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Models of drug-induced epileptiform synchronization in vitro

Massimo Avoli^{a,b,*} and John G.R. Jefferys^c^aMontreal Neurological Institute and Departments of Neurology & Neurosurgery, and of Physiology, McGill University, Montréal, QC, Canada H3A 2B4^bDepartment of Experimental Medicine, Facoltà di Medicina e Odontoiatria, Sapienza University of Rome, Roma 00185, Italy^cDepartment of Pharmacology, University of Oxford, Oxford OX1 3QT, UK

Abstract

Models of epileptiform activity in vitro have many advantages for recording and experimental manipulation. Neural tissues can be maintained in vitro for hours, and in neuronal or organotypic slice cultures for several weeks. A variety of drugs and other agents increase activity in these in vitro conditions, in many cases resulting in epileptiform activity, thus providing a direct model of symptomatic seizures. We review these preparations and the experimental manipulations used to induce epileptiform activity. The most common of drugs used are GABA_A receptor antagonists and potassium channel blockers (notably 4-aminopyridine). Muscarinic agents also can induce epileptiform synchronization in vitro, and include potassium channel inhibition amongst their cellular actions. Manipulations of extracellular ions are reviewed in another paper in this special issue, as are ex vivo slices prepared from chronically epileptic animals and from people with epilepsy. More complex slices including extensive networks and/or several connected brain structures can provide insights into the dynamics of long range connections during epileptic activity. Visualization of slices also provides opportunities for identification of living neurons and for optical recording/stimulation and manipulation. Overall, the analysis of the epileptiform activity induced in brain tissue in vitro has played a major role in advancing our understanding of the cellular and network mechanisms of epileptiform synchronization, and it is expected to continue to do so in future.

Keywords

Animal models in vitro; K⁺ channel blockers; GABA_A receptor antagonists; Epileptiform synchronization

1. Introduction

Li (1959) was presumably first in identifying the intracellular counterpart of an EEG interictal spike generated by feline cortical neurons in response to strychnine application. Interestingly, in collaboration with McIlwain, he had shown two years earlier that cortical

*Corresponding author at: Montreal Neurological Institute McGill University, 3801 University Street, Montréal, PQ Canada H3A 2B4. Tel.: +1 514 998 6790/+39 333 483 1060; fax: +1 514 398 8106. massimo.avoli@mcgill.ca (M. Avoli).

neurons could be maintained alive *in vitro* in a brain slice preparation (Li and McIlwain, 1957). These pioneering studies were followed by several *in vivo* experiments demonstrating that interictal discharges recorded in neocortical or hippocampal foci induced acutely by convulsants (Matsumoto and Ajmone-Marsan, 1964; Prince, 1967; Ayala et al., 1973) or freezing lesions (Goldensohn and Purpura, 1963) correspond to the so-called paroxysmal depolarizing shift (i.e., a large amplitude depolarization with sustained action potential firing that can be followed by a hyperpolarization). However, it was only late in the 1970s that the *in vitro* hippocampal slice preparation was secured as a reliable tool for analyzing the cellular and pharmacological mechanisms underlying epileptiform synchronization (Schwartzkroin and Prince, 1976a, 1977).

During the last four decades we have witnessed the introduction of different *in vitro* preparations that encompass extended brain slices including several interconnected (e.g., thalamocortical or hippocampus-rhinal cortical) areas, the whole isolated hippocampus as well as the isolated whole-brain (reviewed in this special issue by Marco de Curtis). These experiments have firmly demonstrated that epileptiform events resembling the electrographic interictal and ictal activities occurring in the EEG of epileptic patients can be recorded *in vitro*. Here we will review the findings obtained in these studies by focusing on experiments in which chemoconvulsants were used to acutely induce epileptiform synchronization in cortical (and most often limbic) networks. In the first section of our review we will describe the different *in vitro* methodologies. Then, we will analyze the results obtained by applying drugs that target specific pharmacological mechanisms.

2. *In vitro* brain preparations for studying epileptiform synchronization

2.1. Neuronal cultures

Cell culture is a long-established tool in molecular and cellular biology. From the point of view of drug-induced epilepsy models the loss of connectivity inherent in dissociated cell cultures is problematic, although the *de novo* connections formed by such cultures can result in interesting activities, which include in the extreme case the generation of epileptiform discharges by single isolated neurons due to their extensive recurrent synaptic connections. This point was made almost 40 years ago in the pioneering studies performed in Jeff Barker's laboratory at NIH in Bethesda (MD, USA) (Macdonald and Barker, 1978; Barker and MacDonald, 1980). These early studies used spinal cord neurons but were later extended to hippocampal cells cultured *in vitro* (Furshpan and Potter, 1989; Segal, 1991).

A single cell in culture is outside the neuronal synchronization remit of this chapter. However, the fact that this preparation provided “early” important information of the mechanisms underlying epileptiform hyperexcitability grants its mentioning. Typically neurons are dissociated from foetal brain tissue using trypsin and gentle mechanical agitation, and plated in appropriate media, with or without glia. If they are plated at appropriate densities they form interconnected networks that can be used for epilepsy research (Dichter, 1978). As mentioned above, such primary neuronal cultures are susceptible to convulsant treatments (Giachello et al., 2013) or application of low-Mg²⁺ medium (De Lorenzo et al., 1998; Wang et al., 2014). Growing cultures on multi-electrode arrays can help make long-term recordings from this preparation (Potter and De Marse,

2001). High density dissociated neuronal cultures tend to develop synchronized, arguably epileptiform, bursting over periods of a few days. Given the ability of multi-electrode stimulation to prevent this process, most likely the underlying mechanism for the synchronous bursting is deafferentation and subsequent rewiring (Wagenaar et al., 2005).

2.2. Acute brain slices

Per Andersen's laboratory at the University of Oslo (Norway) was the inspiring place for establishing the hippocampal slice as a tool for neuroscience research, and specifically for electrophysiological studies focusing on the fundamental mechanisms underlying neuronal excitability. The choice of the hippocampus stemmed from the assumption that once "sliced" its lamellar organization could warrant interconnectivity approximating what occurring *in vivo* (Andersen et al., 1971). In these experiments "isolated" hippocampal slices were cut at thickness of 350–500 μm employing the original McIlwain chopper, and they were maintained in an interface tissue chamber similar to that originally employed by Li and McIlwain (1957). These studies made the neuroscience community aware of the unique advantages of this preparation that included: (i) stable intracellular recordings; (ii) ability to change the extracellular milieu; and (iii) direct application of known drug concentrations to the brain tissue.

Working in Andersen's laboratory, Phil Schwartzkroin began a series of experiments that were continued in collaboration with David Prince at Stanford University (CA, USA), and established some electrophysiological features of the paroxysmal depolarizing shifts associated to interictal spikes in hippocampal slices (Schwartzkroin and Prince, 1976a, 1977); these early experiments also extended to the use of human brain slices from brain tissue removed by the neurosurgeons (Schwartzkroin and Prince, 1976b). Around this time, several other investigators joined the *in vitro* track either going through the Norwegian training or starting their own "brain slicing procedure". This process made brain slicing be extended to other CNS structures, and led to the design of new recording tissue chambers with faster perfusing features (Haas et al., 1979) including the procedure of keeping brain slices submerged in artificial cerebrospinal fluid rather than lying at an interface (Jefferys, 1979; Nicoll and Alger, 1981). Overall, these experiments exploited the well-known connectivity of the hippocampus proper that was however limited to the classic circuit: dentate gyrus $\rightarrow$ CA3 $\rightarrow$ CA1 $\rightarrow$ subiculum. By doing so they underscored the pacing role of CA3 networks in the generation of short lasting interictal activity that resulted from the recurrent excitatory connections that link CA3 pyramidal cells along with their ability to produce high-threshold Ca^{2+} spikes (Schwartzkroin and Slawsky, 1977; Wong and Prince, 1978; Miles and Wong, 1983).

The successive introduction of the vibroslicer (an instrument similar to what used by histologists) to cut brain tissue for electrophysiological experiments enlarged the application of brain slice to the study of epileptiform synchronization. In fact, besides a better preservation of those neurons that are close to the cut surface, the vibroslicer allowed the introduction in several laboratories of "large" brain slices that could contain more extensive neuronal networks including "horizontal slices" of neocortex (Fleidervish et al., 1998) and hippocampus (Miles et al., 1988; Traub et al., 1993) comprising several, and often

interconnected, brain structures including those of the limbic (Stanton et al., 1987) and thalamocortical (Agmon and Connors, 1991; McCormick et al., 1995) systems. These “more complex” slice preparations have made possible to establish whether different types of epileptiform discharges were structure-specific, how they propagated from one structure to another, and how they could potentially influence each other (e.g., Barbarosie and Avoli, 1997).

2.3. Brain slices in culture

Organotypic slice cultures combine many of the benefits of long term culture with preservation of much of the normal connectivity found in acute brain slices. The slicing procedure causes a partial deafferentation, which in the case of slice cultures leads to sprouting of new synaptic connections. In most cases slice cultures are prepared from young or neonatal rodent brains, and may be cultured for weeks or even months, as long as they have the appropriate sterility, oxygen, incubation media and temperature control. Employing young brain for these experiments means that the tissue still is developing; such a point should be taken into consideration in interpreting the results. Interestingly, the earliest report of the use of slice cultures in epilepsy research come from the early 1970's when paroxysmal activity was shown to be induced by bicuculline, strychnine or repetitive stimulation in hippocampal slice cultures (Zipser et al., 1973). An extensive account of slice culture methods used with human tissue by Mark Cunningham et al. is included in this special issue.

Several methods can be used to prepare slice cultures (Gähwiler et al., 1997). Simple culture dishes will sustain slice cultures for a few days. Placing slices either on semi-permeable membranes at the surface of the culture medium or stuck to a glass coverslip in a roller tube. Semi-permeable membrane cultures retain more 3D structure, flattening to ~150 µm. In roller tubes the slices are alternately immersed and exposed to air on a cycle of ~10 min. This last procedure has the effect of flattening them to a cellular monolayer, which can be useful for visualizing single neurons. Early pioneers were Beat Gähwiler (Zimmer and Gähwiler, 1987) and Phil Schwartzkroin (Malouf et al., 1990). Slice cultures can develop interictal and seizure-like bursts (Malouf et al., 1990; Dyhrfeld-Johnsen et al., 2010), perhaps due to the sprouting of new synaptic connections following the deafferentation inherent in the slicing process. As in conventional slices, epileptiform activity can also be induced in non-epileptic organotypic slice cultures by GABA_A receptor antagonists, glutamatergic agonists, or K⁺ channel blockers (Thompson et al., 1996; Routbort et al., 1999; Bausch and McNamara, 2004). Epileptiform activity in this preparation can also be induced as a result of blocking neuronal activity for several days, presumably due to homeostatic changes in receptors following the silencing of the neuronal population (Bausch et al., 2006).

Organotypic slices share several advantages with other *in vitro* preparations in that they combine the easy access for drugs and other neuroactive treatments along with ease of recording. This provides approaches to investigate long-term processes such as epileptogenesis, neuroprotection, neuronal degeneration and sclerosis (Thompson et al., 1996; Chaturvedi et al., 2012; Kukko-Lukjanov et al., 2006; Ziobro et al., 2011; Koyama,

2013). One special feature of slice cultures is the ability to co-culture structures that are not adjacent *in vivo*, but which are functionally connected, e.g. a slice comprising hippocampal and posterior hypothalamic tissue containing histaminergic neurons to examine the protective effects of histamine (Kukko-Lukjanov et al., 2006). Organotypic slice cultures can also be used to investigate epileptogenesis in mice genetically altered to replicate epilepsy-related channelopathies (Lachance-Touchette et al., 2014).

2.4. Isolated hippocampi

In 1997, Khalilov et al. moved forward in the *in vitro* brain scene by demonstrating that the entire hippocampus (or even two interconnected hippocampi, including the septum) could be maintained alive in a fully submerged chamber with separated compartments to enable the application of drugs separately to each structure. Such preparation is useful for analyzing the generation and propagation of network activities or epileptiform discharges within the hippocampus, the septo-hippocampal system, or between the interconnected hippocampi. In this study, long-lasting ictal discharges (30–250 s) could be induced by applying solutions containing the GABA_A receptor antagonist bicuculline, the glutamate receptor agonist kainate, or high K⁺ while only brief interictal activities were recorded in age-matched slices under similar conditions. In addition to allow measurement of intracellular changes in [Ca²⁺] using confocal microscopy from groups of neurons loaded extracellularly with Fluo 3-AM, Khalilov et al. (1997) demonstrated that electrical stimulation of the septum inhibited the firing of hippocampal CA3 stratum oriens interneurons; this finding thus confirmed the existence of GABAergic projections from the septum to hippocampal interneurons (Freund and Antal, 1988).

This preparation has also allowed the demonstration that ictal discharges induced by bath application of kainic acid originate from the hippocampus and propagate to the septum, since they disappear in the septum following its surgical separation from the hippocampus (Khalilov et al., 1997). Finally, by employing field potential or dual whole-cell recordings from the two interconnected hippocampi it was shown that unilateral application of medium containing high Mg²⁺ or tetrodotoxin blocked the propagation of the ictal discharge generated in the contralateral hippocampus by high extracellular [K⁺]. Shortly after, Khazipov et al. (1999), proposed a further development of this preparation by designing a novel chamber in which two intact hippocampi and the commissural fibers were placed in three independent compartments that were separated by latex membranes and could be perfused selectively with different solutions. This type of preparation has led to the demonstration that a “secondary epileptogenic mirror focus” can be established by applying a convulsant to one hippocampus, allowing the propagation of seizure activity to the contralateral untreated hippocampus, and then blocking the connections between the two hippocampi with application of the voltage-gated Na⁺ channel blocker tetrodotoxin to the commissural chamber (Khalilov et al., 2003).

During the last two decades this preparation has been employed in several laboratories for analyzing the generation and spread of epileptiform discharges (Derchansky et al., 2004, 2006, 2008; Zhang et al., 2012). Isolated hippocampi in most of these studies had to be obtained from neonatal or very young rats (up to 15 days postnatal) but more recently older

animals (up to 22 days postnatal) have been used, even though these experiments have so far addressed the generation of physiological theta rhythms rather than epileptiform synchronization (Gu et al., 2013).

3. Drug-induced epileptiform patterns *in vitro*

3.1. GABA receptor antagonists

Following the tradition established in the 1960s for using drugs such as penicillin to induce focal epileptiform discharges (see Section 1), most of the early *in vitro* studies employed compounds that were, shortly after, demonstrated to be GABA_A receptor antagonists by several investigators (see for instance Alger and Nicoll, 1982). These pharmacological manipulations lead to the generation of epileptiform events that are usually characterized by electrographic features resembling interictal activity, i.e. short-lasting (<3 s) synchronous field events that are intracellularly associated with large amplitude depolarizations and sustained action potential firing. During application of medium containing GABA_A receptor antagonists, interictal events have been recorded in isolated hippocampal slices (Schwartzkroin and Prince, 1976a, 1977; Dingledine and Gjerstad, 1980; Miles and Wong, 1983), neocortical slices (Gutnick et al., 1982) including those obtained from epileptic patients undergoing epilepsy surgery (see for review Avoli et al., 2005) as well as in brain slices that included the entorhinal cortex and hippocampus (Jones and Lambert, 1990).

These studies firmly established that interictal events are accompanied by the reduction or blockade of GABA_A receptor-mediated IPSPs but also demonstrated the need of electrical stimulation to induce such interictal discharge since spontaneous activity rarely occurred with media containing “physiological” [K⁺] (i.e., around 3.5 mM). It has, in fact, been reported that during blockade of GABA_A receptor signaling, spontaneous interictal events in hippocampal slices occur only when the extracellular [K⁺] is set to levels higher than 5 mM (Tancredi and Avoli, 1987; Rutecki et al., 1987).

An additional problem with pharmacological procedures that antagonize GABA_A receptors, at least when brain slices are obtained from adult animals, is the fact that prolonged epileptiform events, resembling ictal discharge, are rarely observed or, perhaps, they may be labile. For instance, Borck and Jefferys (1999) reported robust prolonged epileptiform events in ventral hippocampal slices exposed to bicuculline, including in small segments containing CA3 and adjacent hilus; these ictal events became vanishingly rare following, perhaps coincidental, refinements in husbandry in the early 2000's (J.G. Jefferys, unpublished observations). Immature or juvenile brain tissue slices are, however, capable of generating ictal-like events during application of the weak GABA_A receptor antagonist penicillin, although these discharges as well do not occur spontaneously but rather need to be induced by local electrical stimulation (Swann and Brady, 1984). These investigators have also shown that similar sustained and prolonged ictal-like depolarizations are generated during penicillin application by pyramidal cells in “small isolated segments” of the CA3 subfield of hippocampal slices obtained from 1 to 2 week-old rats (Swann et al., 1993). Khazipov et al. (1999) have also shown that long-lasting (30–250 s long) epileptiform discharges are readily induced by application of bicuculline (as well as of kainate or high K⁺ solution) to the intact hippocampi maintained *in vitro* that were obtained from rats 0 to 15 days postnatal; as

already mentioned in Section 2.4, these authors found that, in contrast to what reported by Swann and Brady (1984), who employed penicillin as convulsive agent, only brief interictal activities could be recorded in slices obtained from age-matched animals under the same experimental conditions.

Epileptiform activity generated by hippocampal slices during reduction/blockade of GABA_A receptor signalling has been used to test the effects of some antiepileptic drugs. Early work has established that penicillin-induced interictal activity generated by neuronal networks in guinea pig hippocampal slices is suppressed by phenytoin, an effect that was proposed to be due to decreased excitatory synaptic transmission (Schneiderman and Schwartzkroin, 1982). Later, these experiments were extended to other antiepileptic drugs while employing different agents that weaken inhibition (e.g., picrotoxin or pentylenetetrazole) to induce interictal bursting in the CA3 region of the *in vitro* hippocampal slice (Piredda et al., 1986; Köhr and Heinemann, 1990). More recently, Armand et al. (1998) have analysed the effects of valproic acid derivatives on pentylenetetrazole-induced epileptiform discharges recorded from brain slices that included the entorhinal cortex and hippocampus. Overall these studies have shown that epileptiform discharges induced *in vitro* by GABA_A receptor antagonists are differentially sensitive to conventional and new antiepileptic drugs.

3.2. K⁺ channel blockers

Galvan et al. (1982) were presumably the first investigators to show that bath application of the K⁺ channel blocker 4-aminopyridine (4AP) could induce spontaneous or stimulus-evoked ictal-like discharges in slices of the guinea-pig olfactory cortex. 4AP was known to cause seizures *in vivo* (e.g., Szenté and Pongrácz, 1979) and to enhance transmitter release at both excitatory and inhibitory synapses in the hippocampal slice preparation (Buckle and Haas, 1982). Shortly after, Voskuyl and Albus (1985) identified 2 types of spontaneous, interictal-like field potentials in isolated hippocampal slices; the first type resembled the spontaneous epileptiform activity induced by GABA_A receptor antagonists and originated in the CA3 subfield, while the second lasted longer, spread at slower velocity and was resistant to glutamatergic receptor antagonism. These results were later confirmed by other investigators (Rutecki et al., 1987; Michelson and Wong, 1991; Perreault and Avoli, 1992; Watts and Jefferys, 1993).

Some of these studies also demonstrated that the “slow” interictal spikes induced by 4AP in the hippocampal slice reflected mainly the synchronous firing of interneurons leading to the postsynaptic activation of GABA, and in particular type A, ionotropic receptors, since these synchronous interictal events, when recorded in the presence of excitatory amino acid receptor antagonists were abolished by application of picrotoxin or bicuculline. The “slow” glutamatergic-independent spikes recorded in the CA3 subfield during 4AP application are associated with transient increases in extracellular [K⁺] that are caused by the activation of GABA_A receptors following the release of GABA from interneurons (Morris et al., 1996). In line with this view, Benardo (1997) has found in rat neocortical slices that interneurons generate periodic action potential firing in the presence of 4AP and excitatory transmission blockers while large amplitude IPSPs occur rhythmically in pyramidal cells; he also suggested that under these pharmacological conditions, interneurons are synchronized

through electrotonic coupling and recurrent collaterals (which release GABA thus causing postsynaptic GABA_A receptor depolarizations as proposed in the hippocampus by Michelson and Wong, 1991).

Epileptiform interictal discharges have also been identified during application of medium containing 4AP to neocortical slices obtained from patients undergoing surgery for pharmacoresistant temporal lobe epilepsy while ictal discharges could be recorded in brain slices from patients presenting with focal cortical dysplasia (reviewed in Avoli et al., 2005). In these experiments as well, synchronous events associated with increases in extracellular [K⁺] continued to occur during application of glutamatergic receptor antagonists and were abolished by subsequent application of GABA_A receptor blockers. Of interest for a methodological review, it has been shown that neocortical slices obtained with the McIlwain chopper respond to 4AP application in a species dependent manner. Specifically, ictal-like epileptiform discharges could be recorded in 80% of the guinea-pig neocortical slices but only in 6% of the neocortical slices obtained from rats; in addition, epileptiform activity was blocked in guinea-pig neocortical slices by antagonizing NMDA receptors while in the rat it disappeared only during perfusion with a non-NMDA glutamatergic receptor antagonist (Mattia et al., 1993).

As discussed above for the epileptiform activity induced by GABA_A receptor antagonists, 4AP application does not disclose ictal-like discharges in the adult isolated hippocampal slice. This is however not true when similar concentrations of 4AP are applied to hippocampal tissue obtained from young (between 10 and 23 day-old) animals (Chesnut and Swann, 1988; Avoli, 1990). Such ictogenetic “propensity” may reflect developmental changes in neuronal excitability and neurotransmitter signalling as well as in a less efficient ability of the juvenile tissue to control changes in extracellular [K⁺]. In line with this view, measurements of the extracellular [K⁺] elevations associated with the “slow” 4AP-induced interictal events occurring during blockade of glutamatergic transmission have shown larger values in juvenile than in adult hippocampal slices (Avoli et al., 1996b). However, in line with what originally reported by Galvan et al. (1982), ictal-like activity is readily generated in response to 4AP application by neuronal networks in several limbic and extralimbic structures such as the rhinal cortices, the amygdala, and the insular or cingular cortices (Avoli et al., 1996a; Brückner and Heinemann, 2000; Klueva et al., 2003; see for review Avoli and de Curtis, 2011). By employing the *in vitro* isolated hippocampus from new-born rats Luhmann et al. (2000) have shown that 4AP can induce interictal and ictal discharges in the CA3 area as well as that these events can propagate to the entorhinal cortex when animals older than 3 days are used. Remarkably, the pharmacological characteristics of the 4AP-induced epileptiform discharges recorded from the isolated hippocampus were very similar to those identified in the young rat hippocampal slice preparation (Avoli et al., 1996b) since they were abolished by non-NMDA receptor antagonists while they were not significantly influenced by drugs that blocked the NMDA receptor. In contrast, ictal discharges generated by adult limbic areas in brain slices bathed in 4AP containing medium are highly sensitive to both NMDA and non-NMDA glutamatergic receptor antagonists (see for review Avoli and de Curtis, 2011).

Slices of the in young hippocampus as well as those obtained from several adult limbic structures generate, during 4AP application, ictal discharges that are initiated by synchronous events that reflect the firing of GABA releasing cells thus suggesting the involvement of GABA in ictogenesis (Avoli and de Curtis, 2011). This hypothesis has recently been tested with optogenetic techniques in the entorhinal cortex during 4AP treatment (Shiri et al., 2015; Yekhlef et al., 2015); these experiments have shown that optogenetic activation of parvalbumin- or somatostatin-positive interneurons can elicit ictal discharges that are similar to those occurring spontaneously, including their association with initial isolated spikes that are caused by the optogenetic stimulation of interneurons. As discussed above, slow interictal discharges induced by 4AP are associated with transient increases in extracellular $[K^+]$ due to the activation of $GABA_A$ receptors (Morris et al., 1996) and more specifically to the activation of the KCC2 co-transporter, which causes the extracellular efflux of both K^+ and Cl^- (Viitanen et al., 2010). As reviewed by Avoli and de Curtis (2011), such increases in extracellular K^+ are expected to depolarize neighbouring neurons, to promote ectopic spike generation, and to shift the reversal potential of $GABA_A$ receptor-mediated IPSPs in a positive direction, thus weakening inhibition. All these mechanisms, which are paradoxically initiated by the activation of $GABA_A$ receptor signalling, are known to increase neuronal excitability and are thus likely to promote ictogenesis in the 4AP *in vitro* brain slice model.

The *in vitro* 4AP model has been used to evaluate the effects of antiepileptic drugs. During application of 4AP, standard antiepileptic compounds can abolish ictal discharges in isolated young rat hippocampal slices at concentrations that are unable to influence shorter, interictal activity (Fueta and Avoli, 1992); in addition, it was shown in this study that antiepileptic drugs reduced interictal discharges in adult hippocampal tissue only at very high concentrations. In partial agreement with these findings Watts and Jefferys (1993) have reported that in rat transverse hippocampal slices exposed to 4AP, carbamazepine abolishes long-lasting interictal events but does not influence shorter discharges. Similar evidence has been later obtained from combined adult rat hippocampus-entorhinal cortex slices in Uwe Heinemann's laboratory. Specifically, Brückner and Heinemann (2000) found that prolonged ictal discharges initiating in the entorhinal cortex during 4AP application are blocked by several antiepileptic drugs, whereas interictal events (identified by these authors as "recurrent short discharges") continue to occur. In addition, Brückner et al. (1999) reported that recurrent, interictal discharges recorded *in vitro* from the entorhinal cortex during application of medium containing 4AP and the $GABA_A$ receptor antagonist bicuculline, are insensitive to antiepileptic drugs. These findings suggested that weakened inhibition should cause refractoriness to pharmacologic treatment in epileptic patients. However, more recently, it was shown that the ability of antiepileptic drugs to control epileptiform synchronization *in vitro* may mainly result from activity-dependent characteristics such as discharge duration (D'Antuono et al., 2010); it should be noted that this *in vitro* evidence is in line with clinical data that indicate that in epileptic patients the interictal spikes are unaffected by antiepileptic drug levels that are effective to control seizures (Gotman and Marciani, 1985; Spencer et al., 2008).

Epileptiform synchronization can also be induced by other K^+ channel blockers such as tetraethylammonium. Rutecki et al. (1990) found that bath application of

tetraethylammonium, like 4AP, induces spontaneously occurring interictal discharges in the CA3 area of isolated hippocampal slices obtained from adult animals; these experiments as well demonstrated that interictal discharges recorded extracellularly were associated with paroxysmal depolarizing shifts that comprised both excitatory and inhibitory currents. Ictal-like events did not occur in these experiments. As shown in the *in vitro* studies performed with 4AP, it was later shown that ictal-like discharges occur in the CA3 subfield of hippocampal slices obtained from young (12–18 day-old) rats (Fueta and Avoli, 1993).

3.3. Muscarinic agonists

Activation of muscarinic receptors blocks the K^+ channels responsible for the M current (Adams et al., 1982; Brown and Adams, 1980) but also induces a Ca^{2+} -activated, non-selective cation current that causes depolarizing plateau potentials that are intrinsically generated by principal cells in the hippocampus and subiculum (Caeser et al., 1993; Fraser and MacVicar, 1996; Kawasaki et al., 1999). These mechanisms contribute to increased neuronal excitability and, *in vivo*, the muscarinic agonist pilocarpine represents a valuable tool for inducing *status epilepticus* thus establishing a chronic epileptic condition that is regarded as a useful experimental model of temporal lobe epilepsy (Curia et al., 2008). In line with this evidence, it has been found that bath application of pilocarpine can induce structure-specific patterns of interictal and ictal discharges in combined hippocampal-entorhinal cortex slices (Nagao et al., 1996).

Prolonged periods of epileptiform synchronization have also been reported to occur in the entorhinal cortex during application of carbachol, another muscarinic receptor agonist (Dickson and Alonso, 1997; Cataldi et al., 2011). Interestingly, in the study by Dickson and Alonso (1997) small-amplitude field spikes could be recorded in the entorhinal cortex during application of carbachol and excitatory glutamatergic antagonists. As in the *in vitro* 4AP model these field events were correlated to large-amplitude IPSPs that were abolished by GABA_A receptor antagonists. It should be, however, noted that the ictal-like discharges induced by carbachol, especially when recorded from limbic areas such as the hippocampal CA3 subfield or the subiculum, are mainly characterized by prolonged series of regular field oscillations at 5–16 Hz, and thus they lack any clear “tonic” and “clonic” component as in the case of 4AP-induced ictal discharges (D’Antuono et al., 2001; Cataldi et al., 2011; M. Cataldi and M. Avoli, unpublished data). These oscillations, which appear to rely on muscarinic receptor-dependent activation, are integrated within the neuronal network via non-NMDA receptor-mediated synaptic transmission and are presumably caused by an intrinsic oscillatory mechanism that is contributed by Ca^{2+} -activated, non-selective cation currents (D’Antuono et al., 2001). In one study, the antiepileptic drugs topiramate and lamotrigine were shown to be capable of reducing these carbachol-induced oscillations in the rat subiculum suggesting that muscarinic receptor mediated excitation represents a target for the action of some antiepileptic drugs (D’Antuono et al., 2007). Carbachol-induced field oscillations similar to those reported to occur in adult rodent limbic structures have been also identified in *in vitro* preparations of the intact cerebral cortex and in cortical slices obtained from neonatal rats (Kilb and Luhmann, 2003).

4. Conclusions

Experiments performed in *in vitro* brain preparations by employing chemoconvulsants to acutely induce epileptiform synchronization have provided much valuable information on the fundamental mechanisms regulating both intrinsic and network neuronal excitability; more specifically, these *in vitro* findings have played a pivotal role in providing much of our current understanding of the initiation and maintenance of epileptiform discharges. While they strictly provide a model of symptomatic seizures, the insights gained from these experiments have laid foundations for our current understanding of the epileptic focus.

Developments from the original hippocampal and neocortical slices have seen more extensive networks maintained *in vitro*; as a result, these preparations can provide us among other advantages: (i) the opportunity of investigating longer range circuitry, (ii) an improved visualization for identifying individual neurons and/or for optical recording and optogenetic stimulation, and (iii) the maintenance in culture for long-term investigations. We have no doubt that we will witness many further exciting developments of these *in vitro* approaches in the near future.

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