Epileptiform synchronization in the human dysplastic cortex

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ABSTRACT − Taylor’s focal cortical dysplasia corresponds to a localized disruption of the normal cortical lamination with an excess of large, aberrant cells. Sustained epileptic discharges originate from the dysplastic neocortex and this tissue retains sufficient connectivity for expressing seizure abnormalities. In this brief review, we summarize the findings obtained by analyzing surgically-resected human tissue with focal cortical dysplasia that was maintained in vitro in a brain slice preparation. These data have been compared with those obtained from human cortex with normal structural organization; such tissue was available from patients undergoing surgery for a variety of neurological disorders, most often for mesial temporal lobe epilepsy. These studies have shown that: (i) slices obtained from focal cortical dysplastic tissue have an intrinsic ability to generate ictal-like epileptiform events when challenged with the convulsant drug 4-aminopyridine; (ii) 4-aminopyridine-induced ictal discharges are not seen in neocortical slices obtained from neocortical samples with no or minimal structural lesion; (iii) these ictal discharges are caused by the activation of excitatory amino acid receptors, and in particular those of the N-methyl-D-aspartate type; (iv) focal cortical dysplastic tissue also generates synchronous potentials that are mainly contributed by GABA_A receptor-mediated conductances.

KEY WORDS: dysplasia, synchronization, 4-aminopyridine, human cortex

Introduction

Taylor’s focal cortical dysplasia (FCD) corresponds to a localized disruption of the normal cortical lamination with an excess of large, aberrant cells [1]. The radiological picture of FCD in high resolution magnetic resonance imaging (MRI), includes an increased cortical thickness, abnormal gyral pattern, blurring of the gray-white matter interface, increased signal in T2-weighted or proton density images, and signal changes in the underlying white matter [2-6]. These MRI characteristics have made it possible to identify FCD in an increasing number of patients who often present with intractable epilepsy. Sustained epileptic discharges originate from the dysplastic neocortex, and this tissue retains sufficient connectivity for expressing seizure abnormalities [6, 7]. Indeed, the inclusion of dysplastic cortex within the resected tissue determines the degree of seizure control that is achieved in patients undergoing surgical treatment.

Over the last two decades, several studies have analyzed the fundamental properties of the human neocortex in vitro. This tissue was obtained dur-
ing epilepsy surgery, most often from biopsy tissue removed from temporal lobe epilepsy patients [8]. These patients’ brains display a rather selective neuronal loss in the hippocampal formation, while the neocortex is characterized by a mild to moderate degree of gliosis with no structural organization abnormalities. Findings obtained from this type of human neocortical tissue have shown cellular and pharmacological features similar to those seen in normal rodent or feline neocortex [8].

Several animal models of cortical dysgenesis exist [9-12]. However, none of them successfully replicate Taylor-type FCD. To circumvent this problem, we have recently used surgically resected human FCD tissue that was maintained in vitro in a brain slice preparation [13, 14]. Here we will review these data that have advanced our knowledge on the cellular and pharmacological properties of FCD neuronal networks, and from this we have identified some mechanisms that may be relevant for epileptiform synchronization in this epileptic disorder. This short review is dedicated to Claudio Munari, a rigorous epileptologist and a wonderful friend for those who were fortunate to know him.

Patterns of synchronous activity in FCD and structurally normal networks

Spontaneous field potential activity is rarely seen in cortical slices maintained in vitro, regardless of their origins (e.g., hippocampus versus neocortex, human versus rodent brain). Hence, in the studies that were performed in human neocortical tissue, we used pharmacological procedures known to increase neuronal excitability in vitro in order to obtain synchronous field potentials with characteristics similar to those recorded in vivo in the EEG of epileptic patient. In particular, in the experiments reviewed here, we bath applied the K+ channel blocker 4-aminopyridine (4AP, 50-100 mM), a drug that does not antagonize GABAergic inhibition, but readily induces neuronal network synchronization by increasing the release of both inhibitory and excitatory transmitters [15, 16].

As illustrated in Fig. 1A, 4AP application to neocortical slices obtained most often from patients presenting with mesial temporal lobe epilepsy (i.e., with no evident structural abnormality), induced the appearance of spontaneous, synchronous activity. As illustrated in figure 1D, these 4AP-induced synchronous events continued to be recorded during bath application of ionotropic, excitatory amino acid receptor antagonists. A recent study performed with [K+]o recordings [18] has demonstrated that the isolated field potentials recorded in the human neocortex during application of medium containing 4AP + ionotropic excitatory amino acid receptor antagonists are accompanied by rises in [K+]o (up to 4.1 mM from a baseline of 3.25 mM) (figure 1D). Evidence obtained in this and previous studies [17] has indicated that the isolated events induced by 4 AP in human neocortical tissue with no structural abnormality represent mainly network, GABA receptor-mediated phenomena that reflect the activation of type A receptors following GABA release from interneurons.

In contrast, we have found that bath application of similar concentrations of 4 AP to human FCD slices can induce two main types of spontaneous, synchronous activity. As illustrated in figure 2, the first type consists of prolonged negative shifts (duration = 14-108 s, interval of occurrence = 61-460 s) with superimposed fast (duration = 35 to 100 ms) transients of negative polarity (figure 2, continuous lines in panels A-C). Typically, these fast events occurred at a high rate (up to 12Hz) during the initial 2-20s of the discharge and later became of larger amplitude while decreasing in frequency. In some cases this synchronous activity progressed toward a clear, clustered pattern (figure 2C). Hence, these discharges (thereafter called ictal) were reminiscent of the electrographic pattern associated with tonic-clonic seizures in situ.

The second type of synchronous activity induced by 4 AP consisted of isolated negative events (arrows in figure 2A-C) that lasted 0.8-3 s and recurred at intervals of 6-40 s. Thus, this type of activity resembled that observed in neocortical slices obtained from patients with mesial temporal lobe epilepsy. However, when analyzed at high speed these isolated events could contain repetitive, fast transients of low amplitude (up to 0.05 mV) (arrow-heads in figure 2Db2).

Overall, these data indicate that 4 AP concentrations that are unable to disclose epileptiform activity in human cortical slices with no structural abnormality [17,18], elicit clear epileptiform events in the FCD tissue maintained in vitro. Moreover, these epileptiform discharges resemble the electrographic seizures recorded in FCD patients during preexcision EcoG [6]. These electrographic patterns are considered to be specific and sensitive indicators of FCD lesions [6, 7].

Intrinsic properties and repetitive firing of neurons in FCD slices

Intracellular recordings obtained from neurons located 800-1 600 µm from the pia in FCD slices have demonstrated resting membrane potentials, apparent input resistances and subthreshold properties that were similar to
those observed in neurons analyzed in neocortical tissue samples with no or minimal structural lesion (i.e., those obtained from mesial temporal lobe epileptic patients) [14]. In these experiments, we also found that FCD neurons generated fast action potentials with amplitudes (calculated from the baseline) in excess of 80 mV. Moreover, when depolarized with pulses of intracellularly injected current (duration = 100 to 300 ms), these neurons generated regular spiking activity with adaptation. Depending on the resting membrane potential, a long-lasting, after-hyperpolarization (160 to 250 ms) could follow the pulse termination (not illustrated, but see Avoli et al. 1999) [14]. Overall, these intracellular properties suggest that neurons recorded in FCD slices do not have any apparent abnormality as regards the intrinsic membrane properties or the ability to generate repetitive firing. However, this conclusion should be treated with caution because of the small sample of neurons recorded so far in FCD slices, and because we did not establish their morphological characteristics (i.e., by injecting them with intracellular dyes). It is indeed possible that we did not succeed in recording from the large, aberrant cells that are characteristic of FCD tissue [1].

Glutamatergic receptor contribution to epileptiform synchronization in FCD slices

Ictal discharges induced by 4 AP in FCD slices are readily abolished by antagonists of the NMDA receptor, while the isolated field potentials continued to occur (figures 3A, 4 AP + CPP). Similar effects were seen during application of antagonists of non-NMDA receptors (not illustrated). However, as seen in slices obtained from patients present-
ing with mesial temporal lobe epilepsy (figure 1D), the isolated field potentials continued to occur during concomitant application of NMDA and non-NMDA receptor antagonists, although they were of shorter duration. These events were abolished by further addition of the GABAA receptor antagonist bicuculline methiodide (figure 3B).

The role played in ictogenesis by glutamatergic receptor-mediated mechanisms, and in particular those of NMDA type, is in line with a recent immunohistochemical study of human FCD tissue [19]. Here it was found that the intensity and distribution of excitatory amino acid receptors in dysplastic tissue is abnormal when compared with adjacent normal cortex, and with previous data from normal human material [20]. Moreover, it has been reported that large neurons located in the deepest portion of the cortex of patients with FCD, preferentially contain the NMDAR1 subunit, while large pyramidal cells express GluR2-3 AMPA subunits [19]. Hence, these immunocytochemical data support the conclusion that an NMDA receptor-mediated mechanism, located in the deep cortical structure, represents a main factor in the occurrence of ictal events in FCD tissue.

The involvement of NMDA and non-NMDA receptors in the initiation and propagation of seizure activity has been demonstrated in many models of epileptiform discharge, both in vivo and in vitro. For instance, ictal discharges induced by 4 AP, or pilocarpine in the normal rat entorhinal cortex are abolished by NMDA receptor antagonists [21, 22], while similar epileptiform events are insensitive to NMDA receptor antagonists in the juvenile rat hippocampus, but readily abolished by antagonizing the AMPA receptors [23, 24].

The pharmacological experiments performed in FCD slices also show that this type of tissue, when challenged with 4 AP, can generate isolated synchronous field potentials that can continue to occur during concomitant application of NMDA and non-NMDA receptor antagonists. Thus, as reported in human tissue with no apparent structural abnormality [17, 18], GABA receptors, mainly type A receptors, can sustain synchronization without any contribution by NMDA and/or non-NMDA receptor-mediated conductances.

**Conclusion**

We have reviewed here some fundamental properties that characterize neocortical networks obtained from FCD pa-
Patients maintained in vitro in a slice preparation during application of the convulsant drug 4 AP [14]. These data were also compared with those seen in human neocortical slices from patients presenting with mesial temporal lobe epilepsy [17, 18]. This evidence indicates that: (i) slices obtained from FCD tissue have an intrinsic ability to generate ictal epileptiform events when challenged with 4 AP; (ii) similar prolonged discharges cannot be recorded in slices obtained from neocortical samples with no or minimal structural lesion (i.e., those from mesial temporal lobe epileptic patients); (iii) ictal discharges in FCD tissue are caused by the activation of excitatory amino acid receptors, and in particular those of the NMDA type; (iv) FCD, as well as mesial temporal lobe neocortical slices, generate isolated synchronous potentials that are mainly contributed by GABA<sub>A</sub> receptor-mediated conductances.

An important aspect that needs to be addressed in future studies, is the role of GABA receptor-mediated mechanisms in the genesis of epileptiform synchronization in FCD tissue, and thus in the generation of seizures in FCD patients. To date, no functional analysis of GABA receptor-mediated potentials has been carried out from FCD slices maintained in vitro and superfused with normal medium. Hence, it is not known whether IPSPs or responses to GABA receptor agonists are different when compared with tissue presenting no structural abnormality.

However, the immunocytochemical data obtained by several authors [19, 25, 26] indicate that in Taylor-type FCD tissue, even though GABAergic cells are decreased in number, they provide an ‘overexpression’ of GAD-positive terminals that surround large, aberrant glutamatergic neurons. This evidence is in line with the ability of 4 AP to induce synchronous potentials that are caused by the activation of GABA, mainly type A receptors. Indeed, these GABA receptor-mediated events may play an important role in initiating seizure activity, as documented in rodent entorhinal cortex in which ictal discharges elicited by 4 AP are initiated by a similar type of GABA receptor-mediated synchronization [21, 27, 28].

**References**


