtypen gegenüber Autopsiekontrollgewebe erkennen. Das Hippokampusgewebe der Patienten mit HS, das einen ausgeprägten Nervenzellverlust und eine Gliose aufwies, zeigte erstens, trotz einer verminderten Färbungsintensität der GABA$_A$-Rezeptor-Untereinheiten in den Regionen mit Nervenzellverlust, eine höhere Dichte an GABA$_A$-Rezeptoren in der Körnerzellschicht des Gyrus dentatus, insbesondere vom α2-Subtyp. Zweitens beobachteten wir Unterschiede in der Anzahl und Morphologie der Interneuronen immunreaktiv für den α1-Subtyp. Unsere Resultate, die eine ausgeprägte Reorganisation von spezifischen GABA$_A$-Rezeptor-Subtypen in den überlebenden Neuronen des Hippokampus aufzeigen, bieten neue Erkenntnisse zur Rolle von inhibitorischen Mechanismen in der Pathophysiologie fokaler Epilepsie.

Schlüsselwörter: Menschlich, Temporallappenepilepsie, GABA, Hippokampus, Gyrus dentatus, Interneuronen

Les récepteurs GABA$_A$ dans l'épilepsie du lobe temporal chez l'homme

Parmi les différentes formes d'épilepsie focale, l'épilepsie du lobe temporal (ELT) associée à une sclérose de l'hippocampe (SH) est une des plus résistantes au traitement pharmacologique. Les patients souffrant de ce type d'épilepsie répondent cependant favorablement à une intervention chirurgicale dans la majorité des cas. Plusieurs études ont montré que l'ELT s'accompagne d'un dysfonctionnement de la transmission synaptique par l'intermédiaire du GABA (acide γ-aminobutyrique), le neurotransmetteur inhibiteur principal du cerveau. Les récepteurs GABA$_A$ qui transmettent l'action inhibitrice synaptique rapide du GABA sont aussi la cible de plusieurs médicaments utilisés lors du traitement des crises épileptiques, notamment les benzodiazepines et les barbituriques. Ce récepteur est composé d'une combinaison de cinq sous-unités codées par une vingtaine de gènes. Cette hétérogénéité est reflétée par la multitude de sous-types du récepteur GABA$_A$ possédant des structures et des fonctions diverses dans les differentes régions du cerveau.

En comparant par marquage immunohistochimique des pièces de résection de patients souffrant d'ELT associée à une SH avec des pièces d'autopsie contrôle, nous avons observé, au sein de l'hippocampe, des changements importants dans la distribution et l'expression de trois sous-types majeurs du récepteur GABA$_A$. Chez les patients atteints d'une SH qui est constituée par une perte neuronale sélective associée à une gliose de l'hippocampe, il a été constaté que, si une diminution des différentes sous-unités du récepteur GABA$_A$ est bien observée dans les régions qui subissent une perte cellulaire, les neurones en grain du gyrus denté qui survivent possèdent un nombre plus élevé de récepteurs GABA$_A$, en particulier du sous-type α2. Nous avons aussi observé des altérations dans le nombre et la morphologie des interneurones qui expriment le sous-type α1. Nos résultats, montrant clairement une réorganisation de certains sous-types du récepteur GABA$_A$ dans les neurones qui survivent au sein de l'hippocampe, contribuent à approfondir nos connaissances du rôle des méchanismes inhibiteurs dans la pathophysiologie de l'épilepsie focale.

Mots clés : humain, épilepsie du lobe temporal, GABA, hippocampe, gyrus denté, interneurones

Introduction

Epilepsy is one of the most prevalent neurological disorders, afflicting approximately one percent of the world population – about 50'000 people in Switzerland. This condition is characterized by an enduring predisposition to generate epileptic seizures and the occurrence of at least one epileptic seizure [1]. Among the many types of epilepsy, the most common in adults is temporal lobe epilepsy (TLE). This form of epilepsy frequently involves the hippocampus, a brain area important for processing memory and emotions (**Figure 1A**). The associated pathological alterations are referred to as hippocampal sclerosis (HS). TLE with HS is particularly refractory to treatment with available antiepileptic medication, but fortunately in recent years it has been recognized that surgical treatment provides a high success rate of seizure freedom [2, 3]. The examination of brain tissue, which had to be excised during surgery, provides a unique opportunity to study the changes caused by repeated seizures and to probe the mechanisms underlying the generation and maintenance of epilepsy. Ultimately, this approach will afford new treatments that offer lasting relief from seizures.

In this brief overview, I report on an investigation in which we analyzed the changes in neurotransmitter receptors mediating inhibition in hippocampal tissue removed at surgery from patients with medically intractable TLE [4]. The study was performed at the Institute of Pharmacology and Toxicology, University of Zurich, in the laboratory of Prof. J.M. Fritschy and conducted in close collaboration with Profs. H.G. Wieser and Y. Yonekawa of the Departments of Neurology and Neurosurgery, respectively, at the University Hospital Zurich.

Temporal lobe epilepsy with hippocampal sclerosis

The symptomatic focal epilepsies can be divided into three categories: 1) those associated with HS, 2) those associated with specific lesions such as tumors, scars, vascular malformations, and dysplasias, and 3) those of unknown etiology [5]. First described in 1825 [6], HS is the most common structural alteration in epilepsy and is characterized by neuronal cell loss and gliosis that selectively affect different regions of the hippocampus (**Figure 1B and 1C**). At the macroscopic level, the neuronal cell loss results in hippocampal atrophy, which, when severe, can be seen with high resolution magnetic resonance imaging (MRI). At the microscopic level, pathological analysis reveals that neuronal cell loss is particularly extensive in the CA1 area and less severe in the CA3 area and in the hilus of the dentate gyrus (endfolium) [7]. Moreover, there is a relative preservation of CA2 pyramidal cells and of the granule cells of the dentate gyrus. Another feature of HS encountered in approximately 50% of TLE patients is granule cell dispersion

defined as a widening of the granule cell layer (see **Figure 3**, first and third columns).

GABAergic synaptic neurotransmission

Epileptic seizures are manifestations of synchronized waves of uncontrolled but transient electrical activity in populations of neurons in the brain. Neurons communicate with each other at specialized contact points termed synapses by releasing neurotransmitters that bind to receptors located on the surface of the receiving neuron. Depending on the neurotransmitter, the type of receptor, and the prevailing ionic gradients, the receiving neuron is either excited or inhibited. In the healthy brain, a delicate balance between excitation and inhibition is maintained that ensures the efficient functioning of neuronal networks. Disease processes that tip the scales in favor of excitation result in agitation or even seizures, while excessive inhibition of large numbers of neurons results in sedation. Accordingly, many antiepileptic drugs act by promoting inhibition in the brain.

In the hippocampus and the cerebral cortex, there are two main types of nerve cells, the pyramidal cells and the interneurons. The pyramidal cells represent approximately 80% of all cortical neurons, are excitatory, and use glutamate as their neurotransmitter. The granule cells of the dentate gyrus, which provide an important input to the hippocampus, are also excitatory and glutamatergic. In contrast, the interneurons, comprising the remaining 20% of neurons, are inhibitory and release γ-aminobutyric acid (GABA). Even though GABAergic neurons are relatively few in number, they often exert control over hundreds of pyramidal cells via strongly divergent and particularly efficacious synaptic connections, and can thereby synchronize activity of neuronal populations.

Fast synaptic inhibition is mediated primarily by the $GABA_A$ class of receptors, which gate the flow of chloride ions across the neuronal membrane. $GABA_A$ receptors belong to the family of ligand-gated ion channels and form heteromeric complexes composed of five subunits. A multitude of subunit variants encoded by at least 19 genes in the mammalian central nervous system have been classified into several families (for example α1-6, β1-3, γ1-3) [8]. Different subunits from these families assemble to form functionally and pharmacologically distinct receptor subtypes, most of which include at least one of each of the α, β and γ subunits [9, 10]. It has been shown that the α-subunit variants are useful markers for the identification of specific subtypes, while the β- and γ-subunits are ubiquitous being present in most receptor subtypes. Furthermore, in animal studies these distinct receptor subtypes were shown to be preferentially expressed in specific regions and neuronal populations [11, 12]. Previous to our study, limited data were available in the human on the
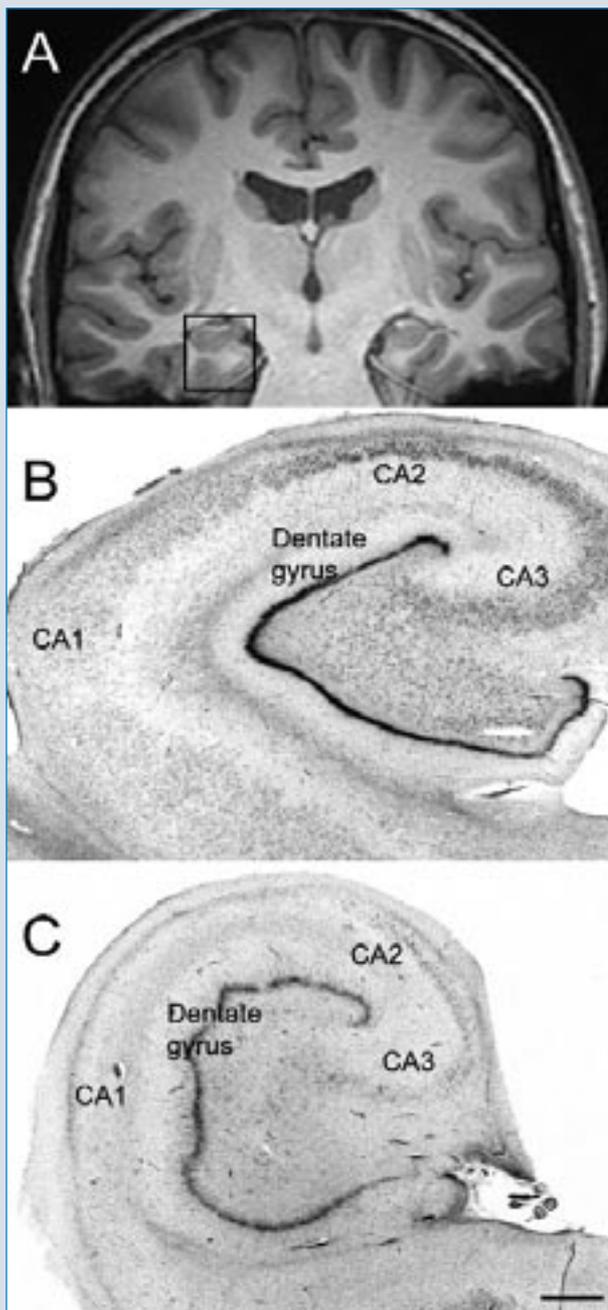

Figure 1: (A) A coronal section of a brain MRI scan showing the position of the hippocampal formation in the mesial part of the temporal lobe (box), which includes the dentate gyrus, the hippocampus proper (Ammon's horn), the subiculum, and the entorhinal cortex. (B, C) Coronal sections of human hippocampal tissue stained with cresyl violet to demonstrate the pattern of neuronal cell loss seen in TLE with HS (C) as compared to a control (B). (B) Normal distribution of neuronal cell bodies in the dentate gyrus and the hippocampus proper, which is divided into three areas and labeled CA1, CA2, and CA3. (C) Marked hippocampal atrophy due to prominent cell loss in CA1, CA3, and the hilus of the dentate gyrus. CA2 pyramidal cells and dentate granule cells are relatively well preserved. Granule cell dispersion, although present, is difficult to see at this low-power magnification. Scale bar, 1 mm. (A) Courtesy of PD Dr. H.J. Huppertz; (B) Modified from Loup et al. [4], with permission of the Society for Neuroscience.

differential expression of GABA$_A$ receptor subtypes in normal or disease states because of methodological limitations. Thus, PET studies using flumazenil, an antagonist at the benzodiazepine/GABA$_A$ receptor complex, although indispensable for tracking changes in living subjects, provide low spatial resolution of GABA$_A$ receptor distribution and do not allow the differentiation between distinct different subtypes.

GABA$_A$ receptors are of particular interest from the medical standpoint in view of their sensitivity to widely used therapeutic agents, such as barbiturates and benzodiazepines. The latter drugs exert powerful antiepileptic, anxiolytic, sedative, and muscle-relaxant actions by potentiating GABA$_A$ receptor function. An interesting direction in recent research has been the attribution of specific subtypes to each of these distinct actions, which will help in the development of more selective medications with less side effects [13]. Blocking GABA$_A$ receptors pharmacologically promotes the generation of seizures in animals and in humans. These findings together with recent studies in animal models of TLE suggest that impairment of GABA$_A$ receptor function contributes to epilepsy [14].

Methodology

For this study we used hippocampal tissue obtained at surgery from patients with pharmacoresistant TLE. Prior to surgery, the patients were subjected to a comprehensive assessment including EEG monitoring, neuropsychological testing and high resolution MRI with special protocols to localize and characterize the epileptogenic foci. Based on the results of these tests, the patients were classified into those with HS and those without sclerosis. Patients from the HS group underwent selective amygdalohippocampectomy in which afflicted parts of amygdala, hippocampal formation, and parahippocampal gyrus were resected [15]. The origin of TLE in the non-HS patients was usually attributed to a tumor or a vascular malformation. In these patients the surgical procedure was adapted to the location and extent of the lesion. For comparison we used hippocampal tissue collected at autopsy from subjects who did not have a history of neurological or psychiatric disorder. All procedures were performed with the informed consent of the patients or legal next of kin and were approved by the Ethics Committee of the University Hospital Zurich in accordance with the Declaration of Helsinki.

The tissue samples obtained at surgery or autopsy were cut into 7 to 12 mm-thick blocks and comprised the anterior and mid levels of the hippocampal formation. They were processed in the laboratory to achieve optimal tissue preservation and frozen. They were cut in very thin slices (thickness of 40 μm, that is 40 x 1/1000 of a 1 mm) perpendicular to the antero-posterior axis of the hippocampal formation, collected in a buffer solu-
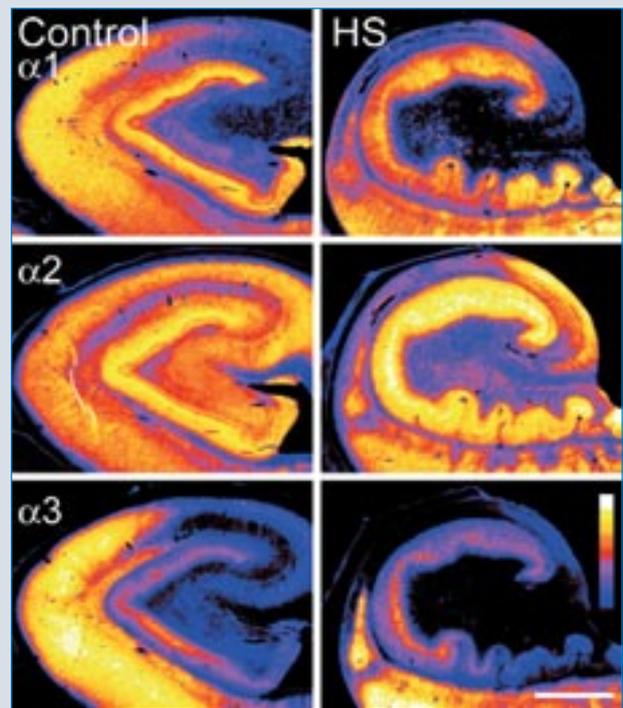

Figure 2: Differential distribution of three major GABA$_A$ receptor subtypes in the hippocampus from a control (left column) and a TLE patient with HS (right column). The color-coding indicates optical density of staining using a normalized scale with the strongest signal in white and no signal (background) in dark blue. In both specimens, adjacent sections were stained for the subunits α1, α2 and α3. In the control, the α-subunit variants demonstrate distinct patterns of immunoreactivity. In TLE with HS, staining is decreased in areas of prominent cell loss (CA1, CA3 and hilus of the dentate gyrus), whereas staining is increased in the dentate gyrus. Scale bar, 2 mm. Modified from Loup et al. [4], with permission of the Society for Neuroscience.

tion and stored at -20°C. This procedure allowed the processing in parallel of 10 to 12 different specimens in one single session, thus minimizing experimental variability.

In a first step we then used a technique called immunoperoxidase staining to visualize the different GABA$_A$ receptor subunits in human brain tissue using light microscopy. This method is based on the specific and selective binding of a high-affinity antibody with the subunit, which acts as the antigen. An amplification procedure followed by an enzymatic reaction catalyzed by peroxidase then leads to the staining of the subunit. We used several specific antibodies directed against the most abundant GABA$_A$ receptor subunits: the subunits α1, α2, α3, β2/3 and γ2. To improve the signal-to-noise ratio of the immunhistochemical staining, an antigen-retrieval method based on microwave irradiation was adapted for human brain tissue [16]. Finally adjacent series of sections were stained with cresyl violet for histopathological examination. The numbers of cells were counted to assess objectively the degree of neuronal

loss in TLE specimens with HS. In addition, the intensity of staining for each subunit in the CA2 area and dentate gyrus was quantified using a computer program for optical density measurements.

Differential distribution of $GABA_A$ receptor subtypes: a comparison between control tissue and TLE specimens with HS

To obtain an initial overview, the subunits $\alpha 1$, $\alpha 2$ and $\alpha 3$, which form distinct $GABA_A$ receptor subtypes, were visualized at low-power magnification in the normal human hippocampus as illustrated in the color-coded images (**Figure 2**, left column). Differences in staining intensity for each subunit were assessed using a normalized color scale revealing that the α-subunit variants showed distinct patterns of distribution. Thus while all three α-subunits are present in CA1 pyramidal cells, they are differentially expressed in CA2 pyramidal cells, and only the $\alpha 2$-subunit was detected in CA3 pyramidal cells.

The distribution of the $GABA_A$ receptor subunits was then analyzed in hippocampal specimens from TLE patients with HS and from TLE patients without HS. While the cytoarchitecture and the staining pattern for $GABA_A$ receptor subunits were largely similar in the autopsy controls and the TLE specimens without HS, prominent changes were observed in TLE specimens with HS (**Figure 2**, right column). Thus two main alterations in $GABA_A$ receptor subunit expression were observed. First, areas of prominent cell loss, such as CA1, CA3, and the hilus of the dentate gyrus showed marked decreases in $GABA_A$ receptor staining for all subunits. Similar changes have been reported in other studies [17-19]. This finding is not unexpected. Where there are no more cells, there are also no more receptors. What was however unexpected was a second observation in our work,
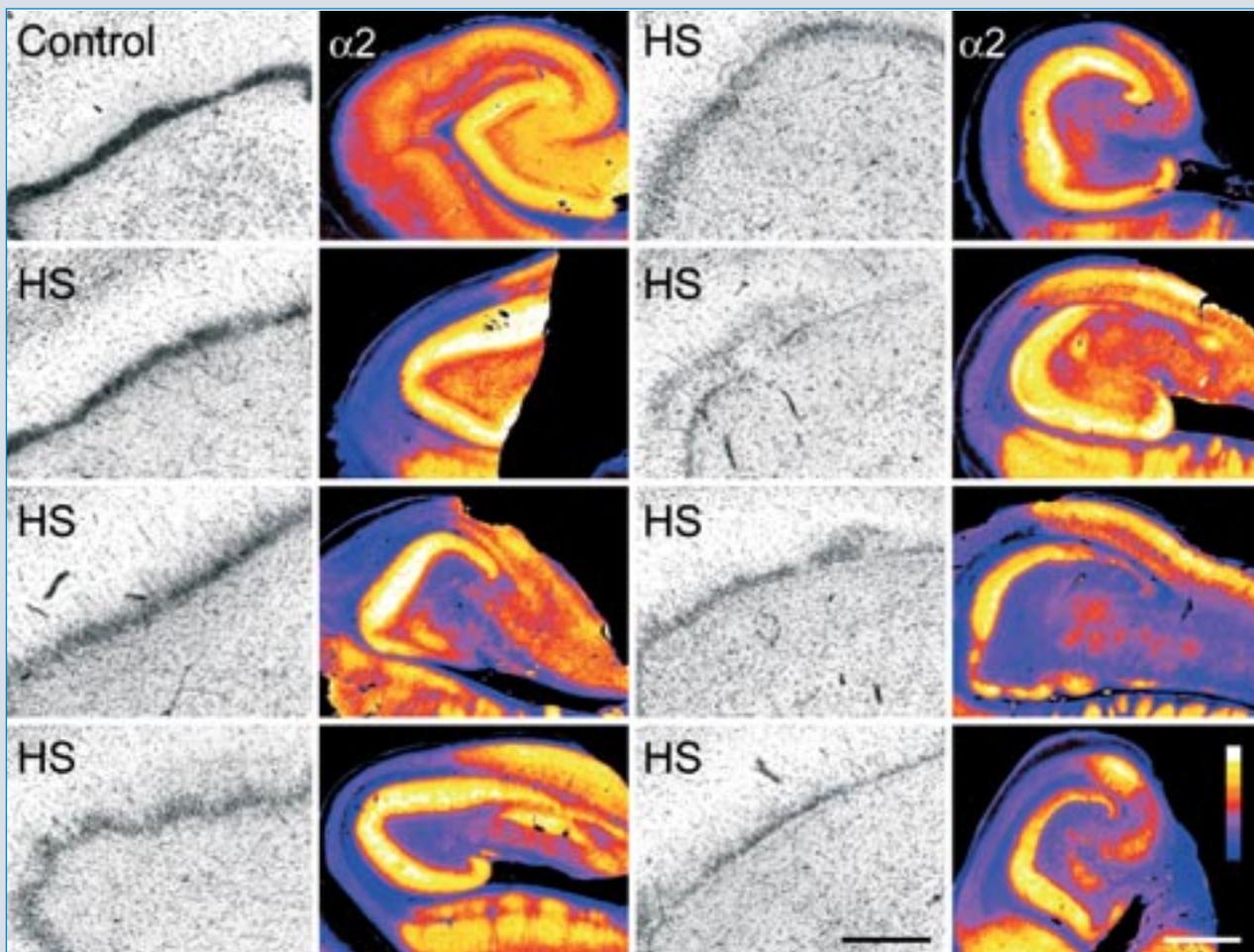

Figure 3: Upregulation of the $GABA_A$ receptor $\alpha 2$-subunit in the dentate gyrus of seven TLE specimens with HS in comparison to a control specimen. Overviews of the hippocampus are shown in the color-coded images. Adjacent sections stained with cresyl violet are represented in black and white at higher magnification, with the upper blade of the dentate gyrus depicted to illustrate granule cell density. In control hippocampus (top left panel), granule cells are tightly packed forming a compact layer. In HS specimens with moderate granule cell loss, staining intensity in the dentate gyrus is higher than in controls. In HS specimens with severe granule cell loss, the apparent intensity is similar to that in controls. Scale bars, cresyl violet 0.5 mm, $\alpha 2$-subunit 2 mm. Modified from Loup et al. [4], with permission of the Society for Neuroscience.

namely that areas of relative cell loss such as the dentate gyrus exhibited an increase in GABA$_A$ receptor subunit staining.

Upregulation of GABA$_A$ receptor subtypes in the dentate gyrus of TLE specimens with HS

We further analyzed the relationship between the loss of dentate granule cells and the changes in subunit expression in each of the TLE specimens with HS. We first counted the granule cells and found on average a more than 50% loss. As illustrated in **Figure 3** for one control and seven patients, there was not only variability in granule cell loss, but also in the degree of granule cell dispersion (**Figure 3**, first and third columns). We then compared the amount of granule cell loss with the intensity of staining for the different GABA$_A$ receptor subunits in the dentate gyrus. At low-power magnification, the dentate gyrus displayed prominent staining, standing out against the relatively weak staining in the rest of the hippocampus (**Figure 2**, right column, and **Figure 3**, second and fourth columns). Because staining appeared conserved or augmented when compared to control specimens despite a more than 50% loss in granule cells (**Figure 3**), we concluded that there were more GABA$_A$ receptors on the surviving granule cells in TLE specimens with HS than in controls. Of all subunits, the α2 exhibited the largest increase in expression as illustrated in **Figure 2** and **Figure 3**.

To show that there were more receptors on the surviving granule cells, we used immunofluorescence staining, which is based on the detection of the different subunits by linking them to a fluorescent dye. When combined with confocal laser scanning microscopy, this technique allows the precise localization of the receptor subunit examined at a much higher resolution than with light microscopy, as illustrated in **Figure 4** for the α1-subunit. This approach demonstrated that surviving granule cells express more GABA$_A$ receptors. At the subcellular level, individual granule cells were outlined by intense staining along the surface of the cell bodies (**Figure 4B**) in contrast to the discrete staining observed in the control (**Figure 4A**). In conclusion, the augmented staining in the dentate gyrus indicates an upregulation of GABA$_A$ receptors in surviving granule cells of TLE patients with HS, despite extensive granule cell loss. Similar results have been obtained in several animal models of TLE [20-23] and our findings have been confirmed in a recent human study [19].

What are the implications of these observations? Receptor upregulation has been proposed to result from hyperactivity at GABAergic synapses onto the surviving dentate granule cells, suggesting the presence of compensatory mechanisms in response to seizures. Thus the surviving neurons appear to cope with the repeated waves of excitation by increasing the number of GABA$_A$ receptors on these neurons, thereby enhancing their sensitivity to inhibitory neurotransmitter signals.

Changes in interneurons immunoreactive for the α1-containing GABA$_A$ receptors

Our analysis of α1-subunit staining in the normal hippocampus revealed that in addition to expression in pyramidal cells, the α1-subunit is also present in numerous hippocampal interneurons, the cells that release GABA and control the activity of dentate granule cells and pyramidal cells. Based on their size and location, several types of interneurons could be distinguished including large and intensely stained multipolar cells lo-
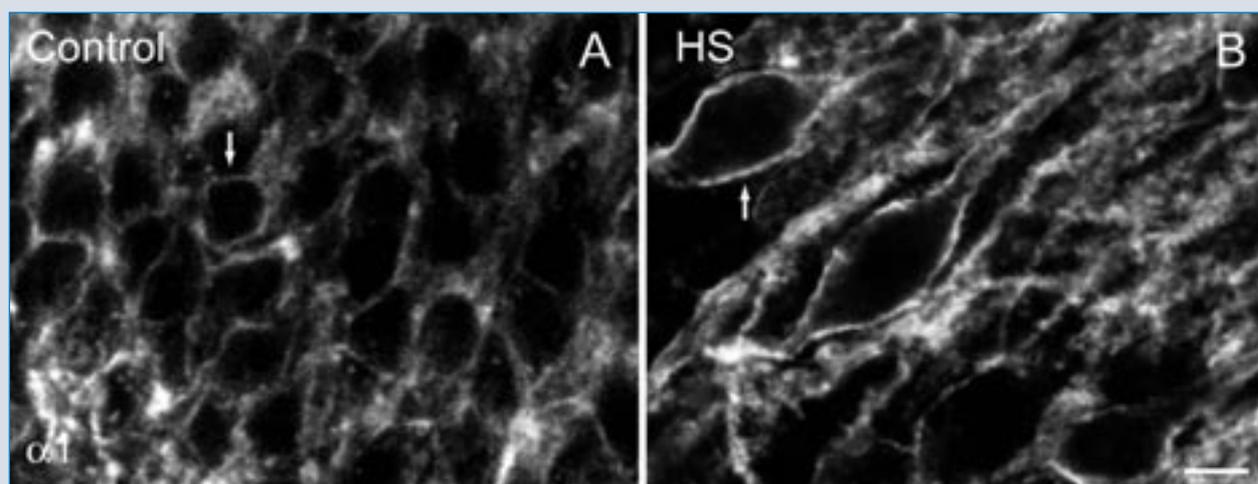

Figure 4: Digital images obtained with immunofluorescence staining and confocal laser scanning microscopy illustrating the increase in GABA$_A$ receptor α1-subunit staining (white) in the dentate gyrus in TLE with HS specimens (B) versus controls (A). (A) In the control, discrete α1-subunit immunoreactivity outlines the cell bodies of individual granule cells (arrow). (B) In TLE with HS, the surviving granule cells are larger and surrounded by intense staining along the surface of the cell bodies (arrow). Scale bar, 10 µm. Modified from Loup et al. [4], with permission of the Society for Neuroscience.
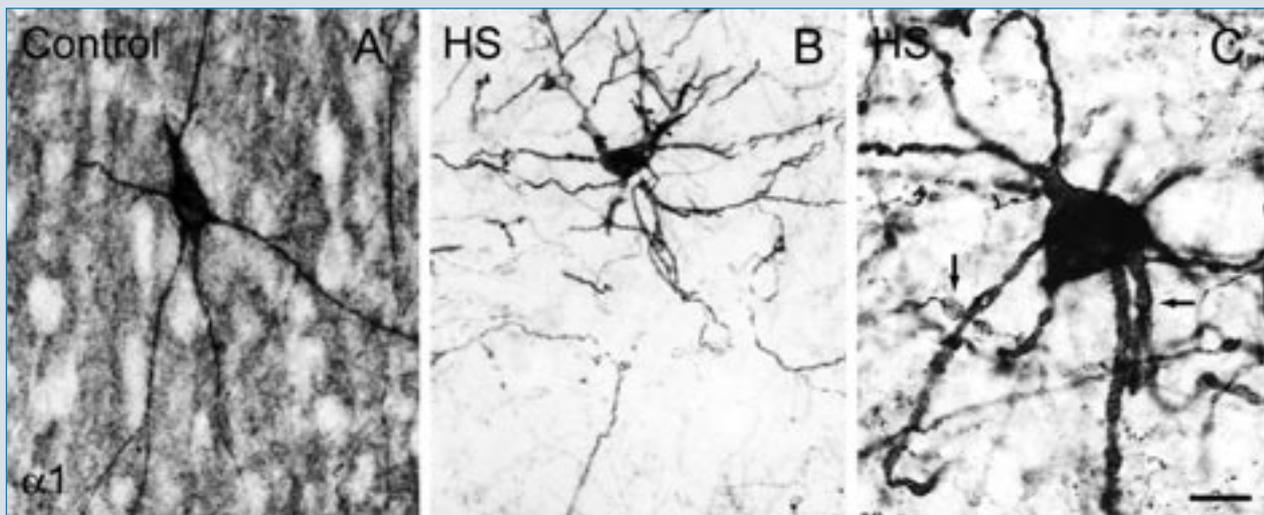
Figure 5: Altered morphology of large multipolar interneurons immunoreactive for the α1-subunit in the CA1 pyramidal cell layer in TLE with HS. (A) In the autopsy control, the cell body contours are smooth with few, long and straight dendrites. (B, C) In HS specimens, the cell body exhibits an irregular shape and dendrites appear tangled, and increased in number. At higher magnification (C), dendritic nodulations are visible (arrows). Note the absence of α1-subunit staining in neuropil reflecting severe pyramidal cell loss in CA1. Scale bar, A and B 50 µm, C 25 µm. Modified from Loup et al. [4], with permission of the Society for Neuroscience.

cated in the pyramidal cell layer. **Figure 5A** shows such an interneuron superimposed on a dense network of immunoreactive dendrites originating from CA1 pyramidal cells. The presence of inhibitory receptors on these interneurons indicates that they not only regulate the activity of the excitatory cells but also that they are themselves kept in check by neighboring interneurons.

In TLE specimens with HS, α1-subunit staining revealed a population of interneurons readily visible in all hippocampal areas but most conspicuous in CA1, because of the absence of staining reflecting CA1 pyramidal cell degeneration (**Figure 5B** and **5C**). We made two novel observations with respect to these interneurons. The first was that a significant number of these cells had died, while others had survived in only certain layers of the hippocampal areas. Second, a proportion of the surviving interneurons, especially the large and multipolar ones, displayed irregularly shaped cell bodies and dendrites that appeared tangled, nodulated, and increased in number (**Figure 5B** and **5C**).

In summary, the severely altered dendritic morphology of surviving α1-positive interneurons, which was not present in TLE specimens without HS or controls, indicates pronounced dendritic reorganization in TLE specimens with HS. The layer-specific loss of a subpopulation of interneurons expressing the α1-subunit, which had not been reported before in human epileptic tissue or animal models of TLE, suggests that inhibitory drive is reduced at specific inputs on surviving pyramidal cells. Earlier studies of human TLE using other markers of GABAergic interneurons such as neuropeptide Y, somatostatin, or calretinin also found loss or preservation of specific subclasses of interneurons [24-29]. The highly differentiated sensitivity to seizure-induced damage underscores the functional and neurochemical specialization of hippocampal interneurons [30].

Conclusions

This brief review describes important alterations in GABA$_A$ receptors observed in hippocampal tissue from TLE patients with HS. Our analysis reveals a complex and differential reorganization in surviving principal cells and interneurons involving the α1, α2 and α3-subtypes [4]. In a recent study, we further investigated the distribution and expression of these subtypes in the temporal neocortex of patients with TLE [31]. In contrast to the data in the hippocampus, we found a downregulation of α3-containing GABA$_A$ receptors in the superficial layers of the temporal neocortex, while there were no changes in the α1 or α2-subtypes. Taken together these results show a subtype-specific reorganization of GABAergic systems in both the hippocampus and the neocortex of TLE patients with HS. Our studies are unique in that they allow direct insight into diseased tissue from patients with medically refractory TLE, and thus circumvent the potential difficulties involved in the interpretation of data from animal models of this complex disorder. Furthermore, the identification of altered GABA$_A$ receptor subtypes in specific neuronal circuits should facilitate the development of subtype-selective drugs with an improved therapeutic profile as compared to classical benzodiazepines.

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