Electrophysiological properties of interneurons from intraoperative spiking areas of epileptic human temporal neocortex

Liset Menendez de la Prida,[CA] Ruth Benavides-Piccione,[1] Rafael Sola[2] and Miguel Angel Pozo

Brain Mapping Unit, Instituto Pluridisciplinar, Universidad Complutense de Madrid, Paseo Juan XXIII, 1. Madrid 28040; [1]Instituto Cajal, CSIC, Madrid; [2]Department of Neurosurgery, Hospital de la Princesa, Madrid, Spain

[CA]Corresponding Author

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We combined visual-assisted whole-cell recordings with Lucifer Yellow labelling to record *in vitro* from interneuron-like cells from human epileptic neocortical tissue that exhibited spiking activity *in situ*. Information from intraoperative electrocorticography, which was performed to tailor resection, were used to select those areas that participate in interictal spiking. Whole-cell recordings from slices prepared from these areas showed that both pyramidal and interneuron-like cells were present and retained their main intrinsic firing patterns, i.e. regular spiking and fast spiking, respectively. Moreover, non-pyramidal interneuron-like cells remained innervated by excitatory inputs as both spontaneous and evoked non-NMDA mediated excitatory postsynaptic potentials were recorded. We conclude that putative inhibitory GABAergic cells are present and functional in the human epileptic tissue. *NeuroReport* 13:1421–1425 © 2002 Lippincott Williams & Wilkins.

Key words: Dormant inhibition; Human epilepsy; Interneurons; GABA; Slices

INTRODUCTION

Alterations in the inhibitory GABAergic system have been implicated in many experimental models of epilepsy (see [1] for review). However, although changes in $GABA_A$ receptor function have been reported in human epileptic tissue [2], electrophysiological and morphological pieces of evidence suggest that inhibition may be operative. In the human hippocampus, certain GABAergic neuronal types were preserved [3] and inhibitory postsynaptic potentials (IPSPs) were recorded in slices prepared from epileptic patients [4]. Similarly, in neocortical tissue, local inhibitory synaptic activity over pyramidal cells was recorded *in vitro* [5–7], even though regions of decreased immunostaining for parvalbumin and somatostatin were reported [8,9]. It is therefore likely that certain GABAergic interneurons may be preserved in the human epileptic tissue and that they remain functional during interictal activity.

Electrophysiological recordings from putative GABAergic human interneurons *in vitro* are scarce. Using neocortical tissue, Foehring *et al.* [10] described the electrophysiological properties of one morphologically identified interneuron. This cell was typically non-pyramidal and aspiny, and the electrophysiological response to depolarizing current injection was characterized by a fast-spiking pattern, similar to GABAergic interneurons from rodents [11–13]. Three other fast-spiking cells were recorded by Foehring's group [10] but their morphological appearance was not evaluated. Similarly, Schwartzkroin and Haglund [14] and Telfeian *et al.* [15] reported single cases of fast-spiking cells without anatomical verification. In these reports a correlation between interneuron location and electrocorticographically normal (non-spiking) or abnormal (spiking) tissue was not explicitly considered.

Here, we addressed whether interneuron-like cells remain functional in human epileptic tissue *in vitro*. We combined visually assisted whole-cell recordings with dye labeling to record from non-pyramidal interneuron-like cells in neocortical slices from human epileptic tissue. Intraoperative electrocorticography (ECoG) was performed to tailor resection. This information was subsequently used to select specific areas of the resected tissue for slicing. We examined several passive and active electrophysiological properties from interneuron-like cells, together with the presence of glutamatergic synaptic activity.

MATERIALS AND METHODS

Human tissue from the lateral temporal neocortex was removed during surgical treatment of drug-resistant temporal lobe epilepsy in two patients. Informed consent was obtained prior to surgery. The ethics committee at the Hospital de la Princesa of Madrid, and at the Instituto Pluridisciplinar at the Universidad Complutense, approved the present study. Intraoperative ECoG recordings were

performed to define the location of the cortical region producing spiking activity and to tailor the cortical resection limits. ECoG recordings were performed using a 4×5 electrode subdural grid placed over the lateral temporal cortex covering gyri T1, T2 and T3 (Fig. 1a). A second grid of 1×4 electrodes was positioned under the mesial temporal cortex to monitor activity adjacent to the hippocampal/entorhinal cortex formation. The reference electrode was placed in nearby scalp. ECoG recordings were obtained under low doses of sevoflurane (0.5%) and remifentanyl (0.1 mg/kg/min) using a 22-channel EEG amplifier, digitized and stored in a PC.

The principal spiking electrodes were identified in the surgery room and their positions were marked *in situ* using both a video camera and a schematic representation (Fig. 1a). Anterior portions of the middle (T2) and inferior (T3) temporal gyri were removed using information from the ECoG. After resection, neocortical tissue was immersed in a cold (0–4°C) solution containing (in mM): 250 sucrose, 2.5 KCl, 2 $MgSO_4$, 1.2 NaH_2PO_4, 2 $CaCl_2$, 22 $NaHCO_3$, 10 glucose and pH 7.4, bubbled with 95% O_2/5% CO_2. The specimen was photographed and the location of the spiking area was identified using information from intraoperative ECoG. A small block of tissue ($\sim 1\,cm^3$) was removed from neocortical spiking areas and transferred to the laboratory for slicing. Slices were cut at 250 µm using a vibratome and maintained at room temperature for ≥ 1 h in a normal solution containing (in mM): 125 NaCl, 3 KCl, 1.2 $MgSO_4$, 1.2 NaH_2PO_4, 2 $CaCl_2$, 22 $NaHCO_3$, 10 glucose and pH 7.4. A thionin Nissl staining of the adjacent tissue showed a normal cytoarchitectonic appearance (Fig. 1b).

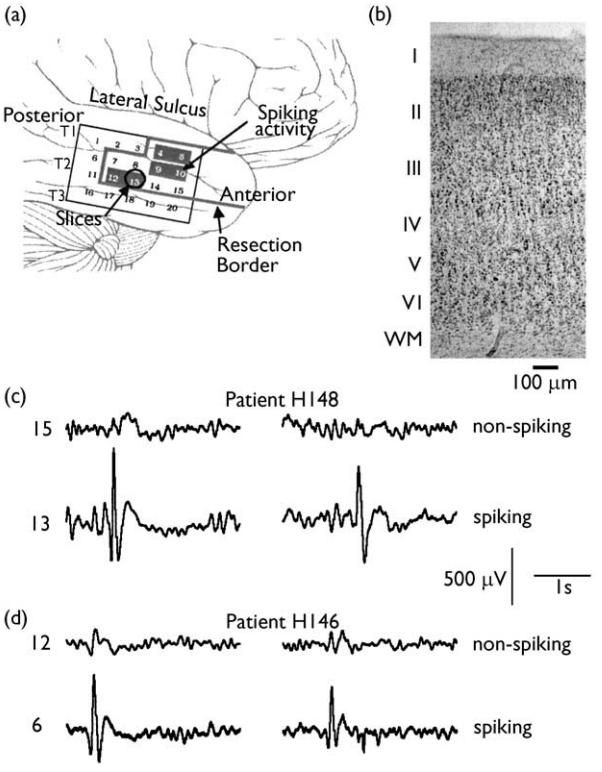

Fig. 1. Intraoperative electrocorticographic recordings from the lateral temporal neocortex. (**a**) Schematic representation of the position of the subdural electrode grid (4×5 contacts), the localization of interictal spiking areas (gray) and the resection border (thick line). Slices were prepared from neocortical areas showing intraoperative spiking activity (circle). TI, T2 and T3 refer to anterolateral superior, middle and inferior temporal gyri. (**b**) Nissl staining from neocortical tissue adjacent to those used for slices showed a normal cytoarchitectonic appearance. (**c**) Two epochs (left and right) of non-spiking and spiking intraoperative activity from patient HI48. Slices were prepared from neocortical areas under spiking electrode 13. (**d**) Non-spiking and spiking intraoperative activity from patient HI46. Slices were prepared from neocortical areas under electrode 6.

Slices were transferred to a submerged type-recording chamber (flow rate 1–1.5 ml/min at 32–34°C) attached to an upright infrared Olympus DIC microscope. Recordings were made at 800–1400 µm from the pial surface, corresponding to middle layers (III–IV) approximately, using a $\times 4$ lens for electrode placement. Cells were visualized using a $\times 60$ objective. Somatic whole-cell recordings in current-clamp mode were made using an Axoclamp 2B amplifier (Axons Instruments), digitized (Digidata 1322A, Axon Instruments) and stored on disk at 10 kHz. Patch pipettes were filled with intracellular solution containing (in mM): 131 K-gluconate, 6 KCl, 1 $MgCl_2$, 1 NaCl, 1 EGTA, 5 HEPES, 2 K_2-ATP; pH 7.4 and osmolarity 290–300 mOsm. For subsequent morphological classification 0.1–0.5% Lucifer Yellow dipotassium salt (Sigma) was added to the internal solution. After recordings, labeled cells were viewed and photographed using fluorescent microscopy.

Passive membrane properties were measured using current pulses of 300–500 ms duration (amplitude of ± 0.05 nA and ± 0.1 nA). Active properties such as action potential amplitude, duration and threshold were measured using 50 ms current pulses. Extracellular stimuli were delivered to the white matter using a monopolar tungsten electrode (0.1 ms duration and 0.5–1 mA). The amplitude and onset delay of the evoked excitatory postsynaptic potentials (EPSPs) were measured at resting membrane potential. The non-NMDA receptor antagonist 6-cyano-7-nitro-quinoxaline-2,3 (CNQX, Sigma) was used to examine synaptic activity. Values are reported as mean $\pm$ s.d. Data were compared using Student's *t*-test at $p = 0.05$.

RESULTS

Spiking ECoG activity in the lateral temporal neocortex was identified intraoperatively by detailed visual inspection (Fig. 1c,d). Interictal spikes were defined as a sharp triangular wave lasting < 70 ms and clearly distinct from the background [16]. Subsequent off-line analysis confirmed this result being the areas from where slices were prepared involved in interictal spiking (spike mean amplitude: 571 ± 112 µV; data from 25 spikes). Patients were followed up at 3–6 month intervals and were seizure-free for > 1 year. This strongly suggests that those lateral neocortical spiking areas removed during resection were likely to participate in epileptic activity.

Visual infrared-assisted examination of neocortical slices allowed the morphological identification of both pyramidal and non-pyramidal neurons. Pyramidal cells had triangular somata and a single main dendrite with apical orientation. Non-pyramidal cells typically had ovoid-like somata and bipolar or multipolar dendritic processes. Whole-cell

recordings were obtained from five non-pyramidal (Fig. 2a1) and three pyramidal (Fig. 2a2) cells. Lucifer Yellow labeling confirmed morphological classification (Fig. 2d).

Electrophysiologically, non-pyramidal cells were characterized by a fast-spiking (FS) pattern in response to depolarizing current pulses (Fig. 2a1). Occasionally, non-pyramidal cells tended to fire spikes interrupted by non-firing periods (see inset in Fig. 2b1), similar to that described in FS cells from normal human neocortical tissue [10]. By contrast, pyramidal cell responses were characterized by a regular spiking pattern (Fig. 2a2). These properties of the firing dynamics were more evident by representing the inter-spike interval (ISI) against the time during repetitive firing for various current pulses (Fig. 2b1,b2; arrows in b1 point to firing interruption exhibited by the non-pyramidal cell shown in a1 for pulses of 0.2 and 0.6 nA). We also investigated the firing rate of the first and the second ISI as a function of current pulse intensity (f–I relationship) in pyramidal and non-pyramidal cells. The fast-spiking nature of non-pyramidal cell firing was typical for all current pulse intensities (Fig. 2c1), in contrast to pyramidal cells (Fig. 2c2). In both cases the f–I relationship was fitted by linear regression with mean slope of 101 ± 11 Hz/nA and 32 ± 3 Hz/nA for the first ISI in non-pyramidal and pyramidal cells, respectively (significantly different at $p = 0.05$). The mean firing frequency of these two cell types were computed from depolarizing pulses of 0.4–0.5 nA and were also statistically different (Table 1). Non-pyramidal FS cells had action potentials of similar amplitude and threshold to pyramidal cells, but of shorter half-width (Table 1). Passive properties such as resting membrane potential (RMP) and membrane time constant were similar in these cell types, with input resistant tended to be higher in non-pyramidal than in pyramidal cells, although not significantly so (Table 1). All these properties of human non-pyramidal FS cells from intraoperative spiking areas were similar to those described in normal human [10] and rodents FS cells [11,12]. Since this cell type has been associated with GABAergic interneurons [13] it is likely that non-pyramidal FS cells reported here belongs to this group.

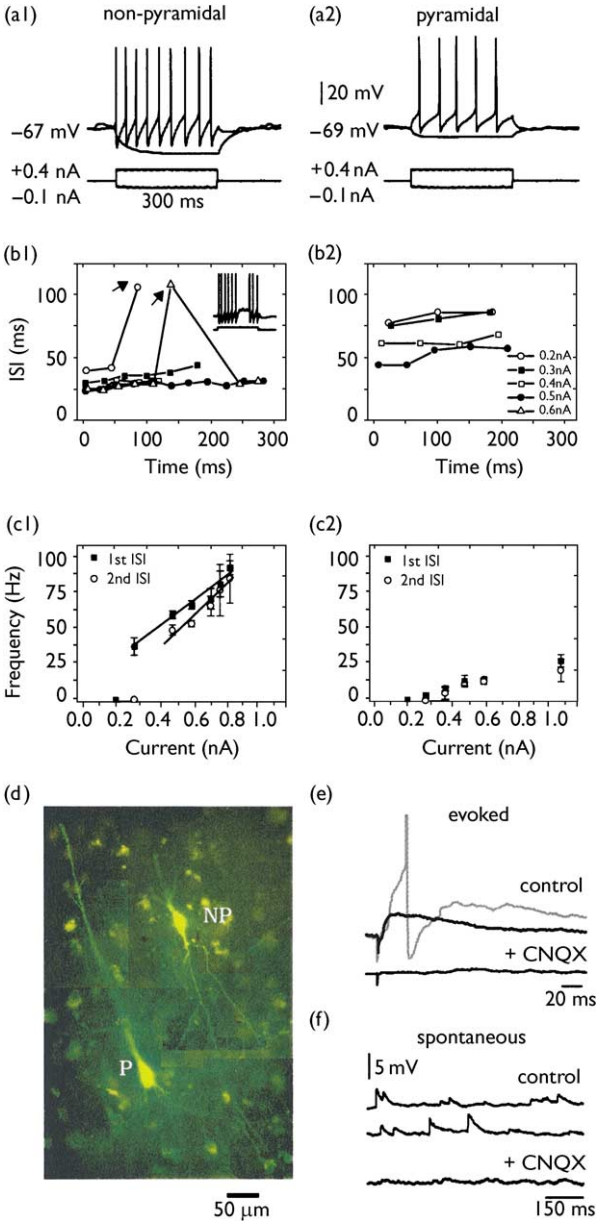

Fig. 2. Whole-cell recordings from non-pyramidal and pyramidal human cells *in vitro*. (**a**) A typical response from non-pyramidal (a1) and pyramidal (a2) cells to depolarizing and hyperpolarizing current pulses. (**b**) Relationship between the inters-spike interval (ISI) and the time during repetitive firing for different current pulses in non-pyramidal (b1) and pyramidal cells (b2). (**c**) Relationship between the firing frequency and the current pulse intensity for the first and the second ISI in non-pyramidal (c1) and pyramidal cells (c2). (**d**) Photomicrograph reconstruction of the non-pyramidal (NP) and pyramidal (P) cells whose recordings are shown in (a). (**e**,**f**) Representative evoked (e) and spontaneous (f) postsynaptic activity recorded from non-pyramidal cells.

Although functional GABAergic interneuron-like cells were present in spiking areas of human neocortex it could be possible that they did not remain innervated by excitatory inputs resulting in their network disconnection, as suggested in the epileptic hippocampus [17]. To explore this possibility we examined both the evoked and spontaneous synaptic activity of non-pyramidal FS cells. Single extracellular stimulation of the white matter evoked EPSPs in non-pyramidal FS cells of 6.1 ± 2.3 mV amplitude at RMP of -70 mV (Fig. 2e, black trace). These EPSPs had a short onset latency of 4.8 ± 1.1 ms, consistent with a direct excitation. Increasing stimulation strength evoked single spikes in FS cells (Fig. 2e, gray trace). The glutamatergic nature of evoked EPSPs was examined by bath application of the non-NMDA receptor antagonist CNQX (10 μM). CNQX blocked evoked EPSPs (Fig. 2e, lower trace). Spontaneous EPSPs were also recorded in all non-pyramidal FS cells (Fig. 2f). These spontaneous EPSP activity had a frequency of 3.3 ± 1.5 Hz and amplitude that ranged from 1.2 to 6.2 mV. Bath application of CNQX blocked spontaneously occurring EPSPs (Fig. 2f).

DISCUSSION

Using visually assisted whole-cell recordings we characterized the electrophysiological properties of interneuron-like cells from intraoperative spiking areas in epileptic patients.

Table I. Electrophysiological properties of human neocortical cells recorded *in vitro*.

	Non-pyramidal	Pyramidal
Active properties		
Mean firing rate (Hz)	43.5 ± 5.7	11.5 ± 2.1
Action potential amplitude (mV)	78.2 ± 12.3	81.5 ± 14.8
Action potential duration (ms)	0.7 ± 0.1	0.9 ± 0.1
Action potential threshold (mV)	−43.2 ± 5.6	−44.1 ± 1.4
Passive properties		
Resting membrane potential (mV)	−65.1 ± 2.3	−60.9 ± 5.3
Input resistance (R_{in}; MΩ)	96.2 ± 36.6	75.1 ± 7.1
Membrane time constant (τ_m; ms)	15.7 ± 3.1	17.5 ± 2.8
n	5	3

The electrophysiological passive and active properties of these cells were similar to those described in FS interneurons from normal neocortical tissue in both human [10] and rodent [11,12]. They were characterized by a fast-spiking pattern in response to depolarizing current injection and the presence of narrow spikes. Human FS interneurons from interictal spiking areas remained innervated by excitatory glutamatergic inputs, since both evoked and spontaneous non-NMDA mediated EPSPs were systematically recorded, as reported in epileptic rats [18]. This result is important in the view of the dormant interneuron hypothesis underlying experimental models of epilepsy in the hippocampus [17]. According to this hypothesis, a loss of excitatory innervation by GABAergic inhibitory cells would results in their disconnection and consequently in network disinhibition. Our results suggest that this does not apply to the neocortex since interneuron-like cells recorded from those areas exhibiting spiking activity *in situ* remained synaptically innervated. Up to our knowledge, this is the first study that looks at the correlation between electrocorticographically abnormal (spiking) tissue and the presence of synaptically functional interneuron-like cells. Also, this report extends the data available on human FS interneurons to those areas involved in spiking activity *in situ*.

The idea of alterations of the GABAergic system underlying epilepsy includes the presence of a decreased inhibitory control of neuronal excitability. Such a loss of inhibition comprises a variety of possibilities including (a) a massive loss of GABAergic interneurons, (b) alterations of their intrinsic firing properties, (c) loss of their excitatory input, (d) presynaptic downregulation of GABA release and (e) postsynaptic alterations due to a decreased GABAergic innervation or down-regulation of the GABA receptor function. Some of these alternatives have being investigated in epileptic human tissue to test hypotheses (a) [3,8,9,19] and (e) [2,4,6,7]. However, there is substantial lack of electrophysiological data from human GABAergic interneurons so that hypotheses (b) and (c) could be investigated. Few human FS cells have been recorded *in vitro* being morphologically identified as interneurons in only one case [10]. These recordings were from non-epileptic tissue, and only in one case was a FS cell was recorded from the epileptic hippocampus during synchronous *in vitro* activity [14]. The electrophysiological properties of this single FS cell were similar to those reported from normal human tissue [10]. In agreement with this, we found that non-pyramidal FS interneurons recorded *in vitro* from those areas that showed intraoperative spiking activity *in situ* shared similar morphological and electrophysiological properties with GABAergic interneurons from normal tissue. These results suggest that there are no changes in the intrinsic excitability of FS interneurons in the epileptic human tissue. Also, because of the excitatory glutamatergic inputs remain functional in these cells, a complete disconnection of GABAergic interneurons is not likely to support disinhibition in the human epileptic neocortex.

However, although FS interneurons were recorded and remained innervated by excitatory inputs they can represent only a particular subpopulation of GABAergic interneurons. Also, disinhibition can be supported by other alternatives such as hypotheses (d) and (e) above. Although postsynaptic GABAergic currents appear to be electrophysiologically normal in human epileptic tissue [4,14], it has been shown that specific subclasses of interneurons, e.g. parvalbumin-immunoreactive chandelier cells [8] or somatostatin-positive cells [19], did degenerate in the epileptic neocortical tissue, concomitantly with a reduction of GABAergic terminals [20]. Furthermore, these changes can occur in relatively small cortical regions [19,20]. We therefore cannot exclude interneuronal loss in the epileptic human tissue examined by us, instead our results demonstrate that at least those interneurons that were present retain their normal firing properties and excitatory synaptic drive (but see [21]). On the other hand, inhibition can be increased or decreased in human epilepsy [2] or be dynamically modulated during interictal states [5], suggesting a role in synchronizing neuronal activity [22]. It would be therefore important to correlate in future studies the electrophysiological properties of FS interneurons with their morphological and neurochemical characteristics in order to understand their role in the puzzle of GABAergic inhibition in epilepsy.

CONCLUSIONS

We showed evidence of the presence of fast-spiking interneurons in the human neocortical tissue *in vitro* prepared from regions exhibiting intraoperative spiking activity. The electrophysiological and morphological properties of these cells were similar to those reported in normal tissue from human and rat. More importantly, both evoked and spontaneous non-NMDA mediated EPSPs were recorded in these cells suggesting that they remained

innervated by excitatory inputs. Whether these cells represent only a subpopulation of neocortical interneurons and whether they participate in population network activity in human epileptic tissue requires further studies.

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