

Heterogeneous distribution of polyamines in temporal lobe epilepsy

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Abstract

Polyamine contents were determined in human temporal lobe epilepsy. In the seven patients studied, stereoelectroencephalography (SEEG) located the epileptogenic focus in Ammon's horn and neuropathological findings were limited to hippocampal gliosis and sclerosis. Each polyamine exhibited a specific regional distribution. The most important variations were observed for spermidine and spermine while putrescine levels varied less. The regional variation was predominant in middle > posterior > anterior parts of the temporal lobe. Spermine contents and the spermidine/spermine (SPD/SPM) index varied especially in the middle and posterior parts of the hippocampus. Metabolic SPD/SPM index and spermidine levels were found to be drastically increased in almost all limbic parts when compared to neocortical regions. The opposite was observed for spermine. The heterogeneous distribution of polyamines was compared to abnormal electrical activities recorded by SEEG: SPD/SPM index and spermidine levels were sharply increased in seizure onset areas and high levels of spermine were detected in temporal cortex propagation areas. The presently reported heterogeneity of polyamine contents might contribute to modulate differentially the local control of excitability in human temporal epilepsy. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

The polyamines, i.e. putrescine (PUT), spermidine (SPD) and spermine (SPM) are biogenic amines widely distributed in eukaryotic tissues.

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Human brain polyamine concentrations have been studied in some developmental and pathological (Sturman and Gaull, 1974; McAnulty et al., 1977, 1977; Harik and Sutton, 1979) as well as in a few control patients (Kremzner, 1970; Shaw and Pateman, 1973). Surgical specimens obtained from untractable epileptic patients undergoing partial lobectomy have already been used for biochemical analyses, but, to our knowledge, polyamine contents in human temporal epilepsy have not yet been determined.

Polyamines play multifunctional roles in tissue growth and cellular mitosis or differentiation (Tabor and Tabor, 1984). These organic polycations are involved in genomic expression by regulating nucleic acid structure and activity (Matthews, 1993). Polyamine neosynthesis is increased during normal and tumoral mitotic growth (Jänne et al., 1978) and cell differentiation (Heby, 1981). Induction of ornithine decarboxylase (ODC), that catalyzes the first step of polyamine synthesis, is observed in cell migration and synaptogenesis in sympathetic and brain development (Slotkin and Bartolome, 1986) and after various damage to mature peripheral and central nervous systems (Dienel and Cruz, 1984).

In animal models of epilepsies, previous studies suggested the involvement of polyamines. Intracerebral microinjection of polyamines induced epileptic electrocortical activities in rat (De Sarro et al., 1986). Polyamine metabolism was increased in a rat kindling model of limbic epilepsy (Hayashi et al., 1992, 1993) and in an electroshock model of convulsions (Zawia and Bondy, 1990). Induction of ODC has been proposed as a possible mediator of seizure-elicited changes in genomic expression in rat hippocampus (Baudry et al., 1986). In astrocytes isolated from the cortex of audiogenic mice, we demonstrated drastic modifications in polyamine transport and metabolism (Laschet et al., 1992a).

In human temporal epilepsy, the most common histopathological finding is hippocampal sclerosis. This pattern is mainly characterized by a loss of principal neuronal cells (Babb and Brown, 1986) associated with a sprouting of the

mossy fibers (Sutula et al., 1989; Houser et al., 1990). These morphological changes are believed to contribute to the epileptogenicity of human cortex together with other modifications in amino acid, catechol (Sherwin and Van Gelder, 1986; Goldstein et al., 1988; Sherwin et al., 1988) and somatostatin contents (Robbins et al., 1991). Changes in the number and distribution of several neurotransmitter receptors have also been reported to be associated to human epileptic focus (Brière et al., 1986; McDonald et al., 1991). In addition, some enzymatic activities in rate-limiting neurotransmitters and excitotoxin quinolinic acid metabolism have been reported (Sherwin et al., 1984; Feldblum et al., 1988). The activity of *S*-adenosylmethionine decarboxylase, an important enzyme involved in polyamine synthesis, was reported to be increased in human epileptogenic cortex (Morrison et al., 1994). In a preliminary study of three case reports, we have previously shown that spermidine levels are increased in the hippocampus in patients with temporal lobe epilepsy (Laschet et al., 1992b).

Besides their trophic role, polyamines have modulatory effects on ion channels, namely a blocking effect on the inward rectifier K^+ channel (Yamada and Kurachi, 1995), and potentiatory effects on receptor channels, especially NMDA (Williams et al., 1990) and GABA_A (Gilad et al., 1992; Laschet et al., 1998) type channels. These ion-channels are considered to be of major importance in the basic mechanisms underlying epileptogenicity. Therefore, the potential role of polyamines in the modulation of those channels must be stressed.

The use of human tissue is necessary to identify any close relationship between biochemistry, morphological changes, and abnormal electrical activity recorded on patients. In the present work, polyamine contents were determined in grey matter samples from patients with untractable temporal epilepsy. We show that polyamine distribution is heterogeneous in the temporal lobe and we propose a possible correlation between biochemical findings and SEEG electrophysiological data.

2. Materials and methods

2.1. Determination of polyamine levels in the brain

Immediately after removal in the operating room, each temporal lobe specimen was cut in two, one part for neuropathological examination, the other for polyamine analysis. For each patient, we obtained seven to 20 tissue specimens. All samples submitted to biochemical analysis were also submitted to histology, but the reverse was not true, as some tissue removals were too small to be divided they were only submitted to histology. For example, when the removal of amygdala occurred, the tissue was never available for biochemistry. In some cases, when the surgeon decided not to cut out the Ammon's horn by entire-block removal, some hippocampal parts were aspirated and were thus 'lost' for both analyses. Thus, all parts (anterior, middle and posterior) of the Ammon's horn were not represented for all patient, but each part was represented with a similar frequency. Samples excised for histological characterization were fixed in formaldehyde 10% (w/v), embedded in paraffin, and stained by the hematoxylin–eosin, thionine and Kluever-Barrera techniques. For biochemical studies, samples were immersed in liquid nitrogen immediately after the block removal. The frozen material was dissected in a cryostat apparatus at -25°C . Cortical superficial blood vessels, pia matter and white matter were carefully removed and aliquots (10–50 mg) of frozen grey matter were placed in 1.5-ml Eppendorf tubes, weighted and stored at -80°C .

Chromatographic analysis of polyamines on perchloric acid extracts were performed by using methods adapted from Bontemps et al. (1984) and Brown et al. (1982). Putrescine, spermidine, spermine, 1,6-diaminohexane hydrochlorides and dansyl chloride were purchased from Sigma. The HPLC-grade acetonitrile was from SFCC (France). All other reagents were of analytical grade.

The HPLC equipment was purchased from Millipore-Waters and consisted of two high-pressure pumps (model 6000A) and a solvent programmer

(model 66). The injection system was a Rheodyne Model 7125. A Kontron spectrofluorometer was used for detection. The optimal excitation and emission wavelengths were 334 and 522 nm, respectively. Electrical signals were processed by a model SP4290 Integrator from Spectra Physics.

A prepacked 5 μm Ultrasphere-ODS column (150 \times 4.6 mm) from Beckman Instruments Inc. was used to chromatograph the dansylated polyamines and was protected by a 10 μm Nucleosil 300-C18 (30 \times 4.6 mm) guard column from SFCC. The temperature of the column was 30°C .

Twenty microliters of 10^{-3} M 1,6-diaminohexane as internal standard and 20 volumes of ice cold 0.2 M perchloric acid were added to the brain samples. The tissue was homogenized and allowed to stand for 15 min at 0°C . It was then centrifuged at $5000 \times g$ for 20 min. The supernatant (200 μl) was used for derivatization by dansyl chloride. The dansylation procedure was based on the method described by Brown et al. (1982).

The chromatographic separation procedure of the dansyl-derivatives was a modification of the method previously described in our laboratory (Bontemps et al., 1984). Peak areas were measured by an on-line computing integrator. Calculations of polyamine contents were based on the ratio of internal standard to wet weight. Results were expressed as nanomoles of polyamine per gram of grey matter tissue (wet weight).

3. Clinical data

Seven epileptic patients (three men and four women) had drug-resistant partial seizures. SEEG recordings were obtained during the presurgical work-up (Talairach and Bancaud, 1974): six to ten depth electrodes were stereotactically implanted in hippocampus, amygdala, temporal neocortex gyri and orbito-frontal region. Except for one patient (E-4) who was implanted bilaterally, the others were implanted in the only hemisphere involved based on lateralized epileptogenesis seen in the scalp-EEG. Three letter-labeled depth-electrodes (right: A, B, C; left: A', B', C') were implanted in the temporal lobe at similar sites from one patient to the other, and the most

mesial contacts of these electrodes were localized, respectively, in the amygdala (A), the anterior (B) and the posterior (C) hippocampus. These were systematically implanted, except for patient E-7 lacking the C-electrode. In all cases, the hippocampal seizure-onset was recorded by at least one of the electrodes B or C. Other optional electrodes were implanted in the temporal lobe and in other parts of the hemisphere (D, F, G, H, O, P, Q, R, T, U); these may have varying implantation sites. The depth of implantation was a function of the target individual anatomy. With this method the clinical and electrical activity can be recorded during spontaneous or evoked seizures and the focus of seizure onset as well as areas secondarily involved in seizure propagation can be located. The ictal onset area was identified by the earliest appearance of fast discharges of low voltage preceding the emergence of a specific hypersynchronous pattern. The propagation pathway was determined from the time course of appearance of fast discharges and/or hypersynchronous pattern on the related electrodes. The number of seizures recorded by SEEG was 6 ± 3 per patient in this population. The propagation

pattern was reproducible, as, in one given patient, if the extent of structural involvement was the same, the pattern of spreading was identical. SEEG were recorded as both bipolar and versus referential.

Antiepileptic drugs (generally carbamazepine or phenobarbital) were withdrawn the morning of the cortectomy. Patients were given pentobarbital and benzodiazepines prior to inducing general anesthesia.

The limits of the 'tailored' resection were inferred from SEEG results and from anatomoelectro-clinical correlations (see Table 1). Details of the surgical procedure have been described elsewhere (Talairach and Bancaud, 1974). Visible traces of previous electrode implantations at the brain surface were precisely recognized: therefore each specimen was rigorously identified at anatomical and electrophysiological levels. Under these conditions, we selected samples from ictal onset and propagation areas.

Control specimens of the temporal lobe were obtained from one adult patient without any history of neurodegenerative or neuropsychiatric disease 10 h after death.

Table 1
Summary of the clinical and neuroimaging data of patients with temporal lobe epilepsy^a

Patient no.	Age at time of neurosurgery (sex)	Etiology	Age at onset of epilepsy (duration)	Neuroradiological imaging	Extent of cortical resection
E-1	27 (F)	Hyperthermic convulsions at birth	11 (16)	Normal (CT-Scan)	Left temporal lobe not T1, T3 ^b
E-2	40 (F)	Hyperthermic hemi-convulsions at birth	–	Right temporal (including hippocampal) atrophy (MRI)	Whole right temporal lobe
E-3	24 (F)	Hyperthermic convulsions at one year	4 (20)	Normal (CT-Scan)	Whole right temporal lobe
E-4	26 (M)	Convulsions at 2 years	15 (11)	Normal (MRI)	Right temporal lobe not T1p
E-5	25 (F)	Hyperthermic convulsions at 2 years	6 (19)	Normal (CT-Scan)	Right temporal lobe not T4p, T5p
E-6	24 (M)	Arteriovenous malformation	21(3)	Right anterior temporal vascular lesion (MRI)	Right temporal lobe not T1p
E-7	19 (M)	Hyperthermic convulsions at 2 years	12 (7)	Normal (MRI)	Whole right temporal lobe

^a CT-Scan, brain computed tomography; MRI, magnetic resonance imaging.

^b Abbreviations of anatomical regions are given in Table 2.

4. Statistical procedures

As the sample size was small ($n = 7$), the biochemical data did not fit a normal distribution, so Student's *t*-test or ANOVA were not suitable. The Mann–Whitney non-parametric *U*-test was fully appropriate and was used for statistical analysis. This distribution-free rank test also gave better accuracy as data from the same site in several patients could be pooled. First, the test was applied to compare neocortical (NC) versus limbic system (LS) and seizure onset areas with propagation areas. Second, this statistical analysis was applied to compare these parameters in temporal lobe subregions: NCa = (P, T1a, T2a, T3a), NCm = (T1m, T2m, T3m), NCp = (T1p, T2p, T3p), LSa = (TSa, AHa), LSm = (TSm, AHm, U) and LSp = (TSp, AHp). Abbreviations are defined in Table 2. These subregions correspond to contiguous analysed areas that were grouped according to the homogeneous nature of their biochemical values ($P < 0.05$), although no average was made to avoid loss of statistical information. As the extent of resection varied somewhat from one patient to another, all areas were not represented for all patients and the frequency of removal (or availability) differed from one site to another.

5. Results

5.1. Clinical, SEEG and neuropathological data

All patients were adults. All had childhood onset epilepsy and, in five cases, hyperthermic convulsions was the only putative etiology (Table 1). Neuropathological examination revealed that the major cellular alterations were localized in Ammon's horn: dense gliosis and variable degrees of pyramidal and granule cell loss were observed. Neuroradiological imaging was normal for all cases except for patients E-2 and E-6. Magnetic resonance imaging (MRI) revealed a temporal (including hippocampal) atrophy for E-2 and a temporal vascular lesion for E-6 which turned out to be an arteriovenous malformation. Although neuronal and glial cells were not quantified, the

Table 2

Stereoelectroencephalographic (SEEG) and anatomical characterizations in the temporal lobe of the epileptic patients ($n = 7$)^a

	Region	SEEG activity
<i>Neocortex (NC)</i>		
Pa:	Temporal pole (anterior part)	Earlier propagation
Pi:	Temporal pole (internal part)	Earlier propagation
Pe:	Temporal pole (external part)	Earlier propagation
T1a:	Superior temporal gyrus (anterior part)	Later ^b or earlier propagation
T1m:	Superior temporal gyrus (middle part)	Later or earlier propagation
T1p:	Superior temporal gyrus (posterior part)	Later propagation
T2a:	Middle temporal gyrus (anterior part)	Earlier ^b or later propagation
T2m:	Middle temporal gyrus (middle part)	Later ^b or earlier propagation
T2p:	Middle temporal gyrus (posterior part)	Later or earlier propagation
T3a:	Inferior temporal gyrus (anterior part)	Later or earlier propagation
T3m:	Inferior temporal gyrus (middle part)	Earlier ^b or later propagation
T3p:	Inferior temporal gyrus (posterior part)	Earlier ^b or later propagation
T4m:	Fusiform gyrus (middle part)	(Not explored)
T4p:	Fusiform gyrus (posterior part)	(Not explored)
<i>Limbic system (LS)</i>		
T5a:	Parahippocampal gyrus (anterior part)	Earlier propagation
T5m:	Parahippocampal gyrus (middle part)	Earlier propagation
T5p:	Parahippocampal gyrus (posterior part)	Earlier propagation
U:	Uncus	(Not explored)
AHa:	Ammon's horn (anterior part)	Seizure onset
AHm:	Ammon's horn (middle part)	Seizure onset
AHp:	Ammon's horn (posterior part)	Seizure onset

^a The temporal lobe is divided into 22 areas by both type of separations, i.e. from anterior to posterior (a, m, p) and from lateral to mesial (P, T1, T2, T3, T4, T5, Ammon's horn, uncus). For example, the superior temporal gyrus (T1) is subdivided into T1a, T1m and T1p. The area T4a was not represented in this study.

^b Most frequent in this population.

outer cortex (temporal neocortex gyri and pole) was anatomically normal, presenting little or no gliosis and neuronal loss.

Stereoelectroencephalography data are presented in Table 2. For all patients, the seizures originated exclusively in Ammon's horn either in the anterior, middle or posterior parts. The onset, with both patterns (low voltage fast followed by hypersynchronous), was recorded in all seven cases. The propagation areas in temporal neocortex were classified as 'earlier' and 'later' propagation of ictal activity, depending on the time course of epileptic discharges. These areas included the temporal pole (P), the superior, middle and inferior temporal gyri (T1, T2, T3) and the parahippocampal gyrus (T5). The fusiform gyrus (T4) and the uncus (U) were not investigated by depth electrodes. In this population, T5 corresponded to earlier (or even earliest) propagation areas since the appearance of hypersynchronous patterns, such as slow spiking activity, occurred with a little delay and these were not always preceded by the fast discharges of low voltage as in the onset area. Therefore, although the propagation pathways may in general differ considerably from case to case, it was possible to use a single scheme to characterize propagation pathways. The low number of patients, in this study, is due to the selection upon two criteria: a same onset localization and a propagation pathway as similar as possible.

5.2. Polyamine levels and the SPD/SPM index

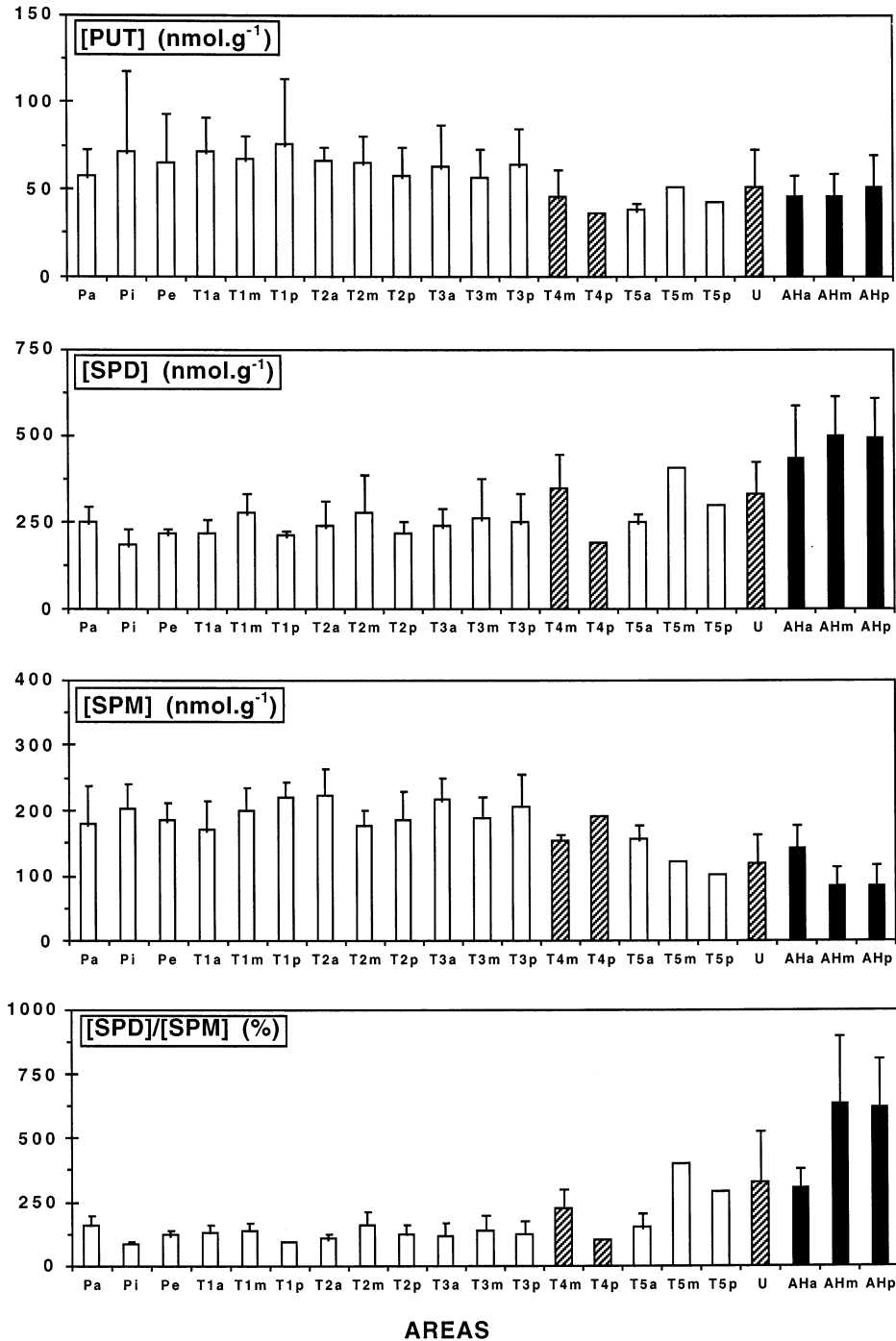
Polyamine contents and the SPD/SPM index were measured in different regions of the temporal lobe. Polyamine concentrations were heterogeneous in epileptic samples (Fig. 1). Two levels of comparison were considered. First, these biochemical parameters were compared between neocortical and limbic areas (Fig. 2). Then, correlations were tested with the SEEG electrical activity recordings, i.e. area of seizure onset and propagation areas (Table 2), including both early and late propagation areas. Descriptive statistics and the non-parametric *U*-test (Mann and Whitney) were applied to polyamine contents and the SPD/SPM index (Table 3).

Regional polyamine contents in the temporal lobe of all epileptic patients are shown in Fig. 1. Comparison of neocortical versus limbic structures revealed highly significant differences for all polyamine measurements: higher putrescine and spermine contents in neocortex and higher spermidine and SPD/SPM ratio in limbic areas. Spermidine contents were significantly higher in the middle part of the limbic structures when compared to the anterior and posterior parts of the neocortex; this was also the case in the posterior limbic areas compared to the anterior neocortex (Fig. 2). In epileptic patients, spermidine content varied greatly in Ammon's horn; this variability was not observed for other polyamines and did not occur in normal tissue. Spermine concentrations were higher in all neocortical regions compared to the middle and posterior limbic areas.

Values of the metabolic SPD/SPM index were found to be two to three times higher in almost all limbic parts when compared to any neocortical region. The most striking differences were observed in the middle and posterior parts of Ammon's horn. Intermediary values were obtained in parahippocampal gyrus, the anterior part of Ammon's horn and the uncus.

Only one control patient was studied (Table 3). Previous studies about regional distribution of polyamines in autopsied human brain have shown that the three polyamines displayed the same distribution profile, and that the SPD/SPM index was unchanged in the different brain areas. Our measurements in grey matter of temporal lobe of one control patient are in good agreement with these studies: the distribution of polyamines was not heterogeneous in control (Kremzner, 1970; Shaw and Pateman, 1973; Morrison et al., 1995). Therefore the heterogeneity in polyamine contents seems to be related to epileptic tissue. However, statistical analysis between normal and epileptic tissue will require more control samples.

In another set of comparisons, we looked for correlations between abnormal electrical activities and polyamine concentrations (Table 3). Polyamine contents differ in epileptogenic and propagation areas. Except for the anterior hippocampus, spermine levels were higher in the propagation neocortical areas than in the other



AREAS

Fig. 1. Regional polyamine contents in the temporal lobe of epileptic patients. Propagation areas are represented in white, non-explored areas in dashed grey and ictus onset area in black. Abbreviations are given in Table 2. As the patients were subjected to selective cortectomy, the extent of the resection was not the same in all. Thus, all of the areas were not represented for all patients, i.e. $n = 6$ for T1m; $n = 5$ for T2m and T3m; $n = 4$ for Pa, Pi, T1a, T3p and AHm; $n = 3$ for T2a, T2p, T3a, U, AHa and AHp; $n = 2$ for Pe, T1p, T4m and T5a; $n = 1$ for T4p, T5m, T5p; whereas the anterior part of T4 (T4a) was never available in this population. Each data point is the average of two or three measurements.

parts of the hippocampus. In the seizure onset area, the metabolic index SPD/SPM was two to three times higher than in the other regions of temporal lobe such as the neocortex which corresponds to the seizure propagation areas. This modification of the index was due to higher spermidine concentration and lower spermine level.

6. Discussion

The aim of the present work was to study the distribution of polyamines in human temporal

lobe epilepsy. One difficulty in comparing among epileptic patients is the interindividual variability of propagation pathways. Our study was therefore limited to fully characterized temporal lobe epilepsies, with similarly located epileptogenic foci. SEEG recordings identified the epileptogenic focus in Ammon's horn in all the patients studied.

The most common neuropathological finding was hippocampal gliosis and sclerosis. Patients with tumors were excluded to avoid interference due to biochemical changes of neoplastic origin, unrelated to epilepsy. Indeed, in another patient (not reported here) we have observed much higher

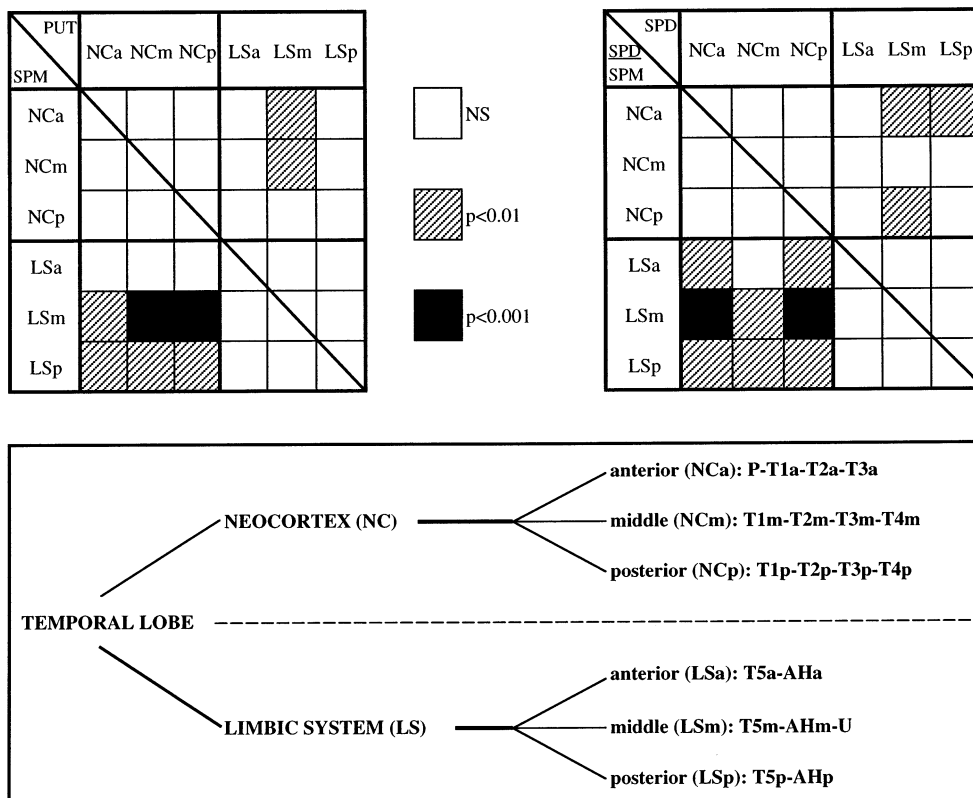


Fig. 2. Statistical analysis using the non-parametric Mann-Whitney U -test. Polyamine contents and the SPD/SPM index are compared in anterior (NCa), middle (NCm) and posterior (NCp) parts of neocortical and limbic (LSa, LSm, LSp) areas of epileptic temporal lobe. These regions correspond to groups of contiguous analysed sites that were found to be homogeneous. These regions also correspond to vertical segments of the temporal lobe. The number of sample sites were: 20 in NCa, 18 in NCm, ten in NCp, five in LSa, eight in LSm and four in LSp. Different degrees of statistical significance are indicated: white, dashed grey and black correspond to NS, $P < 0.01$ and $P < 0.001$, respectively. Schematic definition of the anatomical segments is shown in the lower part of the figure. According to the Paul MacLean's definition of the limbic system, we include the hippocampal formation, parahippocampal gyrus and uncus. But the limbic tissue analysed in this study does not include the extra-temporal part of the limbic system such as the cingulate gyrus.

Table 3
Measurements of polyamine contents in temporal lobe and comparison with SEEG data^a

	Neocortical areas		Limbic areas		Neocortical vs. limbic areas <i>U</i> -test
	Mean	S.D.	Mean	S.D.	
<i>A. Epileptic patients (n = 7)</i>					
[PUT]	68.2	19.5	49.8	12.1	$P < 0.001$
[SPD]	245	66	409	149	$P < 0.002$
[SPM]	200	40	121	41	$P < 10^{-6}$
[SPD]/[SPM]	1.27	0.43	4.08	2.57	$P < 10^{-7}$
	Propagation areas		Onset areas		Propagation vs. onset areas
	Mean	S.D.	Mean	S.D.	
[PUT]	69.9	18.8	51.7	14.1	$P < 0.01$
[SPD]	241	62	487	138	$P < 10^{-5}$
[SPM]	202	40	108	43	$P < 10^{-4}$
[SPD]/[SPM]	1.23	0.36	5.30	2.64	$P < 10^{-6}$
	Neocortical areas		Limbic areas		Neocortical vs. limbic areas
	Mean	S.D.	Mean	S.D.	
<i>B. Control patient</i>					
[PUT]	48.4	4.3	42.1	1.2	NS
[SPD]	260	55	306	98	NS
[SPM]	174	32	162	73	NS
[SPD]/[SPM]	1.50	0.34	1.99	0.63	NS

^a Polyamine contents are measured as described in Section 2. In epileptic patients (panel A), polyamine contents are compared in neocortical versus limbic areas (upper part), and between propagation—earlier or later—and seizure onset areas (lower part). In one control patient (panel B), polyamine contents are compared in neocortical versus limbic areas. Concentrations of polyamines are expressed in nmol g^{-1} . Non-parametric *U*-test (Mann and Whitney) has been used for statistical comparisons. NS, not significant at the level of 1%. The cumulative number of analysed sites for all the epileptic patients ($n = 7$) was, respectively, 48 in the neocortical, 17 in the limbic, 45 in the propagation and ten in the onset areas. For the control patient, the number of sites was, respectively, nine in the neocortical and three in the limbic areas.

putrescine concentrations in the Ammon's horn harboring an oligodendroglioma than in normal and epileptic tissue. It has been suggested that this is related more to neoplasm than to epileptic gliosis (Harik and Sutton, 1979). A possible influence of anticonvulsant drugs on polyamine levels cannot be ruled out but, to our knowledge, no study has shown such an interaction.

The main findings in the present study are:

(1) Polyamine contents are very heterogeneous in the temporal lobe of epileptic patients. We observed a specific pattern of polyamine contents especially in Ammon's horn: spermidine and spermine contents showed distinct regional distributions while putrescine levels varied less. The main modifications in spermidine concentration and metabolic index occurred in the middle and posterior parts of the hippocampus.

This heterogeneity was confirmed by an appropriate statistical procedure, the Mann–Whitney rank test. This non-parametric analysis established with a high degree of confidence that the three polyamines displayed a specific regional distribution. The rank of variations in the temporal lobe was: middle > posterior > anterior part. The degree of variation differed from one polyamine to the other. Putrescine contents varied significantly only in a restricted area. The most striking and widespread differences concerned spermine concentration and SPD/SPM index.

This is in contrast with previous data in control human brain where polyamine levels and SPD/SPM index have shown little variation (Morrison et al., 1995; Shaw and Pateman, 1973). In these studies, the measurements were not performed in all the anatomical regions as defined in the

present work. However, these reported results are in good accordance with our analysis of one control patient which confirmed the lack of notable variation in non-epileptic tissue. For ethical reasons, we were unable to get more suitable control tissue. In the future, it will be challenging to accurately measure the cerebral polyamine distributions in more control patients: this is a prerequisite to better understand the physiological significance of the present findings such as the apparently higher spermine content in the neocortex and spermidine content in the limbic structure of the epileptic temporal lobe.

Spermidine levels were increased in the middle and posterior parts of the hippocampus, where gliosis and neuronal loss were predominant. It is tempting to speculate that this difference simply reflects the greater accumulation of glia in the Ammon's horn, as sclerosis and gliosis are reported as common neuropathological findings. However, spermidine was the only polyamine whose level was increased in areas with gliosis. Spermine concentrations were higher in the neocortex, where the gliosis was absent or weak. Hypotheses concerning seizures and cellular plasticity associated to epilepsy might help to explain these observations.

(2) The heterogeneous distribution of polyamines was compared to abnormal electrical activities recorded by SEEG. The SPD/SPM index and spermidine levels were sharply increased in the seizure onset area and high levels of spermine were detected in temporal cortex propagation areas. In the present study we have calculated the SPD/SPM index since it is widely used as a metabolic index for polyamine synthesis. Its increase reflects an induction of ornithine decarboxylase (ODC) activity, the rate-limiting enzyme for polyamine biosynthesis (Jänne et al., 1978). Our finding of an SPD/SPM index increase associated with relatively stable levels of putrescine suggests an accelerated turnover of putrescine, the precursor of spermidine. Alterations in polyamine metabolism have been suggested in previous studies: the activity of *S*-adenosylmethionine decarboxylase (SAMDC), the rate-limiting enzyme for spermidine and spermine synthesis, is increased in human temporal epilepsy (Brown et al., 1994). In

addition, several observations suggest that putrescine is involved in epilepsy.

Microinfusion of putrescine induces epileptogenic activity in the rat (De Sarro et al., 1986). Putrescine conversion into GABA is accelerated in audiogenic mice during periods of high seizure susceptibility (Laschet et al., 1992a) and putrescine concentration is increased in response to electrical or chemical kindling stimulation in the rat (Hayashi et al., 1992) or in response to convulsant agents (Martinez et al., 1991; Hayashi et al., 1993). However, it has to be stressed, that studies on transgenic mice, in which overexpression of the ODC genes led to an overproduction of putrescine, add support to the notion that the diamine protects against epilepsy (Halonen et al., 1993). Thus, there is some discrepancy between the anticonvulsant and the epileptogenic effects of putrescine. It may be necessary to distinguish between convulsion and epilepsy models since we previously showed that, in audiogenic mice, the maximal anticonvulsant effect of milacemide is higher than its antiepileptic effect (Laschet et al., 1991). It may also be important to make a distinction between putrescine and the other polyamines as they are not significantly increased in response to convulsant agents. Consequently, the role of polyamines in human epileptogenicity remains unclear, i.e. are polyamines involved as protective or epileptogenic agents?

Polyamines exert a modulation on several receptors that contribute to the control of brain excitability. Indeed the presence of a binding site for polyamines on the NMDA receptor is well documented (Ransom and Stec, 1988; Williams et al., 1989, 1991). Spermidine and spermine potentiate glutamatergic responses (Brackley et al., 1990; McGurk et al., 1990; Sprosen and Woodruff, 1990). Moreover, anticonvulsant effects result from combined treatment with glycineB receptor antagonist and a polyamine site antagonist in the amygdala-kindled rat (Ebert et al., 1997). Polyamines also interact with GABA_A receptors on rat membranes (Gilad et al., 1992). Recently, we proposed that polyamines, especially spermine, modulate the endogenous phosphorylation of the purified GABA_A receptor, a mechanism that confers stability on the functional state of the recep-

tor (Stelzer et al., 1988; Laschet et al., 1994, 1998). Thus, the question of a protective or epileptogenic role of the polyamines may be re-addressed to their modulatory roles on those ionotropic receptors.

In summary, the results of this study indicate a heterogeneous distribution of polyamines in temporal lobe epilepsy and support the hypothesis that polyamines can contribute to modulate differentially the local control of excitability.

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