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Central benzodiazepine receptor autoradiography in hippocampal sclerosis

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1 The γ-aminobutyric acid $(GABA)_A$/central benzodiazepine receptor (cBZR) complex is a major inhibitory receptor in the vertebrate CNS. Binding of [^{11}C]-flumazenil to this complex *in vivo* is reduced in hippocampal sclerosis (HS). It has been uncertain whether reduced cBZR binding is entirely due to neuronal loss in HS.

2 The objective of this study was to characterize abnormalities of the cBZR in HS with a correlative autoradiographic and quantitative neuropathological study.

3 Saturation autoradiographic studies were performed with [^{3}H]-flumazenil to investigate relationships between neuronal density and receptor availability (B_{max}) and affinity (K_d) in HS. Hippocampal tissue was obtained at surgery from 8 patients with intractable temporal lobe epilepsy (TLE) due to HS and autopsies of 6 neurologically normal controls. Neuronal densities were obtained by means of a 3-D counting method.

4 B_{max} values for [^{3}H]-flumazenil binding in the subiculum, CA1, CA2, CA3, hilus and dentate gyrus were all found to be significantly reduced in HS compared with controls and significant increases in affinity were observed in the subiculum, hilus and dentate gyrus. In HS, cBZR density in the CA1 region was significantly reduced ($P<0.05$) to a greater extent than could be attributable to neurone loss. In other regions, B_{max} was reduced in parallel with neuronal density.

5 In HS, there is a loss of cBZR in CA1 over and above loss of neurones. This finding and increases in affinity for flumazenil in subiculum, hilus and dentate gyrus imply a functional abnormality of the $GABA_A$/cBZR complex that may have a role in the pathophysiology of epileptogenicity in HS.

Keywords: Benzodiazepine; $GABA_A$ receptors; hippocampal sclerosis; temporal lobe epilepsy; receptor autoradiography; down-regulation

Introduction

γ-Amino butyric acid (GABA) is the major inhibitory neurotransmitter in the vertebrate CNS and the effectiveness of anti-epileptic drugs, that enhance GABA mediated inhibition, supports the view that a functional impairment of GABA neurotransmission may be one mechanism of epileptogenesis (Meldrum, 1979; Olsen *et al*., 1986).

Attempts to determine the inter-ictal concentrations of GABA in epileptic human tissue have been inconclusive but *in vivo* microdialysis has demonstrated a less pronounced glutamate-evoked rise in GABA levels during seizure activity in the epileptic hippocampus compared to the non-epileptic contralateral hippocampus (During & Spencer, 1993). A significant decrease in the number of GABA transporters has been demonstrated in amygdala-kindled rats, a model of mesial temporal lobe epilepsy (TLE), possibly resulting in reduced glutamate-evoked GABA release (During *et al*., 1995). No significant changes in the activity of glutamic acid decarboxylase (GAD), the enzyme responsible for GABA synthesis, were detected in epileptic human hippocampus compared to non-epileptic tissue (Schmidt *et al*., 1984). Immunocytochemical studies have not demonstrated preferential loss of GAD-immunoreactive interneurones in TLE (Babb *et al*., 1989) despite significant losses of other cell types. In electrophysiological studies of pyramidal cells (Knowles *et al*., 1992) and dentate and hilar neurones (Isokawa *et al*., 1991) from sclerotic human hippocampus, fewer stimulus-evoked inhibitory post-synaptic potentials (i.p.s.ps) were elicited when compared with neurones from patients with structural lesions. This would suggest a loss of GABA-mediated inhibition in hippocampal sclerosis (HS). Evidence supporting this paradigm has also been obtained from hippocampal slices from pilocarpine-treated rats which demonstrated diminished GABA-mediated inhibition along with a loss of high-affinity GABA binding sites (Kapur *et al*., 1994).

In vivo positron emission tomography (PET) studies with [^{11}C]-flumazenil, a compound selective for the central-type benzodiazepine binding site, demonstrated significantly reduced binding in HS (Koepp *et al*., 1996). Previous *in vitro* autoradiographic analyses of the benzodiazepine (BZ) binding site in HS have yielded conflicting results. Initial studies with flunitrazepam, which is sensitive to modulation by endogenous GABA, may explain the failure to detect any significant change in binding in the dentate gyrus (McDonald *et al*., 1991). Other receptor autoradiography studies, in which radiolabelled compounds selective for the central-type benzodiazepine receptor (cBZR) were used, have demonstrated significant, region-specific reductions in BZ binding in HS relative to autopsy controls (Johnson *et al*., 1992; Burdette *et al*., 1995). Changes in BZ binding were correlated with changes in neuronal density and binding of flumazenil was found to be reduced to a greater extent than the reduction of neurones in CA1 and CA3 (Johnson *et al*., 1992). Changes in absolute receptor density (B_{max}), affinity (K_d) and relative loss of neurones and receptors have not been quantified. The aim of the present investigation was to carry out saturation autoradiographic analysis of the central BZ receptor by use of [^{3}H]-flumazenil and quantitative neuropathology in hippocampi resected from patients with medically refractory TLE, to define

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abnormalities of the cBZR in hippocampal subregions in HS. These results have, in part, been presented as an abstract to the British Pharmacological Society (Hand *et al*., 1996).

Methods

Subjects

Hippocampal tissue was obtained from 8 patients (median age 32 years, range 22–38 years) with medically refractory TLE and unilateral HS, enrolled in the neurosurgery programme at the National Hospital for Neurology & Neurosurgery, Queen Square, London. Patients had not received any benzodiazepine-type medication in the 2 months preceding surgery. All patients were subject to extensive pre-surgical investigations including MRI and [^{11}C]-flumazenil PET scans; clinical data are summarized in Table 1. Control tissue was obtained at autopsy from 6 subjects (4 male, 2 female), with no evidence of neurological disease. Control subjects were of median age 74.5 years (range 43–77 years) and *post-mortem* intervals ranged from 5.5 to 28 h (median 17 h). Cause of death among the control individuals were as follows: myocardial infarction; left ventricular failure; cancer of the pancreas; ruptured thoracic aneurysm; cancer of the bronchus and unknown in one case. Details of any medication being received by the control group before death were not available. This study was approved by the ethics committee of the National Hospital for Neurology & Neurosurgery.

Autoradiography technique

Temporal neocortex and hippocampi were resected *en bloc* from each patient and frozen within 10–20 min on dry ice, surrounded with Lipshaw embedding matrix and stored at −80°C. Control tissue was obtained at autopsy from patients with no history of neurological disease. Control brains were sliced into 1 cm coronal slices and snap-frozen between pre-cooled metal plates and stored at −80°C. Hippocampi were subsequently dissected from appropriate slices for further sectioning. Cryostat sections were cut at 10 μm at −16 to −20°C, thaw-mounted onto charged microscope slides (BDH Superfrost Plus) and stored with desiccant at −80°C until assayed.

The receptor autoradiography protocol used was based upon the methods published by Houser and colleagues (Houser *et al*., 1988). Briefly, on the day of the assay, slides were allowed to come to room temperature and prewashed twice for 30 min at 0–4°C in buffer containing 170 mM Tris-HCl at pH 7.4. Incubation was for 60 min at 0–4°C in fresh assay buffer containing one of 6 concentrations of [^{3}H]-flumazenil (75.17 Ci mmol^{-1}, Du Pont-New England Nuclear, Brussels, Belgium), ranging from 0.25 nM to 20 nM. Non-specific binding was determined in the presence of 2 μM clonazepam (Sigma, U.K.). Following incubation, slides were vacuum-aspirated, rinsed twice for 1 min in fresh ice-cold buffer, briefly dipped into distilled water and dried under a stream of cold air. Slides were then apposed to ^{3}H-sensitive Hyperfilm (Amersham, U.K.) in X-ray cassettes and co-exposed with ^{3}H-impregnated plastic standards (Amersham, U.K.) for 21 days at room temperature.

Table 1 Clinical data from 8 patients studied with HS and TLE

Patient No	*Sex*	*Age* (y)	*Age at onset of epilepsy* (y)	*Histology*	*Medication*	*MRI finding*
1	F	36	3	HS	CBZ	HS
2	F	33	21	HS,MD	GBP,PHT	HS
3	F	31	7	HS	VPA,LTG	HS
4	F	38	1	HS,MD	CBZ,PHT	HS
5	M	38	23	HS	CBZ,LTG	HS
6	F	26	2	HS,MD	CBZ,LTG	HS
7	F	22	4	HS	CBZ,LTG	HS
8	F	31	7	HS	GBP,PHT	HS

Abbreviations: y – years; HS – hippocampal sclerosis; TLE – temporal lobe epilepsy; MD – microdysgenesis in temporal white matter; GBP – gabapentin; PHT – phenytoin; VPA – valproate; LTG – lamotrigine; CBZ – carbamazepine.

Binding data analysis

Total binding was assessed in at least two sections per concentration (usually four sections) and non-specific binding in one or two sections per concentration, depending on tissue availability, and mean values were used for subsequent analysis.

Hippocampal subregions were identified in sections stained with cresyl violet following autoradiography and optical density values for each subregion were obtained by computer-assisted densitometry, by use of MCID M4 image analysis system (Imaging Research Inc., Ontario, Canada); the entire subregion was outlined and a mean optical density value across the region was obtained. Concentration of bound radioactivity was determined from a standard curve generated from film optical densities produced by the ^{3}H-impregnated plastic standards, corrected for tissue quenching. B_{max} and K_d values were determined by non-linear regression with an interactive curve-fitting package (GraphPad Software, San Diego, CA) by means of a standard two-variable Langmuir equation.

Neuronal counts in the hippocampal subregions were determined in 20 μm paraffin-embedded sections, adjacent to those used for autoradiography, from the patients and control subjects by one of the investigators (WVP) by a three dimensional counting method, as described by Williams and Rakic (1988). Correction for tissue shrinkage was not applicable in this study as neuronal density values were used for direct comparison with receptor binding data.

A Student's t test was used to compare mean B_{max} and K_d values for the sclerotic and control hippocampi. For certain individuals it was not possible to obtain density measurements for a number of subregions, typically CA3 and CA2, due to damage during the resection procedure, and neuronal density data were not available for one of the control individuals and one of the epilepsy specimens.

Results

Neuronal density

Significant neuronal loss was observed in each of five subregions of the specimens of HS, compared to the autopsy controls (Figure 1, Table 2). The dentate gyrus is not represented in Figure 1 in order to avoid loss of clarity due to relatively large differences in scale but mean neuronal density in the dentate gyrus of the epileptic patients was also found to be significantly reduced compared to control (248325 neurones mm^{-3} and 539304 neurones mm^{-3}, respectively – HS 46±9% of control, $P<0.01$, two-tailed Student's t test).

Central benzodiazepine receptor binding density

In the control hippocampi, cBZR binding density was highest in the polymorphic layer of the dentate gyrus, the strata oriens, pyrimidale and lacunosum moleculare of the CA1 region of Ammon's Horn and the subiculum. CA3, CA2 and the hilus showed moderate binding density (Figure 2a).

Figure 3 shows an example of a saturation plot from one region of a control hippocampus (CA3). Data points at each concentration of radioligand represent the mean of binding measurements from a selected hippocampal area in one pa-

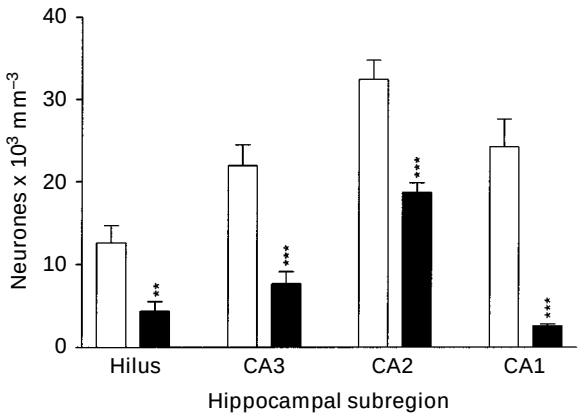

Figure 1 Mean neuronal density values for subregions of hippocampi resected from patients with hippocampal sclerosis and temporal lobe epilepsy ($n=7$, solid columns) and autopsy controls ($n=5$, open columns). Neuronal density measurements were carried out by WVP on 20 μm paraffin-embedded sections adjacent to those used for autoradiography. Error bars represent s.e.mean. Significance of differences between HS and control groups was determined by two-tailed Student's t test (***$P<0.001$, **$P<0.01$).

Table 2 Summary of [^{3}H]-flumazenil binding parameters and neuronal density measurements from HS specimens expressed as a percentage of autopsy controls

	Percentage of control measurements			
Hippocampal subregion	B_{max}	*Neuronal density*	*Relative* B_{max}	K_d
DG	58±5	46±9	120±10	63±8
Hilus	38±6	35±9	114±22	61±9
CA3	50±9	35±7	180±61	70±13
CA2	55±6	58±4	101±15	94±16
CA1	6±1	11±1	55±4*	93±16
Subiculum	79±6			61±6

Data are presented as means±s.e.mean. Abbreviations: DG – dentate gyrus. *$P<0.05$ from control Student's t test.

tient. Specific binding was greater than 95% of total across the concentration ranges used, as determined by area under the curve. Central BZ receptor binding was reduced in all subregions of sclerotic hippocampi when compared with autopsy control specimens (Figure 4, Table 2). The CA1 region of the hippocampus in the HS group showed the most striking changes with binding reduced to 6±0.6% of controls and the hilus, to 38±6% of controls. The subiculum was relatively spared with binding reduced to 79±6% of controls in the HS group.

Central benzodiazepine receptor affinity

Changes in binding affinity for [^{3}H]-flumazenil at the cBZR were apparent in three of the six hippocampal subregions measured in the HS group relative to controls (Figure 5, Table 2). K_d values were reduced to 63±8% in the dentate gyrus, 61±9% in the hilus and 61±6% in the subiculum region of the epileptic group compared to controls. This translates to increases in affinity of 59% in DG, 63% in the hilus and 65% in the subiculum.

Relationship between receptor density and neuronal counts

Comparison of the percentage reduction of cBZR B_{max} and reduction of neuronal density gives an indication of cBZR density per neurone (Figure 6, Table 2). The density of central BZ receptors per surviving neurone in the CA1 subregion (relative B_{max}) was reduced to 55±4% in the HS group, compared with the control group ($P<0.05$, two-tailed Student's t test). Receptor density per neurone was not reduced in the other subregions examined.

Discussion

The present study has demonstrated a loss of cBZR binding associated with HS in all hippocampal subregions examined. This decrease of receptor binding may be primarily attributable to changes in neuronal density. However, in the CA1 region, we have shown a significant reduction of almost 50% of cBZR receptors on surviving neurones. An increase in affinity of the cBZR in HS for [^{3}H]-flumazenil, ranging from 59–65%, was observed in the DG, CA4 and subiculum.

The regional distributions of cBZR binding sites demonstrated in this study are concordant with previously published mapping studies (Faull & Villiger, 1988; Zezula *et al*., 1988). Absolute values for specific [^{3}H]-flumazenil B_{max} were in close agreement with data published by Olsen and colleagues (Houser *et al*., 1988; Olsen *et al*., 1992) for both control and epileptic hippocampi and changes in binding in epileptic tissue relative to control are consistent with previous findings (Johnson *et al*., 1992; Burdette *et al*., 1995). The apparent lack of appreciable binding to the glial cell $GABA_A$/BZR receptor complex, despite glial proliferation, may be due to the presence of $\gamma 1$ receptor subunit in certain glial cell populations which has a negligible affinity for flumazenil. A correlation between changes in cBZR binding and neuronal density in HS has been demonstrated previously (Johnson *et al*., 1992; Burdette *et al*., 1995), but these studies did not measure absolute receptor density (B_{max}) or affinity (K_d). This is the first study to demonstrate significant loss of cBZR binding in all six subregions examined and the observed increases in receptor binding affinity for [^{3}H]-flumazenil in HS may be one explanation for the failure of studies in which single ligand concentrations were used to detect significant loss of binding sites in HS. Johnson and colleagues observed a consistent trend towards lower concentrations of receptors per remaining neurone in HS compared with autopsy control, reaching statistical significance in CA3 and CA1 (Johnson *et al*., 1992). The question of whether loss of central BZ binding in HS represents a reduced functional inhibitory tone or merely reflects a loss of GABA-receptive somata may only be addressed by comparison of receptor density per neurone in HS compared with controls. Analysis of correlation does not detect changes in receptor concentration on surviving hippocampal neurones. Expression of binding data in terms of receptor concentration per neurone may represent a more valid method of comparison between control and epileptic tissue and, from our work, we can definitively conclude that cBZR density is reduced over and above neuronal loss in the CA1 region in HS associated with TLE.

A number of limitations in the use of human tissue must be considered in the interpretation of these results, particularly as far as adequate control tissue is concerned. It is impossible to determine the influence of factors such as age and *post-mortem* interval/agonal state upon the parameters measured. A synaptic membrane binding study of genetically dystonic hamsters; in which [^{3}H]-flumazenil was used, demonstrated an apparent decrease in affinity with age in certain brain regions in control animals but not in all regions (Pratt *et al*, 1995). Affinity constants for the hippocampus were not determined. Lloyd and Dreksler (1979) observed that [^{3}H]-GABA membrane binding was independent of sex, age, *post-mortem* interval or storage time in human brain tissue. However, Burdette and colleagues detected a trend towards increased muscimol binding with *post-mortem* delay which was suggested to be due to *post-mortem* increases in ambient GABA concentration but was not associated with increased BZ binding (Burdette *et al*., 1995). Zezula and colleagues (1988) found no

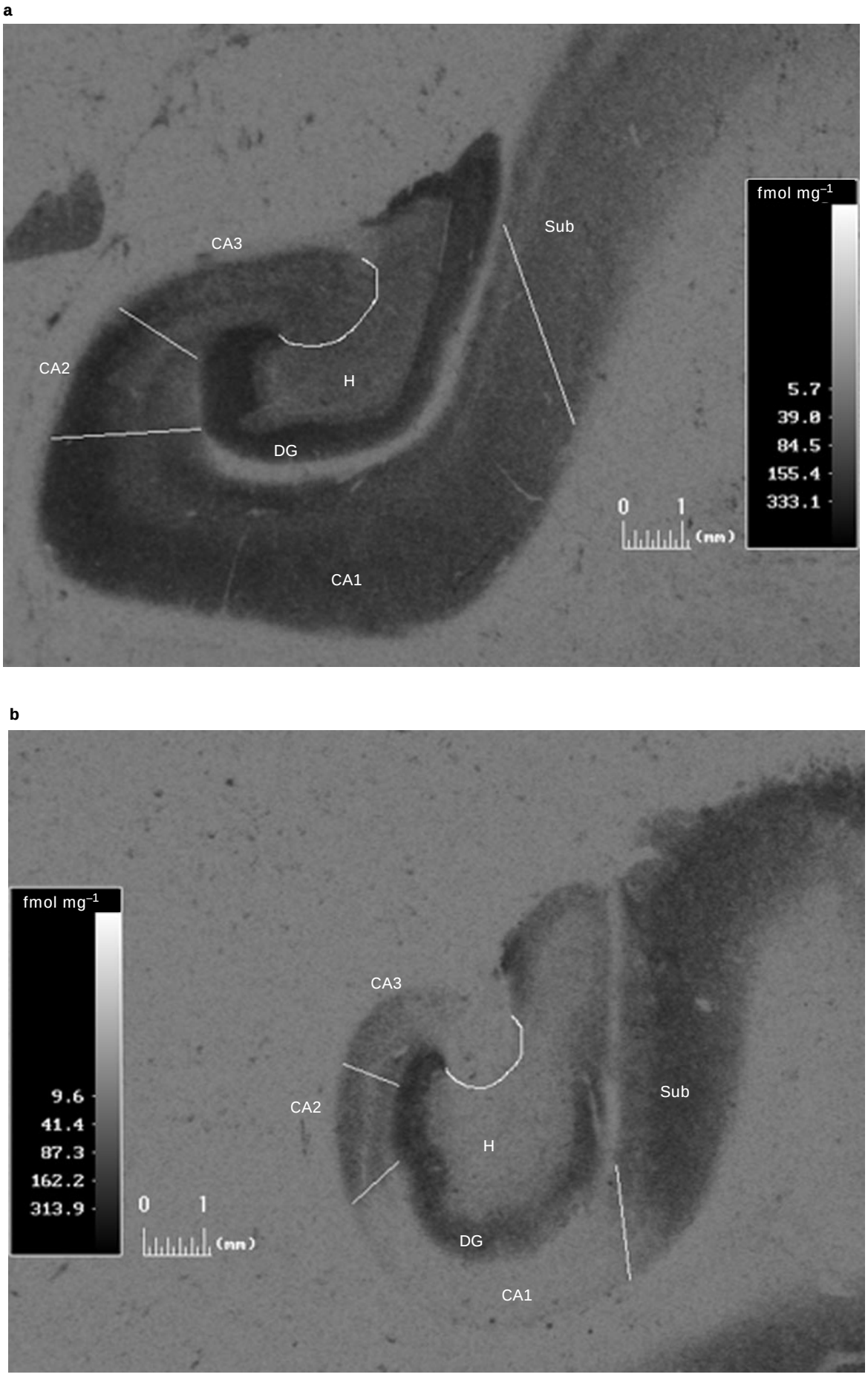

Figure 2 Example of an autoradiographic image of [^{3}H]-flumazenil binding (approximately 5 nM) in a 10 μm hippocampal section taken from a control subject (a) and from a patient with hippocampal sclerosis (b). Abbreviations: DG - dentate gyrus; Sub - Subiculum; H - Hilus.

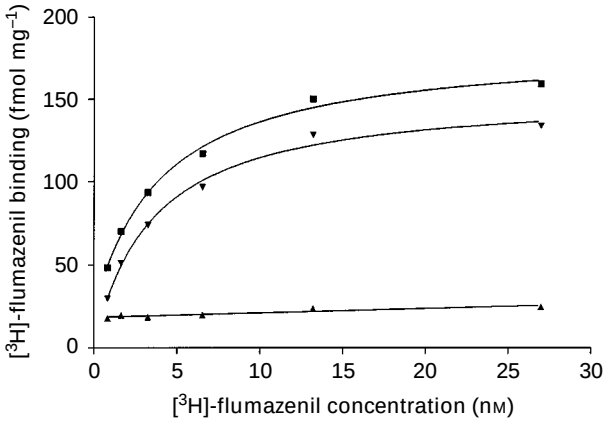

Figure 3 Example of a saturation radioligand binding plot for [^{3}H]-flumazenil in the CA3 region of an autopsy control hippocampus. Following a 60 min prewash, slide-mounted 10 μm sections were incubated for 60 min with a series of differing concentrations of [^{3}H]-flumazenil ranging from 0.25 nM to 20 nM in Tris-HCl buffer pH 7.4 at 0–4°C. Non-specific binding was determined in the presence of 2 μM clonazepam. Slides were subsequently washed in fresh ice-cold buffer, dried and apposed to tritium sensitive film for 21 days. Film optical density for each subregion were obtained by computer-assisted densitometry, by a MCID M4 image analysis system (Imaging Research Inc.). Data were analysed by non-linear regression with an interative curve-fitting computer software package (Graph-Pad Software, San Diego) and a standard two-variable Langmuir equation. Total binding is represented by solid squares, specific binding by inverted solid triangles and non-specific binding by solid triangles. Each point represents the mean of measurements from 2–4 sections (total) and 1–2 sections (non-specific).

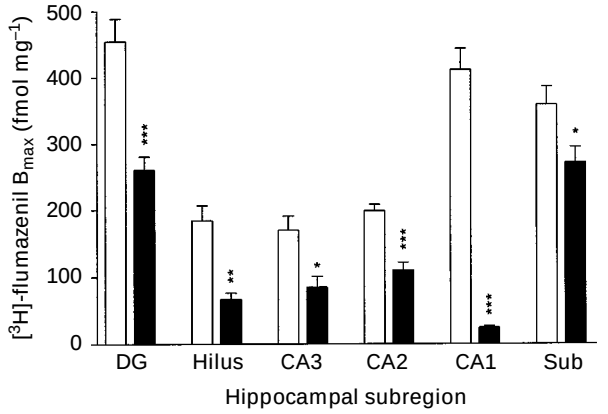

Figure 4 Mean receptor autoradiography radioligand binding density values (B_{max}, expressed as fmol [^{3}H]-flumazenil mg^{-1} tissue dry weight) for subregions of sclerotic hippocampi (solid columns, DG $n=8$, Hilus $n=8$, CA3 $n=5$, CA2 $n=5$, CA1 $n=7$, Sub $n=8$) and autopsy controls (open columns, all areas $n=6$). Error bars represent s.e.mean. Abbreviations: DG = dentate gyrus, Sub = subiculum. Statistical analysis: two-tailed Student's t test (***$P<0.001$. **$P<0.01$, *$P<0.05$).

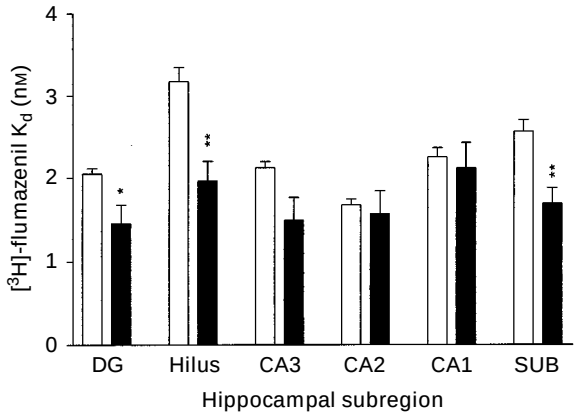

Figure 5 Mean dissociation constant (K_d) values for [^{3}H]-flumazenil binding, as determined by receptor autoradiography, for subregions of sclerotic hippocampi (solid columns, DG $n=8$, Hilus $n=8$, CA3 $n=5$, CA2 $n=5$, CA1 $n=7$, Sub $n=8$) and autopsy controls (open columns, all areas $n=6$). Error bars represent s.e.mean. Abbreviations: DG = dentate gyrus, Sub = subiculum. Statistical analysis: two-tailed Student's t test (**$P<0.01$, *$P<0.05$).

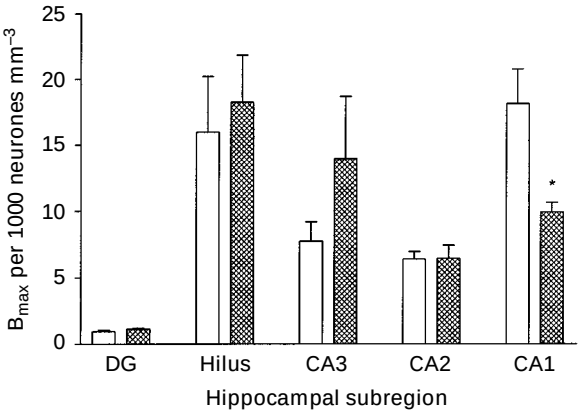

Figure 6 Central benzodiazepine receptor binding ([^{3}H]-flumazenil, fmol mg^{-1} tissue dry weight) expressed as a ratio of neuronal density (neurones mm^{-3}) for regions of hippocampal sclerosis (hatched columns, DG $n=7$, Hilus $n=7$, CA3 $n=4$, CA2 $n=4$, CA1 $n=6$) and control hippocampi (open columns, all areas $n=5$). Error bars represent s.e.mean. Abbreviations: DG = dentate gyrus. Statistical analysis: two-tailed Student's t test (*$P<0.05$).

influence of post-mortem delay, age, gender or *pre-mortem* BZ treatment on [^{3}H]-flunitrazepam binding in human brain. We cannot therefore conclude that the significant difference between the median age of the control and HS groups in the present study did not influence the findings. The influence of chronic medication on receptor binding and affinity must also be considered when interpreting data obtained from human studies. Although the patients selected for this study were receiving chronic anti-epileptic medication not currently thought to act primarily at the $GABA_A$/BZ complex, it is possible that cBZR density and/or affinity may be affected indirectly by the anti-epileptic actions of these drugs. It is not feasible to carry out studies on patients with epilepsy not receiving medication and therefore difficult to assess the influence medication may have on our results. However, flumazenil B_{max} and K_d have been shown to be unaffected by the presence of allosteric modulators at the $GABA_A$ receptor and GABA agonists in the acute instance (Mohler & Richards, 1981). Low non-specific binding, lack of sensitivity to modulation by endogenous GABA and selectivity for neuronal BZ receptors render [^{3}H]-flumazenil and extremely useful and appropriate ligand for receptor autoradiography studies of this type. An anomaly inherent in this method of analysis is the failure to consider the influence of presynaptic $GABA_A$ receptors and possible pathological changes in density of GABAergic terminals in the hippocampal subregions examined. Decreased GABAergic nerve endings have been observed at the epileptic focus in the alumina cream model, by use of glutamate decarboxylase (GAD) immunohistochemical methods in monkey sensorimotor cortex (Ribak *et al*., 1979) and further studies of presynaptic GABA markers are being pursued in order to clarify this issue further.

It has been shown, by use of positron emission tomography with [^{11}C]-flumazenil, that cBZR binding is reduced *in vivo* in patients with HS-associated TLE (Koepp *et al*., 1996). A loss of receptor density on surviving CA1 neurones, in addition to loss of cBZRs due to overall neuronal loss, clarifies the basis for these PET findings. These results are consistent with recent data from our group demonstrating that loss of [^{11}C]-flumazenil PET binding is more significant than changes in hippocampal volume measures on magnetic resonance imaging (MRI), in HS (Koepp *et al*., 1995). These data suggest, therefore, that [^{11}C]-flumazenil PET may be sensitive enough to detect abnormalities *in vivo* when structural imaging by MRI is normal.

Overall losses of cBZRs and reduced density of cBZRs on surviving neurones in the CA1 region in particular, may indicate a functional deficit of GABAergic inhibitory transmission in the hippocampus of patients with HS-associated TLE. It is unclear at present whether changes in cBZR binding and affinity seen in HS are a cause or consequence of epileptogenesis. Reduced levels of a putative competing endogenous ligand at the central BZ receptor in the HS group may explain the increases in affinity observed in this study. Altered gene expression may also provide an explanation for observed changes in affinity. Changes in affinity for [^{3}H]-flumazenil at the $GABA_A$ receptor have been demonstrated by different subunit combinations expressed in *Xenopus* oocytes (Ymer *et al*., 1990). Long lasting changes in the expression of mRNA for various subunits of the $GABA_A$ receptor have been demonstrated in the kindling model of TLE (Kamphuis *et al*., 1995) and loss of the $GABA_A$ α1 subunit has been demonstrated immunohistochemically in epileptic human hippocampus. However, this has been proposed to occur as a result of neuronal loss (Wolf *et al*., 1994). The question of whether neuronal loss in HS precedes the initiation of seizures or develops as a result of seizure-induced excitotoxicity is not yet answered. It is of note that infusion of an antisense of oligonucleotide to the γ2 subunit of the $GABA_A$ receptor into the hippocampus of adult rats was associated with loss of neurones and a loss of central BZ binding (Karle *et al*., 1995). Whether altered BZ binding and [^{3}H]-flumazenil affinity, demonstrated in the current study, represent altered subunit composition of the $GABA_A$ receptor, such as a loss of the γ subunit in HS is a subject of ongoing *in situ* hybridization studies in our group.

In summary, this study has demonstrated significant loss of cBZRs, as determined by saturation autoradiography, in all hippocampal subregions of patients with TLE associated with HS. More importantly, we have shown a clear reduction in cBZR density per remaining neurone in the CA1 region of the sclerotic hippocampus, and a significant increase in affinity for [^{3}H]-flumazenil at cBZRs in the DG, hilus and subiculum subregions of the hippocampus in these patients with HS and TLE. Together, these findings may have considerable implications for the understanding of the pathogenesis of HS. The identification of a particular subunit or subunits of the $GABA_A$ receptor involved in these changes in receptor characteristics may facilitate the development of more selective anti-epileptic agents and ligands for *in vivo* functional imaging.

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References

BABB, T.L., PRETORIUS, J.K., KUPFER, W.R. & CRANDALL, P.H. (1989). Glutamate decarboxylase-immunoreactive neurons are preserved in human epileptic hippocampus. *J. Neurosci*., **9,** 2562 – 2574.

BURDETTE, D.E., SAKURAI, S.Y., HENRY, T.R., ROSS, D.A., PENNELL, P.B., FREY, K.A., SACKELLARES, J.C. & ALBIN, R.L. (1995). Temporal lobe central benzodiazepine binding in unilateral mesial temporal lobe epilepsy. *Neurology*, **45,** 934 – 941.

DURING, M.J., RYDER, K.M. & SPENCER, D.D. (1995). Hippocampal GABA transporter function in temporal-lobe epilepsy. *Nature*, **376,** 174 – 177.

DURING, M.J. & SPENCER, D.D. (1993). Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. *Lancet*, **341,** 1607 – 1610.

FAULL, R.L. & VILLIGER, J.W. (1988). Benzodiazepine receptors in the human hippocampal formation: a pharmacological and quantitative autoradiographic study. *Neuroscience*, **26,** 783 – 790.

HAND, K.S.P., BOWERY, N.G., VAN PAESSCHEN, W. & DUNCAN, J. (1996). Central benzodiazepine receptor autoradiography in human resected epileptic temporal lobe: changes in receptor density and affinity. *Br. J. Pharmacol*., **119,** 66P.

HOUSER, C.R., OLSEN, R.W., RICHARDS, J.G. & MOHLER, H. (1988). Immunohistochemical localisation of benzodiazepine/$GABA_A$ receptors in the human hippocampal formation. *J. Neurosci*., **8,** 1370 – 1383.

ISOKAWA, M., AVANZINI, G., FINCH, D.M., BABB, T.L. & LEVESQUE, M.F. (1991). Physiologic properties of human dentate granule cells in slices prepared from epileptic patients. *Epilepsy Res*., **9,** 242 – 250.

JOHNSON, E.W., DE LANEROLLE, N.C., KIM, J.H., SUNDARESAN, S., SPENCER, D.D., ZOGHBI, S.S., BALDWIN, R.M., HOFFER, P.B., SEIBYL, J.P. & INNIS, R.B. (1992). 'Central' and 'peripheral' benzodiazepine receptors: opposite changes in human epileptogenic tissue. *Neurology*, **42,** 811 – 815.

KAMPHUIS, W., DERIJK, T.C. & DASILVA, F.H.L. (1995). Expression of GABA(A) receptor subunit mRNAs in hippocampal pyramidal and granular neurons in the kindling model of epileptogenesis: An in situ hybridisation study. *Mol. Brain Res*., **31,** 33 – 47.

KAPUR, J., LOTHMAN, E.W. & DELORENZO, R.J. (1994). Loss of GABA(A) receptors during partial status epilepticus. *Neurology*, **44,** 2407 – 2408.

KARLE, J., WITT, M.R. & NIELSEN, M. (1995). Antisense oligonucleotide to GABA(A) receptor gamma 2 subunit induces loss of neurons in rat hippocampus. *Neurosci. Lett*., **202,** 97 – 100.

KNOWLES, W.D., AWAD, I.A. & NAYEL, M.H. (1992). Differences of in vitro electrophysiology of hippocampal neurons from epileptic patients with mesiotemporal sclerosis versus structural lesions. *Epilepsia*, **33,** 601 – 609.

KOEPP, M.J., RICHARDSON, M.P., BROOKS, D.J., FREE, S., SISODIYA, S. & DUNCAN, J.S. (1995). [^{11}C]Flumazenil PET and volumetric MRI in mesial temporal lobe epilepsy. *Epilepsia*, **36,** S167 (Abstract).

KOEPP, M.J., RICHARDSON, M.P., BROOKS, D.J., POLINE, J.B., VAN PAESSCHEN, W., FRISTON, K.J. & DUNCAN, J.S. (1996). Cerebral benzodiazepine receptors in hippocampal sclerosis – An objective in vivo analysis. *Brain*, **119,** 1677 – 1687.

LLOYD, K.G. & DREKSLER, S. (1979). An analysis of [3H]gamma-aminobutyric acid (GABA) binding in the human brain. *Brain Res*., **163,** 77 – 87.

MCDONALD, J.W., GAROFALO, E.A., HOOD, T., SACKELLARES, J.C., GILMAN, S., MCKEEVERPE, TRONCOSO, J.C. & JOHNSTON, M.V. (1991). Altered excitatory and inhibitory amino acid receptor binding in hippocampus of patients with temporal lobe epilepsy. *Ann. Neurol*., **29,** 529 – 541.

MELDRUM. B. (1979). Convulsant drugs, anticonvulsants and GABA-mediated neuronal inhibition. In *GABA-Neurotransmitters*. ed. Krogsgaard-Larsen, P., Scheel-Kruger, J. Kofod, H. pp. 390 – 405. Copenhagen: Munksgaare.

MOHLER, H. & RICHARDS, J.G. (1981). Agonist and antagonist benzodiazepine receptor interaction in vitro. *Nature*, **294,** 763 – 765.

OLSEN, R.W., WAMSLEY, J.K., LEE, R.J. & LOMAX, P. (1986). Benzodiazepine/barbiturate/GABA receptor-chloride ionophore complex in a genetic model for generalised epilepsy. *Adv. Neurol*., **44,** 365 – 378.

OLSEN, R.W., BUREAU, M., HOUSER, C.R., DELGADO-ESCUETA, A.V., RICHARDS, J.G. & MOHLER, H. (1992). GABA/benzodiazepine receptors in human focal epilepsy. *Epilepsy Res. Suppl.*, **8,** 383–391.

PRATT, G.D., RICHTER, A., MOHLER, H. & LOSCHER, W. (1995). Regionally selective and age-dependent alterations in benzodiazepine receptor binding in all genetically dystonic hamster. *J. Neurochem.*, **64,** 2153–2158.

RIBAK, C.E., HARRIS, A.B., VAUGHN, J.E. & ROBERTS, E. (1979). Inhibitory, GABAergic nerve terminals decrease at sites of focal epilepsy. *Science*, **205,** 211–214.

SCHMIDT, D., CORNAGGIA, C. & LOSHER, W. (1984). Comparative studies of the GABA system in neurosurgical brain specimens of epileptic and non-epileptic patients. In *Neurotransmitters, Seizures and Epilepsy, II*, pp. 275–282. New York: Raven Press.

WILLIAMS, R.W. & RAKIC, P. (1988). Three-dimensional counting: an accurate and direct method to estimate numbers of cells in sectioned materials. *J. Comp. Neurol.*, **278,** 344–352.

WOLF, H.K., SPANLE, M., MULLER, M.B., ELGER, C.E., SCHRAMM, J. & WIESTLER, O.D. (1994). Hippocampal loss of the GABA(A) receptor alpha(1) subunit in patients with chronic pharmacoresistant epilepsies. *Acta Neuropathol.*, **88,** 313–319.

YMER, S., DRAGUHN, A., WISDEN, W., WERNER, P., KEINANEN, K., SCHOFIELD, P.R., SPRENGEL, R., PRITCHETT, D.B. & SEEBURG, P.H. (1990). Structural and functional characterisation of the gamma 1 subunit of $GABA_A$/benzodiazepine receptors. *EMBO J.*, **9,** 3261–3267.

ZEZULA, J., CORTES, R., PROBST, A. & PALACIOS, J.M. (1988). Benzodiazepine receptor sites in the human brain: autoradiographic mapping. *Neuroscience*, **25,** 771–795.

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