In vivo demonstration of altered benzodiazepine receptor density in patients with generalised epilepsy

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Abstract
Electrophysiological data suggest that an abnormal oscillatory pattern of discharge in cortical and thalamic neurons may be the major mechanism underlying primary generalised epilepsy. No neurochemical or anatomical substrate for this theory has hitherto been demonstrated in humans and the pathophysiology of primary generalised epilepsy remains unknown. By means of PET and the benzodiazepine (BZ) ligand [^{11}C]flumazenil it has been previously shown that the BZ receptor density is reduced in the epileptic foci of patients with partial epilepsy. In the present study the method was further developed and used in a comparative analysis of cortical, cerebellar, and subcortical BZ receptor binding in patients with primary generalised tonic and clonic seizures (n = 8), and healthy controls (n = 8). Patients with generalised seizures had an increased BZ receptor density in the cerebellar nuclei (p = 0·006) and decreased density in the thalamus (p = 0·003). No significant changes were seen in the cerebral and cerebellar cortex or in the basal ganglia. The observed alterations suggest that the γ-amino-butyric acid (GABA)-BZ system may be affected in the cerebello-thalamo-cortical loop of patients with generalised epilepsy and indicate possible targets for selective pharmacological treatment.

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Generalised epilepsy is a common, yet enigmatic disease. Our knowledge about its pathophysiology is based mainly on electrophysiological experiments, the results of which have led to the centrencephalic[1] and the corticoreticular theories.[2] The first attributes generalised epilepsy to pathological changes in the thalamic nuclei, whereas the second postulates that ascending impulses from the thalamus impinge on a diffusely hyperexcitable cortex, inducing the generalised epileptic discharges. Thus in both theories a neuroanatomical substrate is hypothesised (thalamus, cerebral cortex), but no such substrate has hitherto been shown in humans.

The major factors underlying the proposed disturbances in the thalamocortical oscillations could be changes in the excitation, inhibition, or synchronisation of the involved neuronal circuits. The major inhibitory neurotransmitter in the human brain is γ-aminobutyric acid (GABA). Its action is mediated by the GABA$_A$-benzodiazepine (BZ) receptor complex.

With the PET technique we recently showed that the measured BZ receptor density was reduced in the epileptic foci of patients with partial epilepsy.[3 4] In a follow up study on patients with primary generalised epilepsy no similar reductions were demonstrated, but in comparison with patients with partial epilepsy there was a non-significant tendency towards lower mean cortical BZ receptor density.[5] The receptor affinity was not changed. These findings encouraged further studies on BZ receptor density in generalised epilepsy giving special attention not only to the cortical, but also subcortical and cerebellar structures.[6 7]

Methods
SUBJECTS
Eight patients, aged 30 (SD 8) years, with primary generalised tonic and clonic seizures according to the International Classification of Epilepsies[8 9] were studied. The patients had generalised tonic and clonic seizures without any signs of partial onset and with immediate alteration of consciousness. The seizures could occur at any time of the day. There were no signs of mental retardation, myoclonic syndromes, or cerebellar dysfunction, and no history of epileptic status. Apart from having epilepsy, the patients were healthy. Three conventional scalp EEG recordings (10/20 system) were performed on each patient which showed generalised bilaterally synchronous, symmetrical, and sometimes irregular spike and wave complexes. The CT scans and magnetic resonance tomographies (MRT) were normal. The current medication was phenytoin and/or carbamazepine, drugs considered not to interact with the flumazenil binding to the GABA$_A$-BZ receptor complex.[10] There was no history of phenytoin or carbamazepine intoxication and plasma concentrations were within the recommended therapeutic range. Chronic phenytoin treatment, however, may have a toxic effect on Purkinje cells and cause alterations in cerebellar BZ receptors.[11] Thus if cerebellar changes were observed, it would be difficult to decide whether they were related to generalised epilepsy or to a possible phenytoin toxicity. Therefore, for the assessment of possible phenytoin-related changes in the cerebellar

BZ receptor binding, nine phenytoin treated patients with partial epilepsy served as a control group supplementary to the healthy subjects.

The patients with partial epilepsy were aged 34 (SD 8) years, had histories of phenytoin treatment, and phenytoin doses that were compatible with those of patients with generalised seizures. Three patients had frontal foci, five temporal, and one had an occipital focus. Four of the foci were left-sided and five right-sided.

The healthy controls consisted of eight male volunteers, aged 30(SD 5) years, with normal physical state, routine laboratory tests, CT scans, and EEGs. They had no hereditary predisposition for epilepsy and no history of benzodiazepine, phenobarbitone, carbamazepine, or phenytoin treatment. They were free from medication for at least 10 weeks before the experiments. The study was approved by the local ethics and radiation safety committees.

EXPERIMENTAL PROCEDURE

The PET studies were performed on a PC 384–7B system (General Electrics, USA), which measures the brain radioactivity in seven 10 mm slices with spatial resolution of 7·6 mm.[12] The attenuation correction was performed with contour finding.[12]

The MRT examinations were performed on a 0·5 Tesla Siemens Magnetom instrument with spatial resolution of 1·5 mm and slice thickness of 10 mm. T1-weighted, T2-weighted and inversion recovery images were obtained for each patient. The spacing between slice centres was 13·5 mm during both CT scans and MRTs to comply with the orientation of the slices of the PET camera. Two scanning series were performed with 6·75 mm axial displacement to obtain more detailed anatomical information. In all tomographic studies a stereotaxic helmet fixation of the head was used.[13] This device made it possible to select comparable imaging planes for the PET scans in all subjects and to avoid artifacts due to head movement. The subjects were positioned so that the cerebellum and the thalamus were optimally disclosed. The patients were examined interictally and at least 32 hours after the last seizure. During each PET scan (56 minutes) EEG was recorded continuously from four scalp electrodes.

The BZ receptors were studied with [^{11}C] flumazenil,[14] a selective and non-subtype specific BZ receptor antagonist.[15 16] The kinetics and binding properties of this well established PET ligand have been described earlier.[3–5 17–22] Each subject received two intravenous bolus injections of [^{11}C]flumazenil (5 mCi), with high (100–503 Ci/mmol) and low (about 1 Ci/mmol) specific activity preparations, respectively. The concentration of [^{11}C]flumazenil and its metabolites in the arterial plasma was determined from automatically and manually withdrawn blood samples.[21 23]

COMPUTERISED ANATOMICAL BRAIN ATLAS

For the anatomical identification of the PET data the individually adjustable computerised anatomical brain atlas of Bohm and Greitz was used.[24] In each subject the atlas was first transformed to conform with the anatomy of the subject to be studied. The transformation was made by an interactive fit of the contours of the standard atlas brain to the subject's MRT/CT images using special indices for transformation of the size and form of the brain. After this procedure the anatomical structures were retrieved directly from the data base and presented on the PET images (see later).

QUANTIFICATION OF REGIONAL BZ RECEPTOR BINDING

Original B_{max} images

The BZ receptor density (B_{max}) was calculated with a kinetic compartment model[17] in which the radioligand in the tissue is considered to be in two pools, either unbound or specifically bound. Here, no distinction is made between the pools of non-specific binding and truly free (F) radioligand. The estimate of B_{max} is, however, not affected by this simplification, because this variable is determined by the time courses of the specifically bound and unbound ligand. By contrast, the estimates of the equilibrium dissociation constant, K_d (not presented here) depend strongly on the fraction of non-specific binding in the pool of unbound radioligand. White matter (devoid of BZ receptors) was used as a reference region to estimate the time course of unbound ligand (free-non-specific).[3 4 5 22] The data between 10 and 50 minutes were used in the algorithm both for the reference and the target (receptor containing) region. The B_{max} values were quantified pixel by pixel, enabling construction of B_{max} images showing regional distribution of the estimated BZ receptor density. The error in regional B_{max} estimation was for small regions such as the thalamus, calculated to be below 10%.[17]

Reformatted B_{max} images

The reformation of individual B_{max} images was applied to obtain PET images showing the mean B_{max} values (pixel by pixel) of the patients with generalised epilepsy and controls, respectively (so called averaged images). For this purpose, each subject's set of B_{max} images was, by inversion of the individual transformation variables, reformatted into a corresponding set of reformatted images that were anatomically identical with the standard atlas brain, but where the original regional B_{max} values remained unchanged.[25] As all the brains thereby became anatomically alike, it was possible to construct averaged B_{max} images for the groups for comparison. Averaged B_{max} images were not constructed in patients with partial epilepsy, because they did not represent a homogeneous population, due to varying localisation of the epileptic foci.

Subtraction images

By subtraction of the averaged B_{max} images from each other, a new set of PET images (so

Table 1   Mean region/pons and region/white matter ratios (SD) based on [¹¹C]flumazenil uptake images

Region	Region/pons ratios		Region/white matter ratios	
	Generalised epilepsy	Healthy controls	Generalised epilepsy	Healthy controls
Semilunar cerebellar lobe	4·3(1·2)	4·6(1·0)	4·5(0·9)	4·5(1·1)
Quadrangular cerebellar lobe	4·3(1·2)	4·6(1·0)	4·5(0·9)	4·5(1·1)
Cerebellar nuclei	2·4(0·7)	1·7(0·3)	2·4(0·8)	1·6(0·5)
Cortex	4·8(1·0)	6·3(1·2)	5·0(1·0)	6·2(1·2)
Amygdala + hippocampus	3·8(0·9)	4·8(1·2)	3·9(0·9)	4·7(1·2)
Thalamus	2·3(0·9)	3·5(0·7)	2·4(0·8)	3·2(0·4)
Caudate + putamen	3·0(1·0)	3·0(0·6)	2·8(0·9)	2·8(0·4)

The ratios are based on the uptake images representing average concentration of [¹¹C]flumazenil between 10 and 56 minutes after the high specific activity bolus. The differences are not statistically significant after the Bonferroni correction (p = 0·028 for the thalamus and p = 0·076 for the cerebellar nuclei).

called subtraction images) was obtained. As the computer program allowed only positive values, the subtraction was performed in both directions (controls—patients with generalised epilepsy, and patients with generalised epilepsy—controls). This procedure yielded an overview of the regional increases as well as decreases in the calculated BZ receptor density in the patients with generalised epilepsy. Statistical evaluation was based on individual B_{max} values from each investigated region of interest. The subtraction images were, however, important for the visual analysis of possible alterations in BZ receptor density outside the predefined regions of interest.

ANALYSIS OF REGIONAL DIFFERENCES IN BZ RECEPTOR BINDING

The comparisons between patients with generalised epilepsy and controls were performed on regions of interest in the following (tables 1 and 2):

(1) the mesial temporal lobe consisting of amygdala and hippocampus.

(2) the thalamus; due to limited spatial resolution of the camera this region of interest consisted of all the thalamic nuclei including the thalamic reticular nucleus where BZ receptors are sparse.

(3) the caudate + putamen.

(4) the cerebellar nuclei (including the dentate and emboliform nuclei—the two nuclei involved in the cerebellocerebral connections).

(5) the quandrangular cerebellar lobe (including the anterior and posterior part).

(6) the semilunar cerebellar lobe (including the superior and inferior part).

(7) the big, multilobar cortical region.[4]

Extremely small structures, such as the substantia nigra, were not investigated due to the limited spatial resolution of the PET camera.

In the patients with partial epilepsy only the cerebellar regions were analysed.

Each region of interest was delineated over the contours of the corresponding atlas structure and was congruent with it. The atlas structures were retrieved from the data base of the atlas program and presented directly on three types of PET images: (1) the [¹¹C]flumazenil uptake images, representing average, and decay corrected, radioligand concentration between 10 and 56 minutes after injection of the tracer dose. The value from each region of interest was divided with the value from the corresponding white matter and pons (another reference region for non-receptor bound flumazenil).[17 19 26] This procedure enabled a preliminary evaluation of regional differences between the groups based on the raw data images—that is, before application of any mathematical model; (2) the original B_{max} images; (3) the reformatted B_{max} images. The reason for including both the original and reformatted B_{max} images was to obtain a quality control of the reformation method.

STATISTICS

The comparisons between patients with generalised epilepsy and healthy controls were based on the individual original as well as reformatted B_{max} images; the comparisons with patients with partial epilepsy were confined to the original B_{max} images, as the reformatted images were not constructed in this group. Possible side differences in each group and each region were tested with paired t tests. Comparisons between healthy subjects and patients with generalised epilepsy were based on the mean values from the right and left side. In patients with partial epilepsy each region of interest was defined as contralateral or ipsilateral in relation to the focus site (table 2). Values from both contralateral and ipsilateral regions of interest were included in the statistical analyses.

The regional differences between the groups were evaluated with the Mann-Whitney U test and Bonferroni correction. A p value below 0·007 was considered as statistically significant.

Results

The plasma metabolites (47(12)% after 36 minutes) and the elimination $T_{1/2}$ (27(8) minutes) in patients with generalised epilepsy were not significantly different from the

Table 2   Mean (SD) of regional B_{max} values (pmol/ml) in patients and controls

Region	Generalised epilepsy (n = 8)		Healthy controls (n = 8)		Partial epilepsy (n = 9) Reformatted B_{max}	
	Original B_{max}	Reformatted B_{max}	Original B_{max}	Reformatted B_{max}	Ipsilateral	Contralateral
Semilunar cerebellar lobe	29(12)	29(7)	27(10)	28(8)	27(16)	25(12)
Quadrangular cerebellar lobe	29(13)	33(10)	26(7)	32(6)	29(18)	24(16)
Cerebellar nuclei	16(12)*	16(7)	5(3)	6(3)	6(4)	9(5)
Cortex	40(8)	41(10)	54(13)	57(10)		
Amygdala + hippocampus	12(5)*	16(7)	23(10)	28(12)		
Thalamus	2(1)*	3(1)	6(2)	7(3)		
Caudate + putamen	7(5)	7(4)	8(4)	7(5)		

*Significant differences between patients and controls. For generalised epilepsy and controls the values are from the means of the two sides. The values from reformatted and non-formatted B_{max} images are similar despite a slightly different procedure (see text).

corresponding indices in the healthy subjects.[21]

The regional B_{max} values from reformatted and original B_{max} images corresponded, which supported the previously reported accuracy of the reformation process.[25] No significant asymmetries were found in any of the analysed regions in the three groups.

In relation to the healthy controls, the patients with generalised epilepsy had a high cerebellar nuclei/pons ratio and low thalamus/pons ratio (table 1). The same was valid also for the corresponding regions of interest/white matter ratios (table 1). Also the results from quantified data were in the same direction: Patients with generalised epilepsy had significantly increased B_{max} values (p = 0·006) in the cerebellar nuclei, and significantly reduced B_{max} values in the thalamus (p = 0·003; fig 1 and table 2). No significant differences were seen in the other investigated regions of interest (table 2).

The averaged and subtraction images showed no pronounced alterations of the B_{max} values outside the predefined regions of interest.

There were no significant differences in the regional B_{max} values between patients with more than one seizure monthly (n = 4) and patients with less than one seizure monthly (n = 4) (Mann-Whitney U test).

As pointed out in the methods, in patients with partial epilepsy only the cerebellar structures were analysed. These patients showed no significant differences from the controls in any of the cerebellar regions of interest, including both sides (table 2).
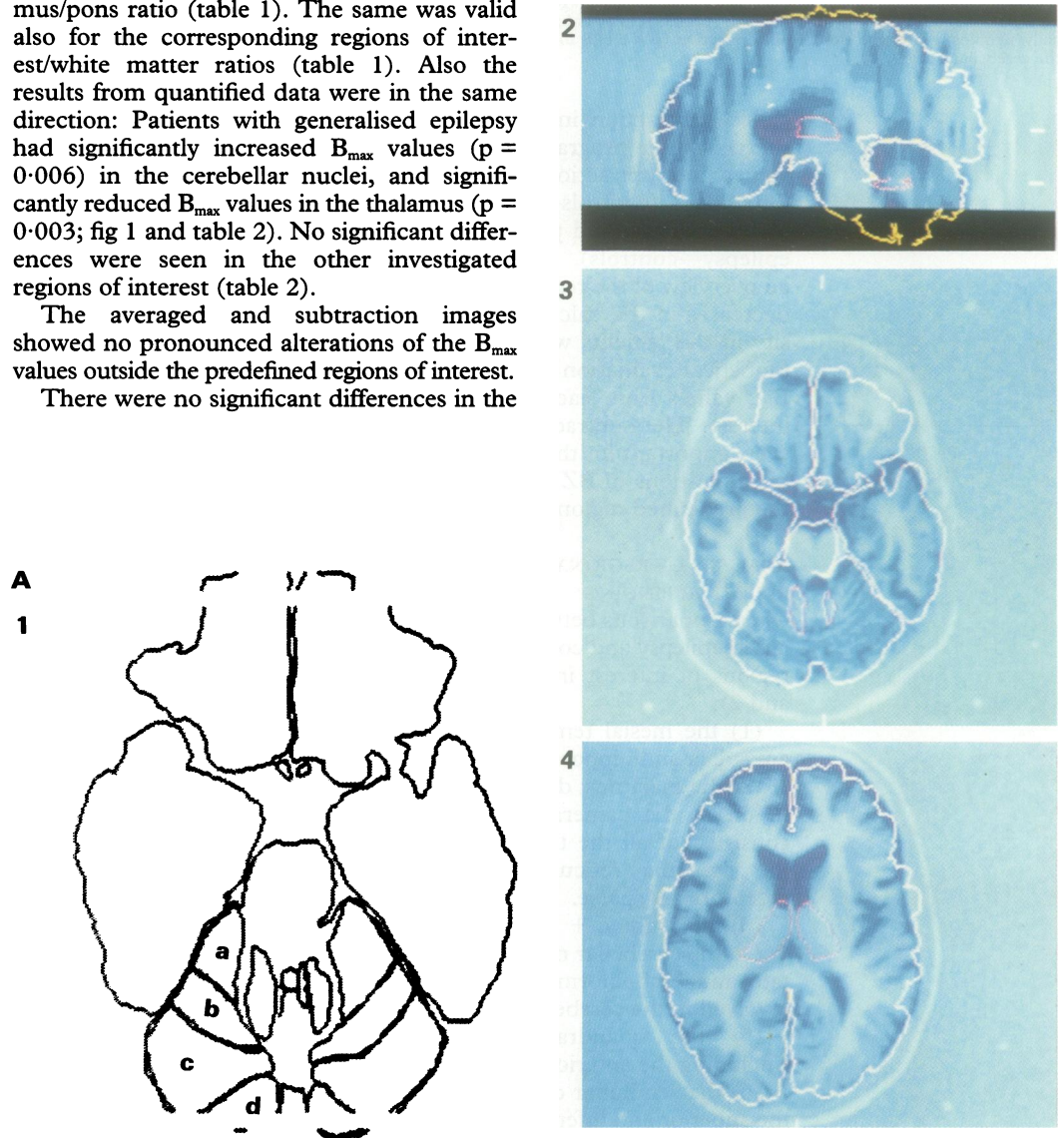

Figure 1 (A) 1: Various structures in cerebellum retrieved from the atlas program; (a) anterior quadrangular lobe; (b) posterior quadrangular lobe; (c) superior semilunar lobe; (d) inferior semilunar lobe; the dentate, emboliform, and fastigius nuclei are in the middle. 2–4: MRT images (sagittal and horizontal sections) with atlas contours. The thalamus and dentate nuclei are indicated. White lines outside the brain indicate the level of horizontal sections of the PET images (see fig 1B). (B) 1–4: Average B_{max} images of controls (Av1 and Av3) and patients with generalised epilepsy (Av2 and Av4). Each pixel value represents the mean of the corresponding pixel values from the individual reformatted images within the group (see methods). (C) 1–4: Corresponding subtraction images. Images Sub 1 and Sub 3 were obtained as averaged B_{max} images of controls—averaged B_{max} images of patients with generalised epilepsy. The procedure for the images Sub 2 and Sub 4 was the reverse. In both av and sub images the dentate nuclei and the thalamus were retrieved directly from the atlas program. The reason for the slight asymmetry in the dentate nuclei is that the postmortem brain that the atlas program is based on was slightly asymmetrical in the posterior part. As pointed out in the results, no systematic asymmetries were found in the patients or controls. Note that in both B and C the colour code denotes only positive values. Thus in C only the relative increases are shown. Therefore, the subtractions were performed both ways and the decreases as well as increases in BZ-B_{max} of patients with generalised epilepsy were visualised. The subtraction images (C 1–4) show the numerical (pmol/ml) differences between the groups. Thus although the numeric differences are lower in the thalamus than in the cortex, the significance level is reached only in the former region as the thalamus has lower BZ receptor density than the cortex. The same is valid for the seemingly increased density in the left quadrangular lobe in the patients (image C2); this difference was not significant. The colours denote approximate pmol/ml values (the first value is for images B 1–4 and C 3–4 and the second value for images C 1–2) as follows: Black 0, 0; dark blue 15, 10; light blue 30, 20; green 40, 30; yellow 65, 40; orange 80, 50.
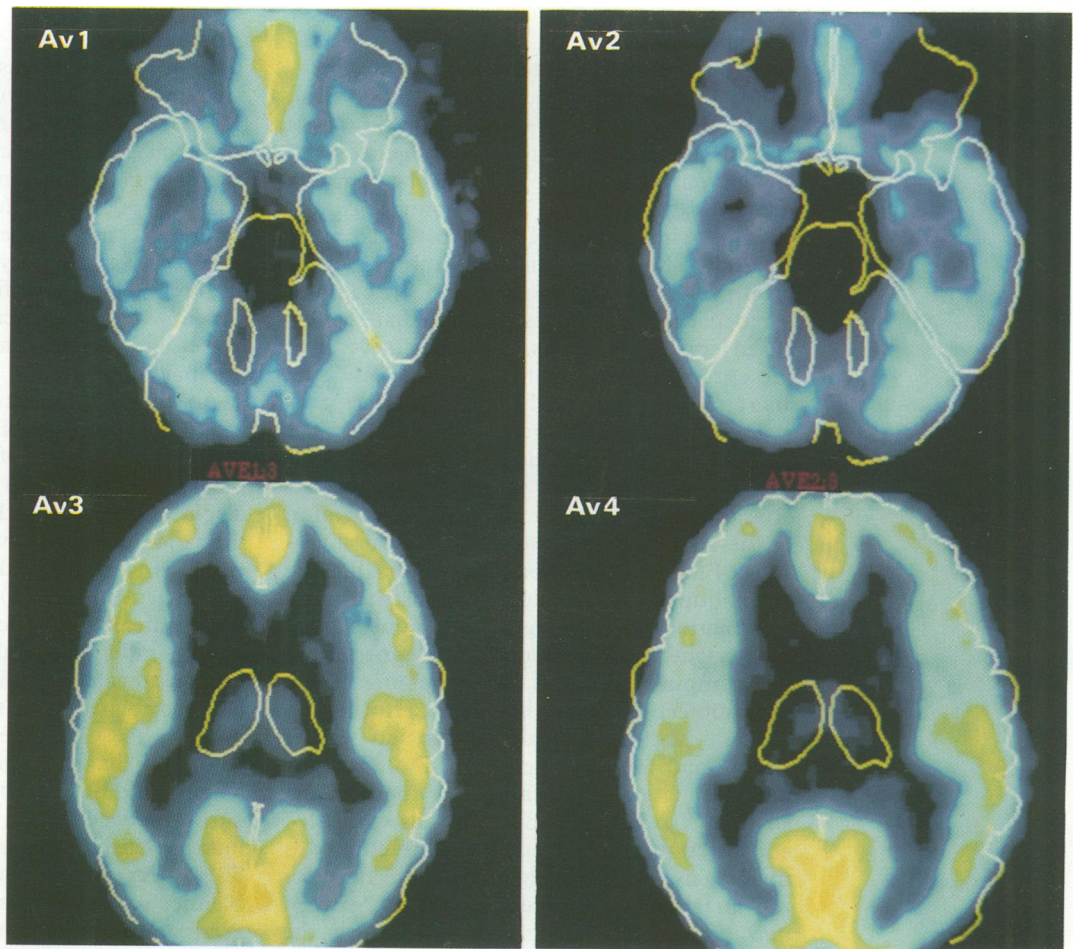
(B)
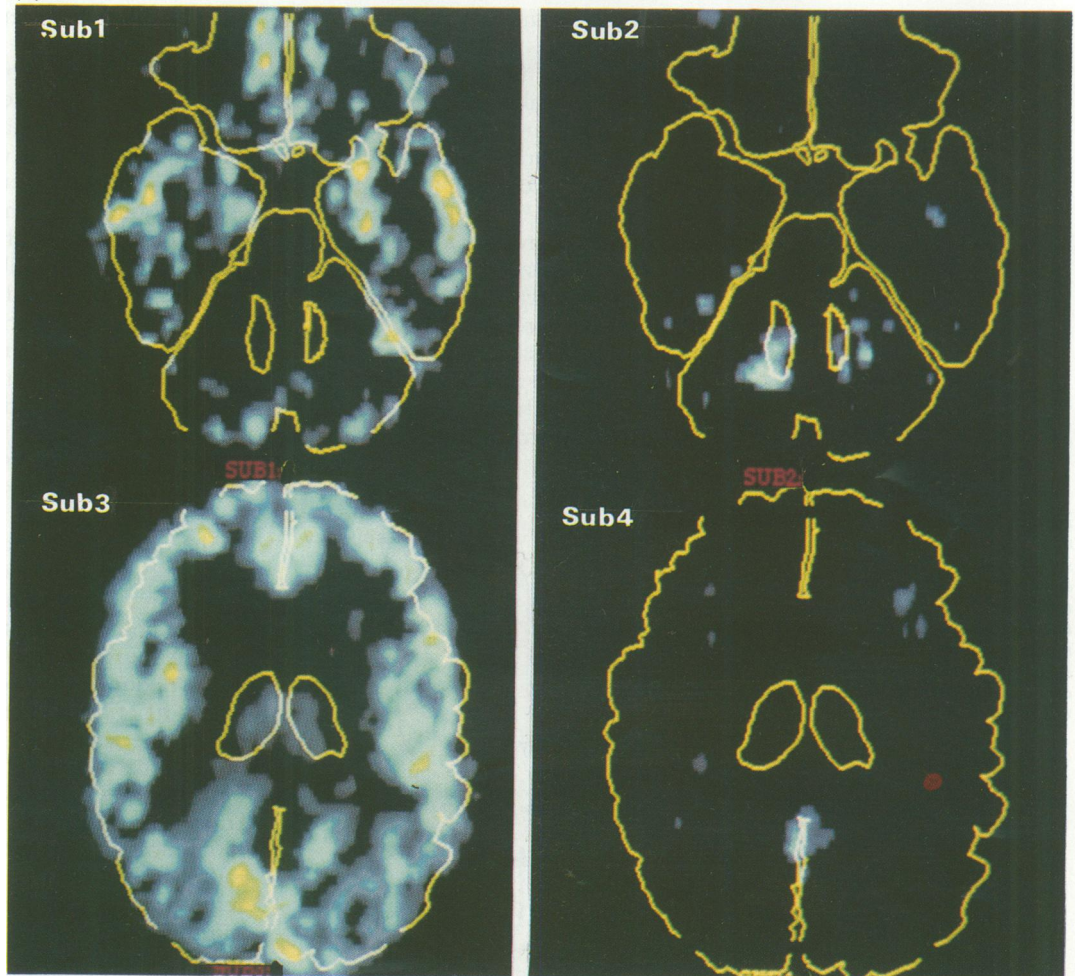
(C)

Discussion

METHODOLOGICAL CONSIDERATIONS

In the present study the cortical, subcortical, and cerebellar BZ receptor binding was analysed in patients with primary generalised epilepsy and healthy controls. The working hypothesis was that the BZ receptor density of patients with generalised epilepsy was either diffusely altered in the cerebral cortex or locally changed in specific subcortical and cerebellar regions suggested to mediate generalised epileptic seizures.[1][2] Because several of these regions are small, the importance of anatomically predefined regions of interest, as well as an exact anatomical correlation to the PET images was especially emphasised. This was accomplished by means of a computerised anatomical brain atlas. The application of the atlas was advantageous for several reasons: Firstly, it enabled corrections for the errors due to tilt. Secondly, by construction of reformatted B_{max} images the values from exactly the same structure, predefined from the computerised atlas, were obtained from each subject. The precision of the reformation process has been evaluated in an earlier study[25] and found to be high (SD of about 1 mm for lateral dislocation of the midline structures). Finally, the atlas also enabled construction of averaged and subtraction B_{max} images, in which the signal to noise ratio for the analysis of regional differences between two groups was increased. Therefore, the alterations in small structures such as cerebellar nuclei (about 2–3 camera–voxels) could be regarded as reliable.

The most prominent finding in the present study was that the calculated B_{max} values were increased in the cerebellar nuclei and decreased in the thalamus of patients with generalised epilepsy. According to the present data and earlier results,[19][21] these changes can not be ascribed to differences in the pharmacokinetics of [^{11}C]flumazenil between patients and controls. Neither can they be effects of variations in regional cerebral blood flow.[3][27] It is unlikely that the differences were caused by a systematic difference in the white matter concentration of [^{11}C]flumazenil as, in relation to the healthy controls, the patients with

generalised epilepsy had both regional increases and decreases in the B_{max} values. Also, the use of the pons as a reference region did not change the results. Theoretically a major change in the concentration of free + non-specific radioligand in patients with generalised epilepsy could explain the observed differences; it is, however, from the biological point of view, unlikely that the concentration of free + non-specific radioligand in these patients would be increased in the cerebellar nuclei and at the same time be decreased in the thalamus.

Another methodological pitfall to be considered is the possibility of a systematically different adaptation of the anatomical atlas in the axial direction in patients v controls. A displacement of the atlas contours by 6 mm in the axial direction, however, changed the individual B_{max} values of the cerebellar nuclei and the thalamus by 9(5)% and 4(3)%, respectively (mean difference between highest and lowest individual value). The changes in numerical values obtained were not systematically correlated with the type of shift (up or downwards), and the changes in the mean values for the whole group were less than 4%. This should be related to the calculated differences between the groups (about 300% increase in the cerebellar nuclei and about 50% decrease in the thalamus; table 2). Neither may such pronounced differences be ascribed to scatter from the surrounding cerebellar cortex, as the B_{max} values in this region did not differ between patients and controls (table 2).

Studies of BZ receptor binding by means of PET and [^{11}C]flumazenil are well established, yielding values of relatively similar orders of magnitude[3–5][18–20][26] at various PET centres. In the present study, the previously applied pseudoequilibrium method was abandoned because it has been shown that the condition of an absolute equilibrium does not prevail and that the B_{max} and K_d estimates are time-dependent.[19][20] Therefore, the full time courses of ligand concentration (except the initial 10 minutes) were used both in the target and the reference regions. Use of a reference region to estimate the time course of unbound radioligand implies a simplification that needs a comment. With this procedure, the pool of unbound radioligand serves as input function to the pool of specifically bound radioligand, and a determination of the transport kinetics across the blood brain barrier becomes unnecessary. The drawback is the difficulty in estimating the concentration of unbound ligand in the region with specific binding. Even if the transport of ligand across the blood brain barrier is the same in the reference and target regions the course of the unbound ligand will be different in the two regions. This difference, as well as the blood-flow-related difference in the time course of unbound radioligand in the reference and target regions, is most pronounced during the first minutes after a bolus injection. At a sufficiently long time after the injection, however, the ratio between the concentrations of

Figure 2 Bold lines represent enhanced/ synchronised input or output. The increased inhibition from the thalamic reticular nucleus is assumed to result in a synchronisation of the excitatory thalamocortical volleys.
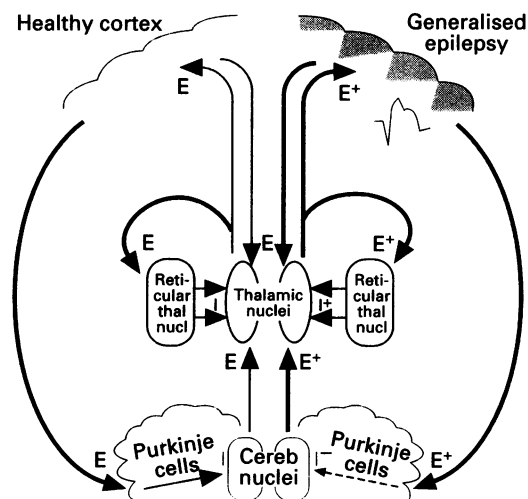
unbound radioligand in the tissue and the plasma will approach the same volume of distribution whether there is specific binding or not.[28] The data from the initial 10 minutes were therefore always excluded as this is the period of ligand distribution, when the effects of the different kinetics of free ligand in white and grey matter are most pronounced. Also, after the first 10 minutes, the effects of possible differences in the cerebral blood flow between patients and controls are negligible.[3]

In our earlier papers only data from 15–36 minutes were included. As both the B_{max} and the K_d estimates decrease with time after a bolus,[19] [20] the B_{max} values in the present study are systematically lower, but the B_{max}/K_d ratios are about the same. It should, however, be emphasised that the kinetic method, from a theoretical point of view, is considered to be more adequate than the equilibrium method, and that present B_{max} values correspond better to the postmortem values.[28]

The BZ receptor binding in the cerebellar nuclei has not been evaluated with PET before. The B_{max} values in the control subjects, however, were in accordance with human postmortem data.[29]

The values in the thalamus and caudate putamen were probably underestimated in the present study and should be interpreted with some caution. One possible reason is that the thalamic regions of interest included all nuclei, even the thalamic reticular nucleus which has few receptors. This was not the case in other reports.[26] [29] Another is that the applied pixel by pixel method is extremely sensitive for positioning errors, which are inevitable even with the helmet fixation of the head.[17] This does not affect large regions of interest, but in small, low density regions that are surrounded with low activity areas both the B_{max} and the K_d values (but not the B_{max}/K_d ratios) may be systematically underestimated. In the present study, however, the relative differences were more important than the absolute values. Furthermore, the uptake image-based ratios of thalamus and caudate putamen with pons and white matter were in accordance with corresponding values from other studies,[26] [29] and yet differences in the same direction as those based on B_{max} images were obtained (tables 1 and 2). Thus it can be concluded that both unquantified (table 1) and quantified (table 2) values reflect biological changes in patients with generalised epilepsy.

THEORETICAL CONSIDERATIONS

To interpret the present findings some details from previous experiments[1] [2] [30–33] should be considered:

The prerequisite for generalised spike-and-wave discharges is considered to be an enhancement and synchronisation of the thalamocortical and corticothalamic impulses, volleys.[30] This synchronisation is exerted by recruitment of the cortical GABA interneurons and the GABA neurons of the thalamic reticular nucleus, the second acting via the GABA$_A$-BZ receptors localised on the thalamic relay cells.[31]

Interestingly, the thalamic nuclei involved in generalised seizures,[32] are activated by excitatory output of the dentate nuclei in the cerebellum (fig 2).

The major input to the cerebellar nuclei is inhibitory and derives from the GABAergic Purkinje cells.[6] [7] During cortical epileptic activity, an initial enhancement and a subsequent cessation of the Purkinje cell activity (interpreted as a fatigue phenomenon) has been shown.[33] In the same experiment it was also shown that the sudden cessation of Purkinje cell firing precedes an enhancement of cortical epileptic discharges and development of seizures.

The GABA-BZ system thus seems to be involved in the development and maintenance of generalised seizures at several anatomical sites in the brain. The exact mechanisms behind the BZ receptor changes in the present study are, however, unknown and several possible mechanisms might be considered:

(1) Effect of phenytoin medication

No changes were demonstrated in the phenytoin treated patients with partial epilepsy (table 2). Hence, by contrast with the results from rat experiments,[11] present data suggest that therapeutic doses of phenytoin do not alter the BZ receptors in the cerebellum. This is in accordance with a recent PET report on [^{11}C]flumazenil uptake in cerebellar hemispheres of patients with epilepsy treated with phenytoin, in whom no alterations could be found.[34] It is also compatible with an earlier report of Dam[35] who stated that frequent and long-lasting generalised seizures rather than phenytoin medication may cause Purkinje cell destruction in human generalised epilepsy.

(2) Cellular loss or migrational disturbances

Microdysgenesis and necrosis of the Purkinje, thalamic, and cortical neurons have been reported in a postmortem study on patients with generalised epilepsy,[36] as well as in experiments on rats with long-lasting generalised seizures.[37] Thus the increased BZ B_{max} values in the cerebellar nuclei could be an effect of Purkinje cell denervation, whereas the low B_{max} values in the thalamus could be ascribed to a destruction of the postsynaptic thalamic relay cells. Our patients had moderate seizure frequency, however, and no histories of long-lasting seizures or epileptic state. Furthermore, the contours of the thalamus and cerebellar regions of interest derived from the data base fitted well with the contours of corresponding structures in the individual MR images, indicating that patients with generalised epilepsy had no visually detectable atrophies in these regions. Finally, a reduced BZ receptor density was not found in the cerebellar cortex, which would have been expected when considering that the Purkinje cells themselves expose BZ receptors[6] [7] and that the applied method allows detection of such a reduction.[38]

Functional disturbances leading to a "vicious circle"

In such a circle, an impaired Purkinje cell control over the cerebellar nuclei could lead to an activation of the thalamus, and a facilitation of cortical epileptic discharges leading to a further increased drive of the already exhausted Purkinje cells (fig 2). This chain of effects could then result in a compensatory increase of BZ receptors in the cerebellar nuclei and a down-regulation/blockade[39] of BZ receptors in the thalamus and perhaps also cerebral cortex (table 2).

The cited alternatives are not mutually exclusive. Additional studies are, however, needed to confirm the findings and evaluate the validity of possible explanations.

To our knowledge this is the first time that a neurochemical-anatomical substrate has been delineated in vivo in human generalised epilepsy. The results seem consistent with the centrencephalic theory of Jasper and Penfield and are supported by experimental data showing reduced BZ binding in the thalamus of Mongolian gerbils.[40] There is presently no way, however, of knowing whether the thalamic and cerebellar findings are the main pathological feature, or an effect of a disorder primarily affecting the cerebral cortex but subsequently leading to a Purkinje cell dysfunction and alterations in the cerebello-thalamocortical loop, according to the proposed "vicious circle" theory (fig 2). This important issue will be considered in future studies.

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