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BRIEF COMMUNICATION

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Electrocorticographic and neurochemical findings after local cortical valproate application in patients with pharmacoresistant focal epilepsy

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Abstract

Because oral pharmacological treatment of neocortical focal epilepsy is limited due to common systemic side effects and relatively low drug concentrations reached at the epileptic foci locally, application of antiepileptic agents directly onto the neocortical focus may enhance treatment tolerability and efficacy. We describe the effects of cortically applied sodium valproate (VPA) in two patients with pharmacoresistant neocortical focal epilepsy who were selected for epilepsy surgery after a circumscribed epileptic focus had been determined by invasive presurgical evaluation using subdural electrodes. Local VPA modified epileptic activity as electrocorticographically recorded from the chronic focus in both patients. In addition, VPA induced local increase of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) in cortical tissue samples, whereas the excitatory glutamate was possibly decreased. In this clinical pilot study, we could show antiepileptic effects of cortically applied VPA in humans by electrocorticographic and neurochemical parameters.

KEYWORDS

electrocorticography, human, local pharmacotherapy, neocortical focal epilepsy, sodium valproate, γ-aminobutyric acid (GABA)

Dirk-Matthias Altenmüller and Jonas M. Hebel contributed equally to this work.

1 | INTRODUCTION

Oral administration of antiepileptic drugs (AEDs) at doses high enough for adequate seizure control may be associated with intolerable systemic side effects. Therapeutic strategies aiming at enhanced efficacy and tolerability, therefore, include the local application of AEDs directly onto the individually identified epileptic focus,[1] for example, by subdural polycaprolactone implants. Neocortical delivery of different agents has been shown to be able to modify epileptic activity in rats and nonhuman primates.[2-7] In humans, only the antiepileptic effects of subdural lidocaine were described.[8] In contrast to lidocaine, sodium valproate (VPA) is widely used clinically for oral treatment of epilepsy.[9] In a rat model of neocortical epilepsy, locally applied VPA has shown efficacy in decreasing interictal epileptiform potentials and enhanced survival.[7,10] In the present study we tried to transfer and replicate these findings in patients with pharmacoresistant neocortical focal epilepsy who were selected for epilepsy surgery.

2 | METHODS

2.1 | Patients

Two patients with a circumscribed neocortical epileptic focus distant from eloquent areas were recruited to the study. Both patients had nonlesional right-sided frontal lobe epilepsy refractory to multiple oral pharmacotherapies. *Patient A* (50 years, male) had focal versive seizures and focal to bilateral tonic-clonic seizures. The last treatment consisted of phenytoin and levetiracetam. *Patient B* (48 years, female) had focal impaired awareness seizures and focal to bilateral tonic-clonic seizures. The last treatment consisted of lamotrigine and levetiracetam. Histopathological examination showed focal cortical dysplasia type Ib according to International League Against Epilepsy (ILAE) classification[11] in both patients.

2.2 | Experimental design

After multimodal noninvasive presurgical evaluation, both patients underwent extraoperative invasive video–electroencephalography (EEG) monitoring with subdural electrodes (10 mm contact spacing) placed on the right hemisphere (see Figure 1A,B for electrode position) in order to delineate the epileptogenic area by interictal and ictal long-term electrocorticography. By visual analysis, the neocortex underlying the electrode contact with the most prominent interictal epileptic activity (clear-cut spikes or bursts of low amplitude fast activity) was defined as the "epileptic focus" (EF) (Figure 1A,B).

After presurgical work-up, patients were scheduled for explantation of the subdural electrodes and an individually tailored resection in the right frontal lobe (see Figure 1A,B for extent of resection). For additional cortical VPA application during epilepsy surgery, appropriate consent was obtained from each patient. The proof of concept study was approved by the local ethics committee. It is important to note that the neocortical area intraoperatively exposed to local VPA was subsequently completely removed surgically as planned before. The extent of the overall resection as defined by extraoperative epilepsy evaluation was not influenced by the study.

2.2.1 | Intraoperative electrocorticography (ECoG)

Stable anesthesia was performed with desflurane (Patient A) and sevoflurane (Patient B), respectively, and remifentanil. Propofol and benzodiazepines were not administered. Pancuronium was used for muscle relaxation. Prior to resection, in both patients the EF and its surroundings were examined by intraoperative ECoG under different conditions using subdural electrodes. For this purpose, a new small grid was centered on the EF, and interictal epileptic activity was recorded during 20 minutes (ECoG baseline). Thereafter the grid was removed and a standard cotton pad (25 mm diameter, 1.5 mm thickness) saturated with 500 μL NaCl solution (0.9%, 37°C) was positioned above the EF for 20 minutes. Afterward the ECoG procedure started again (another 20 minutes, NaCl condition). Finally, a cotton pad saturated with 500 μL VPA solution (Orfiril injection, 37°C; patient A 100 mg/mL, patient B 10 mg/mL) was applied exactly over the EF for 20 minutes, and thereafter ECoG recording was repeated (another 20 minutes, VPA condition) and serum VPA levels were determined.

For each patient and each condition, epileptic activity (spikes and burst suppression patterns, respectively) within the EF was visually analyzed and quantified by two blinded and independent reviewers. In order to evaluate the chronologic course, for both patients the total intraoperative ECoG was divided into 60 segments of 1-minute duration each, which were randomly presented for analysis.

2.2.2 | Neurochemical examinations

After local VPA application and ECoG procedures, tissue samples (at least 10 mg, each) were taken from the resection area for neurochemical analysis, five samples from the cotton pad area (including the EF plus four samples located 10 mm distant from the EF, *inside*), and five tissue samples located 15-20 mm distant from the EF, *outside* the pad area.

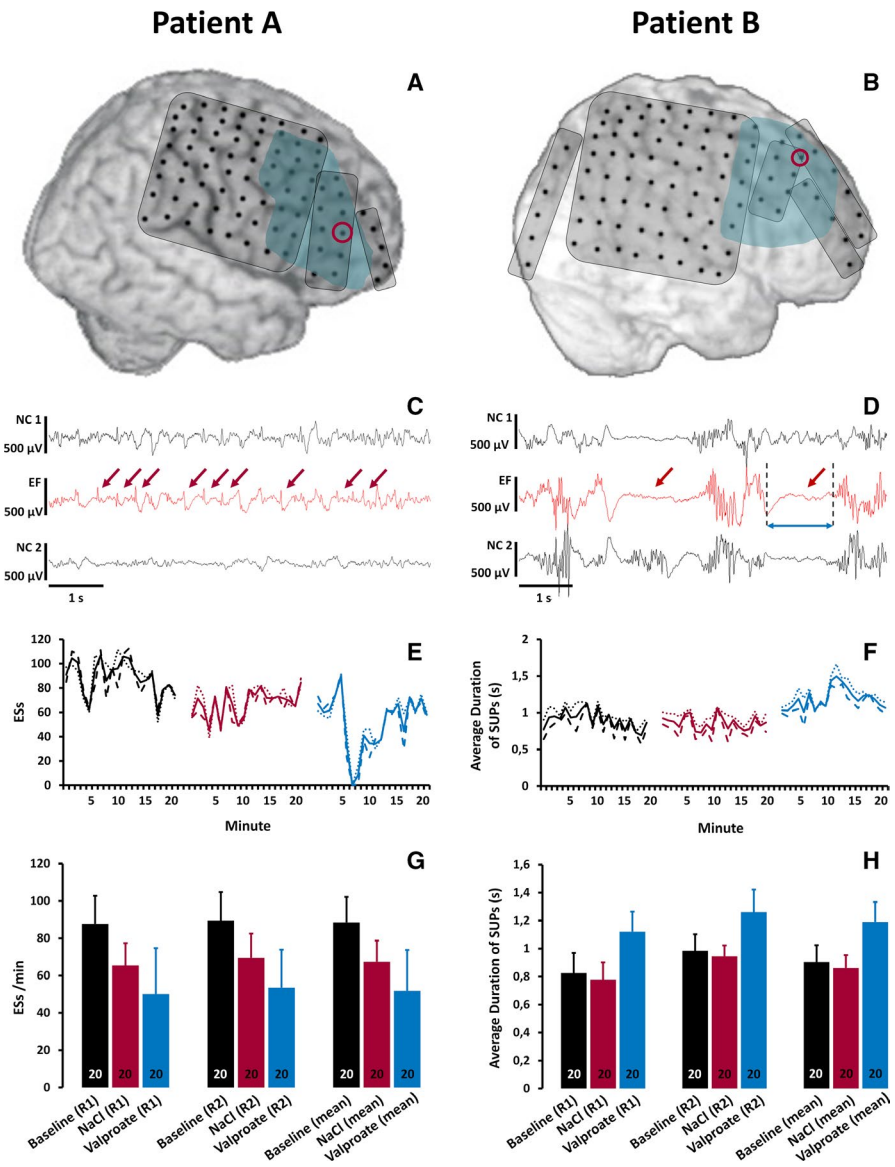

FIGURE 1 Position of subdural electrodes for presurgical evaluation in Patients A (A) and B (B), respectively. "Epileptic focus" (EF, red circle) and extent of tailored resection (green area) were determined according to extraoperative interictal and ictal recordings. Typical examples of intraoperative electrocorticography at EF and neighbor electrode contacts (NCs) with distinct epileptic spikes (ESs, red arrows) in Patient A (C) and burst suppression patterns (BSPs, red arrows) in Patient B (D); blue arrow: suppression phase of BSP (SUP). Chronological course of number of ESs in Patient A (E) and of average duration of SUPs in Patient B (F) for the different intraoperative conditions (black: baseline, red: NaCl, blue: sodium valproate); dashed line: reviewer 1 (R1), dotted line: reviewer 2 (R2), continuous line: mean of both independent reviewers. Mean values + standard deviations of ESs/min in Patient A (G) and of average duration of SUPs in Patient B (H) for the different intraoperative conditions (20 min each)

All samples were frozen at −80°C. Thereafter, the resection of the epileptogenic area was accomplished.

Tissues were thawed and homogenized (10 strokes) in an ice-cold solution of 10 mmol/L phosphate-buffered saline (PBS, 1:10, wt/vol.), pH 7.4 (previously perfused with 95% oxygen, 5% carboxygen), kept at 4°C, centrifuged at 2500 *g* for 10 minutes at 4°C. A total of 80 µL supernatant was diluted with PBS as appropriate and then injected into the high-performance liquid chromatography (HPLC) system and every tissue sample was analyzed three times.

After pre-column derivatization with o-phthaldialdehyde and sodium sulfite for 30 minutes, we measured glutamate (Glu), glutamine (Gln), and γ-aminobutyric acid (GABA) values using HPLC with electrochemical detection as described previously.[12] The HPLC system consisted of a C18 column (Eurospher 100, 5 µm, column size 250 × 4 mm) and a pre-column (30 × 4 mm). The isocratic mobile phase (0.1 mol/L PBS, pH 4.5, containing 0.5 mmol/L EDTA and 25% methanol) was previously degassed by helium and pumped at a flow rate of 1.0 mL/min. The compounds were detected electrochemically using a glassy carbon electrode set at a potential of 900 mV relative to an Ag/AgCl reference electrode.

2.3 | Statistical analysis

Epileptic activity was given as mean of epileptic spikes (ESs, Patient A) or burst suppression patterns (BSPs, Patient B) per minute ± standard deviation (SD) for each condition. In Patient B, in addition, the mean duration of suppression phases of BSP ± SD was calculated for each condition.

Levels of GABA, Glu, and Gln were expressed in nmoles/mg tissue wet weight, as mean ± SD. Furthermore, ratios of Glu/GABA (excitation index) and Gln/Glu (glutamine supply) were calculated.

Since in this preliminary study only two patients were included, we renounced to perform further statistical analysis.

3 | RESULTS

Intraoperatively, ECoG recordings at the EF presented epileptic activity with distinct ESs in Patient A and BSPs in Patient B, respectively (Figure 1C,D). Because quantitative analyses of the two independent reviewers led to equal results (Figure 1E,F), here we report only the values of reviewer 1.

In Patient A, frequency of epileptic activity was 87.7 ± 15.01 ESs/min under baseline condition and slightly decreased to 65.5 ± 11.9 ESs/min with NaCl condition. After VPA application frequency declined to 50.1 ± 24.6 ESs/min (57.13% as compared to baseline; Figure 1G); temporarily, ESs were completely suppressed (Figure 1E).

In Patient B, BSP frequency did not show any apparent difference between the different conditions (baseline 14.95 ± 3.76 BSPs/min; NaCl 16.14 ± 2.93 BSPs/min; VPA 16.88 ± 3 BSPs/min). However, the average duration of suppression phases of BSPs increased during the VPA condition (1.12 ± 0.14 seconds) as compared to baseline (0.83 ± 0.14 seconds) and NaCl (0.78 ± 0.12 seconds) condition (Figure 1H).

As shown in Figure 2, free GABA levels were 1.52 ± 0.38 nmoles/mg in Patient A and 3.07 ± 1.43 nmoles/mg in Patient B, respectively, around the EF. In both patients, GABA levels were higher in those tissue samples located inside the cotton pad area in comparison to that outside. Free glutamate tissue levels did not differ according to the tissue sample location (Patient A: 7.3 ± 1.9 versus 6.8 ± 1.0 nmoles/mg. Patient B: 12.5 ± 6.3 versus 7.9 ± 2.2 nmoles/mg; inside vs outside).

In Patient A, the excitation index given by the Glu/GABA ratio was decreased inside the pad area, whereas the glutamine supply given by the glutamine/glutamate (Gln/Glu) ratio was slightly increased. In contrast, the excitation index and also the glutamine supply did not change in Patient B (Figure 2).

Serum levels of VPA after cortical VPA administration were below the detection limit in both patients.

4 | DISCUSSION

In this pilot study, we studied the effects of cortical VPA on epileptic activity recorded by intraoperative ECoG and inhibitory/excitatory neurotransmitters in two patients with pharmacoresistant neocortical focal epilepsy in whom a circumscribed epileptogenic focus had been determined by invasive presurgical evaluation using subdural electrodes. Despite the low number of patients, the experimental design provided significant results. To our knowledge, we hereby for the first time provide data on local application of a commonly used AED onto a chronic, clinically relevant epileptic focus in humans.

It has been shown that locally applied VPA clearly reduced epileptic activity recorded from the preoperatively defined epileptic focus in one patient and possibly increased focal inhibitory mechanisms (as measured by the duration of suppression phases) in the other patient. The seemingly less pronounced effect in Patient B is probably related to a lower VPA concentration. In both patients, serum levels of

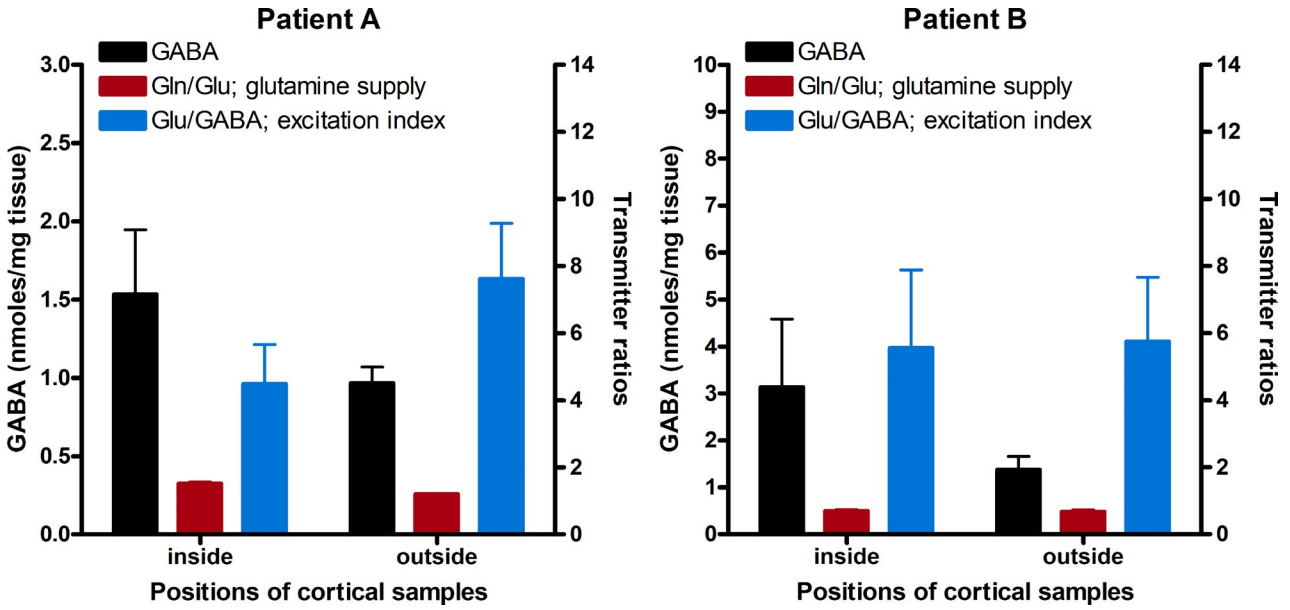

FIGURE 2 Mean values + standard deviations of free γ-aminobutyric acid (GABA) levels, of glutamine/glutamate ratio (Gln/Glu), and of glutamate/GABA ratio (Glu/GABA) according to the position where tissue samples were acquired (*inside/outside* the cotton pad area)

VPA were below the detection limit, supporting the hypothesis that systemic side effects may be avoided through local administration.

The ECoG findings are in line with the neurochemical results from tissue samples obtained shortly after cortical VPA application. In the central nervous system (CNS), inhibitory transmission is primarily achieved through GABA acting mainly via ionotropic $GABA_A$ receptors that form ligand-gated chloride channels.[13] As recently shown,[14] VPA exposure enhances endogenous GABA levels in the rat brain, for example, through GABA uptake inhibition. Thus, our GABA findings in humans appear indeed as an anticonvulsant VPA effect. This assumption is, additionally, underscored by a decrease in the excitation index and increase in the glutamine supply, at least in Patient A.[15] Since by definition epileptic activity was most prominent in the EF, we would expect enhanced glutamate levels in the cotton pad area compared to its surroundings.[16,17] However, in our study, glutamate levels did not differ regarding the sampling points. In line with an increase in glutamine supply in Patient A, this might be explained by a counteracting effect of VPA on focally elevated glutamate when applied locally on the EF, since VPA was found to increase the oxidative deamination of glutamate in vivo resulting in glutamate decline.[18] Again, the overall lesser effect on relevant neurochemical parameters in patient B could be explained by the lower VPA dose applied.

Taken together, our preliminary combined electrocorticographic and neurochemical findings led us to suggest that cortical application of VPA reduces focal epileptic activity and potentially may also prevent spontaneous seizures by local GABA enhancement and, possibly, by glutamate decrease. In addition, our proof of concept study in humans provides further evidence that targeted local pharmacological treatment of neocortical focal epilepsy might be a promising therapeutic strategy in select epilepsy patients.

4.1 | Limitations

Due to the strict inclusion criteria, only two patients could be recruited.

Within the scope of the initial dose-finding process, the VPA dose applied differed between patients. Unfortunately, a timely measurement of VPA concentrations in the tissue samples was not performed.

Because anesthesia conditions remained stable throughout ECoG and an impact of focal cooling can be excluded, the effect of local NaCl in Patient A is not easily explained.

In Patient B, the appearance of the circumscribed epileptic focus switched to BSP intraoperatively, which might have been facilitated by sevoflurane.

Of course, prior to any therapeutic clinical application, further extensive studies are necessary to confirm our findings in a larger number of patients and to additionally investigate possible local side effects and pharmacokinetic details using more sophisticated systems (eg, intracranial drug-releasing polymers and responsive or nonresponsive neuroprosthetic devices) for local VPA delivery.

CONFLICT OF INTEREST

None of the authors has any conflict of interest to disclose.

ETHICAL PUBLICATION STATEMENT

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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