Kainate, a double agent that generates seizures: two decades of progress

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Studies using kainate, an excitatory amino acid extracted from a seaweed, have provided major contributions to the understanding of epileptogenesis. Here we review pioneering and more recent studies aimed at determining how kainate generates seizures and, in particular, how inhibition is altered during seizures. We focus on target and subunit-specific effects of kainate on hippocampal pyramidal neurons and interneurons that lead to an excitation of both types of neurons and thus to the parallel increase of glutamatergic and GABAergic spontaneous currents. We propose that kainate excites all its targets, the net consequence depending on the level of activity of the network.

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ANIMAL MODELS of epilepsy do not provide all the complex etiologies and variety of syndromes that have been identified in humans. Nevertheless, because of similar basic features, experimental models have allowed the determination of the basic molecular and cellular mechanisms of epileptogenesis and its relation to brain damage. This is particularly exemplified in the field of temporal lobe epilepsy (TLE). Indeed, studies using the kainate model of epilepsy have considerably improved our understanding of important issues in the field of the epilepsies: are seizures the cause or the consequence of brain damage? Does hyperactivity *per se* lead to cell loss? And, if so, how? Where are seizures generated and why? Since the publication of an earlier comprehensive review[1], the combined use of molecular biology, patch clamp and imaging techniques as well as novel *in vitro* preparations have significantly improved our understanding of the mechanisms of action of kainate. Here we compare recent and earlier models proposed to explain how kainate generates seizures in the hippocampus. In particular, this review concentrates on the effects of kainate on the multiple facets of GABA-mediated inhibition and their contribution to epileptogenesis. We propose a model in which kainate excites all its targets in the hippocampus, i.e. pyramidal neurons and interneurons via different kainate receptor subtypes. The excitation of interneurons leads to a massive increase of tonic GABA-mediated inhibition in principal cells. This, however, fails to prevent epileptiform activities because of the strong excitation of CA3 pyramidal neurons, first by the selective activation of kainate receptors at mossy fiber synapses and second by glutamatergic recurrent collateral synapses that have a low threshold for the generation of synchronized activities. Thus, the generation of seizures by kainate is not caused by a collapse of inhibition, a conclusion that has been reinforced by observations made in chronic models of epilepsy in which there is no general failure of action-potential driven inhibition.

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The kainate experimental animal model of TLE

Studies performed two decades ago have shown that systemic or intracerebral injections of kainate cause epileptiform seizures in the CA3 region of the hippocampus. These seizures propagate to other limbic structures and are followed by a pattern of cell loss that is similar to that seen in patients suffering from TLE[1,2].

CA3 pyramidal neurons are indeed amongst the most responsive neurons to kainate in the brain, because they readily degenerate following local or distal injections of kainate. However, studies using the kainate model have also shown that CA3 pyramidal neurons are highly vulnerable to network hyperactivity *per se* and readily degenerate following recurrent seizures probably because of a sustained release of glutamate leading to an activation of kainate receptors. Thus, injections of kainate in structures that are distal from the hippocampus, at concentrations that do not diffuse to the hippocampus, are sufficient to generate a seizure and brain damage syndrome that includes CA3 damage[1]. In addition, the neuronal damage in CA3 following distal injections is prevented by blockade of seizures using diazepam injections suggesting that this damage is caused by the repetitive activation of afferent pathways during seizures. This is further supported by the direct relationship between epileptiform activities and the extent of damage in CA3 and by the fact that lesion of hippocampal afferent pathways abolishes most of the damage in the hippocampus. The conclusion that damage in that region is selectively caused by seizure *per se* is confirmed by the direct measure of local blood flow and oxygen consumption *in situ*, which shows that there is no imbalance between oxygen supply and neuronal activity (reviewed in Ref. 1). Furthermore, repetitive high-frequency stimulation of CA3 pyramidal neurons induces a selective cell loss in this region also suggesting that excessive activation of synaptic inputs to CA3 pyramidal neurons is toxic[3]. Therefore, CA3 pyramidal neurons are susceptible to repetitive synchronized activities that leads to cell loss probably as a result of the sustained release of glutamate.

The CA3 region is also the hippocampal pacemaker for the generation of synchronized activities. This is largely the consequence of the dense network of recurrent collateral glutamatergic axons (associated to AMPA receptor-mediated synapses) that interconnect

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pyramidal neurons. More recently, extensive physiological and modeling studies have shown that activation of even a small percentage of recurrent excitatory collateral synapses that interconnect pyramidal neurons is sufficient to generate synchronized activities[4]. Because of this feature, which is unique in the hippocampus, a wide range of convulsive agents with different modes of action, such as bicuculline, kainate, carbachol or 4-AP, generate seizures in CA3 but not in the isolated CA1. Therefore, the CA3 region is the pacemaker for the generation of synchronized activities that subsequently propagate to CA1 and other brain regions.

Several observations suggest that the epileptogenic effects of kainate in CA3 are largely caused by the activation of high-affinity kainate receptors that are preferentially expressed in the mossy fiber synaptic region. This was first suggested by the earlier observation that in both humans and various animal species, the mossy fiber synaptic region (stratum lucidum) is enriched with high-affinity kainate receptors (K_d within the range of 5–20 nM)[5,6] that can be activated even by the small concentrations of kainate crossing the blood–brain barrier during seizures induced by systemic injections of the toxin[7]. Furthermore, selective lesion by neonatal irradiation of the granule cells and their mossy fibers eliminates the epileptogenic effects of kainate but not that of high K^+ concentrations[8] (Fig. 1b). Parallel developmental studies show that the neuronal damage induced by kainate is only observed once granule cells and mossy fiber synapses are operational, at approximately the third postnatal week[1]. In addition, *in vivo*[9] and slice recordings[10–12] indicate that low concentrations of kainate (50–250 nM) that selectively activate kainate and not AMPA receptors[13] generate seizures in CA3 pyramidal neurons that propagate to CA1 and to other limbic structures[10,12]. Even high concentrations of kainate (greater than micromolar) do not generate seizures in the disconnected CA1 area suggesting that the epileptiform activities observed in CA1 following local infusions of kainate[14] are in fact generated in CA3 following diffusion of the toxin. This has also been directly implemented in the intact hippocampus preparation *in vitro*[15].

These studies suggest that local or systemic injections of kainate first activate CA3 pyramidal neurons via high-affinity receptors present in mossy fiber synapses. The activation of CA3 recurrent collateral synapses generates synchronized network-driven glutamatergic currents that propagate to other hippocampal regions. It is important to stress that the crucial role of the mossy fiber synapses is also confirmed by the observations that episodes of status epilepticus, such as those generated by kainate, also lead to the formation of novel aberrant mossy fiber synapses on CA3 pyramidal cells and on granule cell neurons[16–18], to an increased density of kainate receptors and to a reduction of seizure threshold (Fig. 1a). Therefore, 'seizures beget seizures' because although seizures induce damage through neuronal hyperactivity, the neuronal hyperactivity will facilitate seizure generation via the formation of novel mossy fiber synapses.

However, to unravel the precise mechanisms of the action of kainate, it is essential to analyze its action on ionotropic receptor-mediated currents and on voltage-gated currents. Studies performed primarily in CA1 pyramidal neurons have reported a plethora of effects of kainate including: (1) a reduction of the amplitude of evoked GABAergic IPSCs and of the frequency of miniature IPSCs (but see below) – effects that are thought to facilitate seizure generation; (2) an increase of tonic inhibition that might have opposite consequences; (3) a blockade of two currents that are important in regulating cell excitability: I_{AHP} and I_H (Ref. 19) and (4) a reduction of voltage-gated Ca^{2+} currents[20] and of glutamate release[21] (but see below). However, the relevance of these effects to the epileptogenic action of kainate is not clear as a wide range of concentrations of kainate were used (50 nM to 27 μM) and the effects are not consistently directly associated to kainate receptor-mediated synaptic currents. The following discussion first concentrates on the effects induced by low concentrations (submicromolar) of kainate that generate

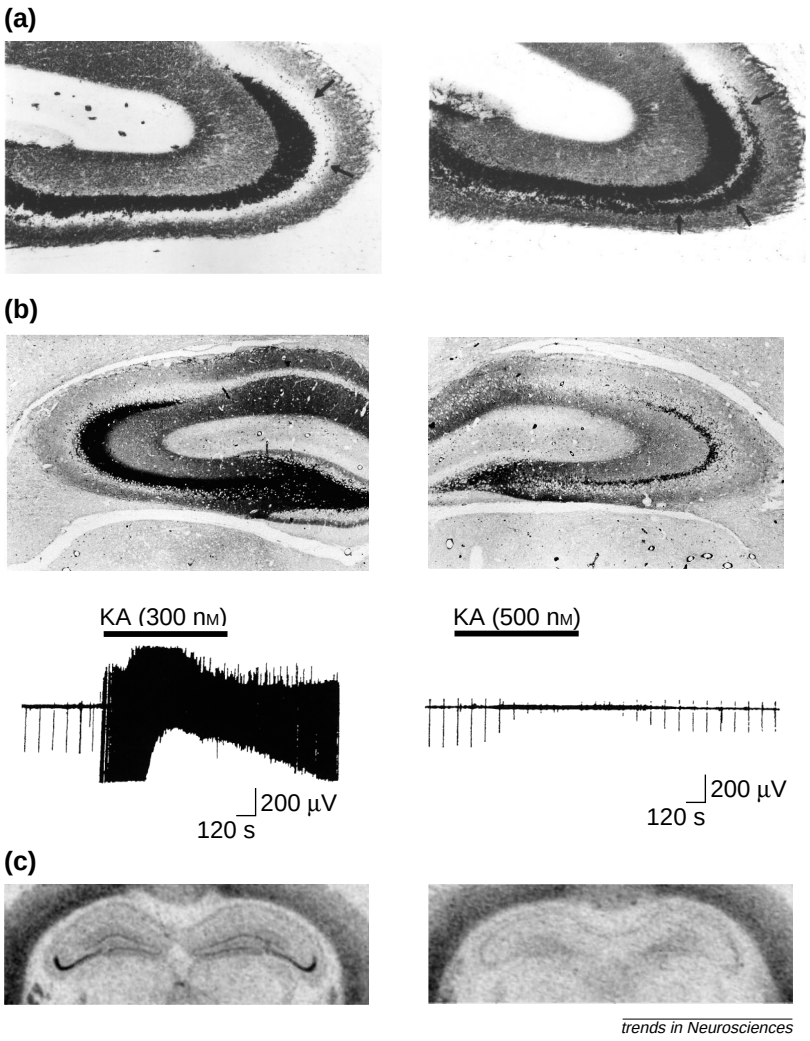

Fig. 1. ***Seizures are generated in CA3 by activation of kainate receptors located at mossy fiber synapses.*** *Seizures induce collateral sprouting of mossy fibers in the CA3 region*[45]*,* **(a)** *shows photomicrographs depicting the sprouting of mossy fibers (Timm-stained) in the CA3 region of a control (left) versus an epileptic rat (right). Note the aberrant infrapyramidal band of mossy fibres (arrows).* **(b)** *Photomicrographs of Timm-stained mossy fibers from control (left) and irradiated (right) hippocampi of an adult rat (P40). The traces below show the field-potential recordings. In the non-irradiated CA3 region (left trace), bath application of kainate (KA) (300 nM) induced spontaneous and evoked epileptiform discharges. In the right trace from the irradiated CA3 region, KA (500 nM) decreased the amplitude of the field potentials and did not induce bursts. Neonatal irradiation reduces the density of Timm-stained mossy fibers and prevents the epileptic action of kainate*[8]*.* **(c)** *Photomicrographs from receptor autoradiography using* ^{3}*[H]kainate on coronal sections from wild-type (left) and GluR6 mutant mice (right). GluR6 deficient mice are less susceptible to systemic administration of kainate (20 mg kg*$^{-1}$*) than control mice as revealed by the number of mice showing seizures*[25]*, 6 out of 6 for control and 1 out of 17 for GluR6 knockout mice.* (a) Adapted, with permission, from Ref. 45, (b) adapted, with permission, from Ref. 8 and (c) adapted, with permission, from Ref. 25.

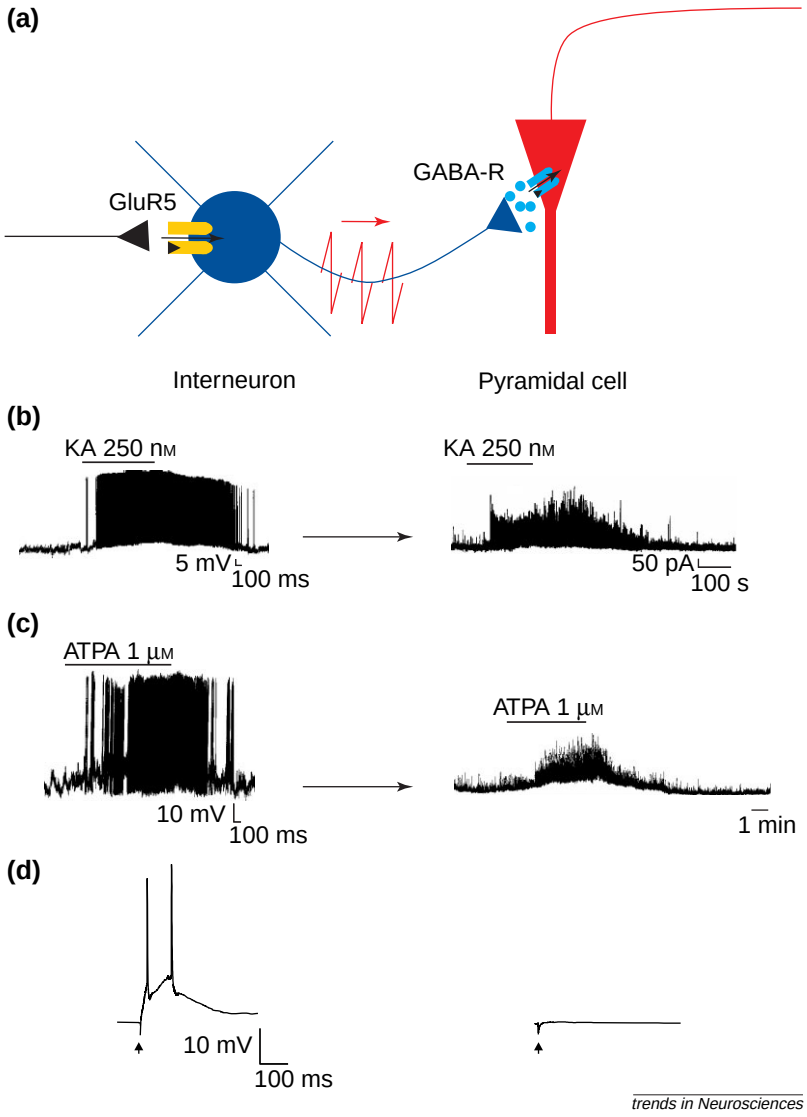

Fig. 2. ***Activation of GluR5-containing kainate receptors located on CA1 interneurons increases tonic GABA-mediated inhibition on pyramidal cells[13].*** **(a)** *Diagram showing CA1 interneuron as the blue cell and the CA1 pyramidal neuron as the red cell.* **(b)** *and* **(c)** *Left traces shown kainate (KA) depolarization in CA1. Current clamp recordings (I holding: 0 pA) were measured in the presence of GYKI53655 (30 μM) and D-APV (50 μM). KA (250 nM) or ATPA (1 μM), the selective agonist for GluR5-containing kainate receptors, caused a reversible depolarization of the membrane potential and repetitive action potential firing. Right traces show that kainate increases tonic inhibition in CA1 pyramidal cells. Voltage-clamp recordings of spontaneous IPSCs at the reversal potential for glutamatergic currents (Vhold: +10 mV). In the presence of GYKI53655 (30 μM) and D-APV (50 μM), KA (250 nM) or ATPA (1 μM) reversibly increases the spontaneous IPSCs frequency.* **(d)** *Left trace shows that the kainate-receptor-mediated EPSP evoked after stimulation (shown by arrow) in stratum radiatum in the presence of GYKI53655 (30 μM), D-APV (50 μM) and bicuculline (20 μM) triggers a burst of action-potentials. Right trace shows that the EPSP evoked in CA1 pyramidal cells by stimulation (arrow) in s. radiatum is completely blocked by GYKI53655 (30 μM) and D-APV (50 μM).* Adapted, with permission, from Ref. 13.

paroxysmal discharges in the hippocampus. These effects are associated to synaptic currents and are, at least in part, target and subunit selective.

Target and subunit-selective effects of kainate associated to synaptic currents

Activation of GluR6-containing kainate receptors at mossy fiber synapses located on CA3 pyramidal neurons

Repetitive electrical stimulation of the mossy fiber pathway generates slow EPSCs in CA3 pyramidal neurons that are mediated by kainate receptors and not AMPA receptors because they are resistant to the selective AMPA receptor antagonist GYKI53655 (Refs 22,23). The mossy fiber synapses are located close to the soma of pyramidal neurons and should therefore generate EPSCs that will efficiently propagate to the cell body and its intracellular machinery. These EPSCs are not generated in CA1 pyramidal neurons, confirming the specific involvement of high-affinity kainate receptors for synaptic transmission in CA3 pyramidal cells[24].

Cloning of several kainate receptor subunits and generation of knockout mice have made it possible to identify the subunit involved in kainate receptor-mediated synaptic currents. Thus, granule cells and CA3 pyramidal cells are enriched with GluR6-containing kainate receptors and the synaptic currents generated by the stimulation of mossy fibers, after blockade of AMPA receptors, are eliminated in GluR6 knockouts[25] (Fig. 1c). In GluR6 knockouts higher concentrations of kainate are also required to generate seizures. These observations provide direct evidence that GluR6 subunits mediate the epileptogenic actions of kainate in CA3. Collingridge and colleagues suggested that mossy fiber synapses also include pre- and postsynaptic GluR5 containing kainate receptors[26]. However, the selective GluR5 ag-onist ATPA does not generate a postsynaptic current in CA3 pyramidal cells[26] and the mRNAs encoding GluR5 are weakly expressed in granule cells or CA3 pyramidal cells[27]. In addition, these data are based on the inhibition by a GluR5 antagonist of EPSCs that are considered to be mediated by mossy fiber synapses. However, stimulating the fascia dentata not only activates the mossy fibers but also activates the extensively arborized glutamatergic recurrent collaterals of the CA3 pyramidal cell axons. Because mossy fiber EPSCs are larger than recurrent collateral ones[28], it will be interesting to determine the effects of GluR5 antagonists on identified mossy fiber-mediated EPSCs.

These observations and the dramatic effects of granule cell and mossy fiber lesion by neonatal irradiation (see above) suggest that the epileptogenic actions of kainate are mediated at least in part by GluR6-containing kainate receptors present on mossy fiber synapses. The secondary activation of the CA1 region, the major output gate from the hippocampus, leads to the propagation of seizures to other limbic structures, notably to the entorhinal cortex and other cortical structures and thus to the generation of a limbic partial type of seizure. Therefore, postsynaptic kainate receptors containing GluR6 subunits and located on CA3 mossy fiber synapses are key players in the generation of seizures by kainate. In spite of this, evidence also exists for the presence of presynaptic kainate receptors in mossy fiber synapses, but their role in the epileptogenic effects of kainate is presently unclear (see below).

Activation of GluR5 containing receptors located on interneurons

Two recent studies have shown that applications of low concentrations (submicromolar) of kainate in the presence of selective NMDA and AMPA receptors antagonists produce a massive long-lasting depolarization of CA1 interneurons and a powerful and sustained barrage of action potentials[13,24]. As expected, the consequence of this strong excitation of interneurons is an increase of the spontaneous inhibition recorded in CA1 pyramidal neurons (Fig. 2); indeed, an eightfold increase of the frequency of tonic IPSCs was attained using 250 nM kainate. This effect is selective for interneurons because similar or higher concentrations of kainate do not significantly depolarize

CA1 pyramidal neurons. Morphological identification of the recorded interneurons indicated that a wide range of GABAergic neurons in strata oriens, radiatum or lacunosum moleculare that project to the cell body or to the apical dendrites of pyramidal neurons are activated by kainate (Fig. 3). This suggests that this is a widespread property of various interneurons types. Preliminary observations suggest that CA3 interneurons are also depolarized massively by kainate. Most importantly, kainate receptor-mediated synaptic currents could be generated by electrical stimulation of stratum radiatum in CA1 inter-neurons but not in pyramidal neurons. Kainate receptor-mediated EPSCs have slower kinetics than AMPA receptor-mediated EPSCs in the same neurons (rise time, 6 ms versus 2 ms; decay time, 30 ms versus 13 ms, respectively), which appears to be a general property of kainate receptor-mediated synaptic currents. The mechanisms underlying this difference are unknown, but it is possible that it results from a distal distribution of kainate receptors at the edge of the postsynaptic density. Interestingly, at low concentrations, kainate did not alter other parameters of inhibition in pyramidal neurons, including miniature and evoked inhibition (see below). Therefore, there is a network of postsynaptic kainate receptors present in interneurons but not in pyramidal cells. Activation of this network produces a paradoxical 'overinhibition' of the target neurons that might reduce seizure generation.

Cossart *et al.*[13] also reported that the effects of kainate are mediated in part by GluR5-containing receptors because they could be mimicked by the selective GluR5 subunit agonist ATPA and blocked by the relatively selective antagonist, LY293558. Most interneurons in stratum oriens responded to ATPA (Fig. 3). By contrast, only 20% of stratum radiatum interneurons were affected by the GluR5 agonist, although most of them were depolarized using kainate (250 nM). Therefore, kainate excites most interneuron types, some via GluR5 subunit-containing receptors, others via different receptor subtypes. Indeed, a recent study suggested that kainate was still able to depolarize stratum radiatum interneurons in mice that lacked the GluR5 subunit[29]. Therefore, it is probable that in addition to the GluR5-mediated network of interneurons, other interneurons 'overinhibit' principal neurons via activation of different conformations of kainate receptors. Nevertheless, activation of GluR5 subunit-containing receptors appears to be restricted to interneurons in CA1 (and also in other hippocampal regions) and thus might act to reduce the propagation of seizures and the generation of synchronized activities. The heterogeneity of interneuron types results in a wide-range of selective modulation by kainate of GABA-mediated inhibition and of network excitability. Future studies are required to determine whether the selective activation of the inhibitory network is sufficient to prevent epileptogenesis.

Other effects of kainate

Presynaptic effects of kainate on the release of GABA

The observation that kainate enhances spontaneous $GABA_A$-mediated inhibition was unexpected because intuitively epileptogenesis should be associated with a reduction of GABA-mediated inhibition. In fact, a collapse of inhibition has been repeatedly suggested to underlie the epileptogenic effects of kainate[11,14,30]. Two

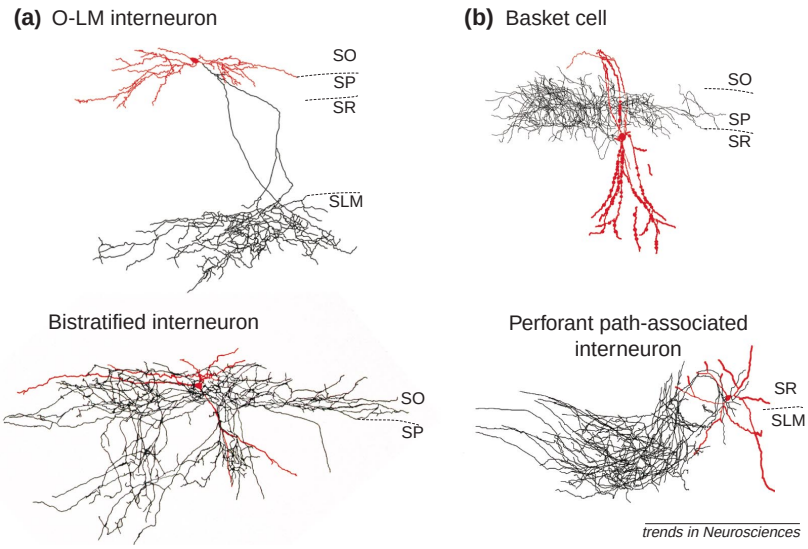

Fig. 3. ***Different types of interneurons tested for their sensitivity to kainate .*** *Reconstructions using the Neurolucida system of four types of interneurons depolarized by kainate (cell body and dendrites are shown in red and axons are shown in black).* **(a)** *100% of stratum oriens interneurons are depolarized by kainate (250 mM) or by ATPA (the GluR5 agonist).* **(b)** *85% of stratum radiatum interneurons are depolarized by kainate (250 nM) and 20% of stratum radiatum interneurons are depolarized by APTA (Ref. 13). Abbreviations: SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum; SLM, stratum lacunesum moleculare.*

parameters have been examined in detail: evoked GABA-mediated IPSCs and miniature TTX-insensitive IPSCs. However, in contrast to the clear cut effects of kainate on tonic inhibition, these effects are controversial and require high concentrations of agonist (Box 1) even if they can be mimicked by glutamate released during repetitive high-frequency stimulation[31]. The decrease of evoked GABA-mediated inhibition has been proposed to be mediated by a presynaptic subtype of kainate receptor involving a metabotropic function[32]. This interpretation has recently been challenged in a study showing that the effects of kainate on evoked IPSCs could be explained by indirect mechanisms resulting from the high amount of GABA released by interneurons during kainate application[33] [(Box 1) but see also Ref. 32].

Another important point to stress is that there are several parameters to consider in order to evaluate the strength of inhibition and the IPSC evoked by electrical stimulation is not necessarily the most relevant. Also, the hypothesis that epileptogenesis is associated to a general fall of the GABA-mediated inhibitory drive has not been confirmed in acute and chronic models of epilepsy (Box 2). Indeed, the notion of kainate-induced decrease of inhibition depends upon the parameter used to assess the level of inhibition. For example, in the most recent studies examining the fate of GABA-mediated inhibition in the kainate model of TLE, the modifications of inhibition are specific for each inhibitory pathway and locus-dependent within each specific pathway. Thus, measuring only one parameter might point to a deficit of inhibition even though, globally, inhibition is enhanced. This stresses the necessity to check all the parameters characterizing inhibition and not one in particular (Box 2).

Presynaptic effects of kainate on the release of glutamate

Early morphological and biochemical studies suggest that kainate receptors are located presynaptically on mossy fiber terminals[8,17,34]. Thus, the selective

Box I. Does kainate presynaptically reduce GABA-mediated 'inhibition'?

Kainate has repeatedly been shown to depress GABA-mediated inhibition in CA1 pyramidal cells, but both the mechanisms and the physiological relevance of this effect are unclear.

(1) The 'disinhibitory action of kainate' hypothesis relies mainly on the decrease of the evoked IPSC amplitude by kainate. However, evoked responses cannot conclusively distinguish either a mechanism (pre- or postsynaptic, or direct or indirect) or a general level of inhibition (see Box 2). First, a decrease of the amplitude of the evoked IPSCs can occur as a result of a pure postsynaptic phenomenon [i.e. a change in postsynaptic membrane resistance (see Fig. I)], or to an indirect presynaptic phenomena such as exhaustion of the terminal, a change in the probability of transmission failure in the axon or activation of presynaptic $GABA_B$ receptors [see points (3) and (4) and Fig. I]. Thus, the strongest evidence for a presynaptic population of kainate receptors on GABAergic terminals should be based on the study of miniature IPSCs or on the study of the effects of kainate on evoked IPSCs obtained with paired recordings from connected neurons. The latter experiment has not been performed and the former has generated contradictory results[a–c]. Furthermore, the decrease in the evoked IPSC amplitude observed only with high concentrations of kainate does not imply that there is a reduction of GABA quantal release. Marty *et al.*[d] have shown that a reduction of evoked IPSC can be associated with an increase of the miniature IPSCs frequency.

(2) Reduction of evoked IPSCs by kainate requires micromolar concentrations (Fig. I). Thus, in the study of Rodriguez-Moreno *et al.*[c], the depressant effect of kainate on evoked IPSCs was shown to follow a bell-shaped curve, with optimal concentrations of 10–30 μM. This bell-shaped curve effect was suggested to occur as a result of the fact that low and high concentrations of kainate hinder steady-state receptor activity: low concentrations are unable to activate receptors and high concentrations rapidly desensitizes the receptors. However, in the same study, kainate was bath applied for 30 min, which is long enough for the receptors to be largely desensitized. In the study of Cossart *et al.*[a], the reduction of evoked inhibition by kainate is also observed at high (greater than micromolar) but not at low (submicromolar) concentrations (Fig. I). By contrast, the target and subunit-specific effects of kainate have been reported at low concentrations.

(3) Nicoll and co-workers[e] suggested that the reduction of the evoked IPSCs by kainate could be explained by indirect mechanisms: kainate increased firing in interneurons leading to an enhanced release of GABA. The resulting massive activation of postsynaptic $GABA_A$ receptors augments passive shunting of the postsynaptic membrane, and the increase of GABA release activates presynaptic $GABA_B$ receptors that in turn depress GABA release[e]. The involvement of $GABA_B$ receptors in the depressant action of kainate could account for the 'metabotropic' action of kainate proposed by Lerma and co-workers[f,g].

(4) An additional problem is caused by the fact that in most experiments the connections between CA3 and CA1 were not surgically removed, thus enabling the propagation of seizures from CA3 to CA1. For example, in the pioneering study of Alger and Fisher[h], the reduction of the evoked IPSP occurs concomitantly with propagated epileptiform activities.

(5) Finally, we have recently shown that activation of presynaptic kainate receptors, selectively located at inhibitory synapses on CA1 interneurons, does not decrease but instead increases GABA quantal release (R. Cossart *et al.*, unpublished observations).

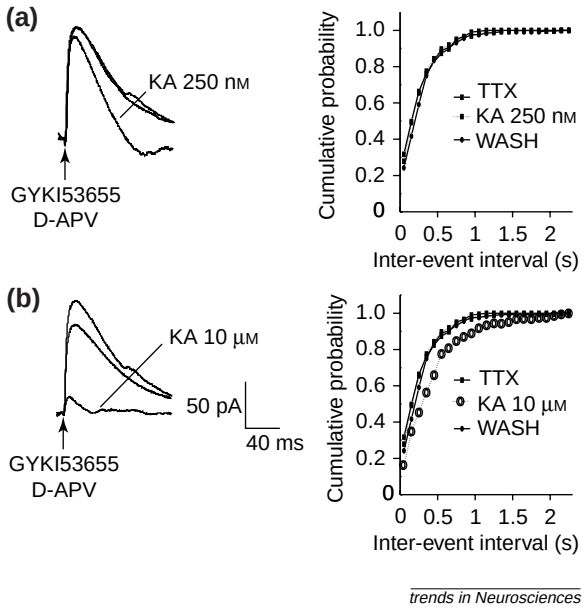

Fig. I. ***Does kainate presynaptically reduce GABA-mediated 'inhibition'?*** *Kainate-induced 'disinhibition' of CA1 pyramidal neurons requires high concentrations of agonist[13].* **(a)** *Kainate (KA) (250 nM) has no effect on the amplitude of the evoked IPSC in CA1 pyramidal cells or on the frequency of miniature IPSCs (shown in cumulative probability plot, right) recorded in the presence of GYKI53655 (30 μM) and D-APV (50 μM) (Vhold: +10 mV).* **(b)** *Increasing the concentration of kainate to 10 μM depresses the evoked IPSC amplitude and reduces the frequency of miniature IPSCs from 60% of the CA1 pyramidal cells recorded. n= x cells/y.*

References

a Cossart, R. *et al.* (1998) GluR5 kainate receptor activation in interneurons increases tonic inhibition of pyramidal cells. *Nat. Neurosci.* 1, 470–478

b Frerking, M. *et al.* (1998) Synaptic activation of kainate receptors on hippocampal interneurons. *Nat. Neurosci.* 1, 479–486

c Rodriguez-Moreno, A. *et al.* (1997) Kainate receptors presynaptically downregulate GABAergic inhibition in the rat hippocampus. *Neuron* 19, 893–901

d Glitsch, M. and Marty, A. (1999) Presynaptic effects of NMDA in cerebellar Purkinje cells and interneurons. *J. Neurosci.* 19, 511–519

e Frerking, M. *et al.* (1999) Mechanisms underlying kainate receptor-mediated disinhibition in the hippocampus. *Proc. Natl. Acad. Sci. U. S. A.* 96, 12917–12922

f Rodriguez-Moreno, A. and Lerma, J. (1998) Kainate receptor modulation of GABA release involves a metabotropic function. *Neuron* 20, 1211–1218

g Rodriguez-Moreno, A. *et al.* (2000) Two populations of kainate receptors with separate signaling mechanisms in hippocampal interneurons. *Proc. Natl. Acad. Sci. U. S. A.* 97, 1293–1298

h Fisher, R.S. and Alger, B.E. (1984) Electrophysiological mechanisms of kainic acid-induced epileptiform activity in the rat hippocampal slice. *J. Neurosci.* 4, 1312–1323

lesion of dentate gyrus neurons and of mossy fibers markedly reduced the high-affinity binding sites in stratum lucidum (Fig. 1) and immunolabeling of GluR6/7 subunits was observed in unmyelinated axons of the CA3 region[34]. Furthermore, it was recently shown that low concentrations of kainate augment the presynaptic afferent volley recorded in CA3 following stimulation of mossy fibers, an effect suggested to result from depolarization of mossy fiber axons[35]. By contrast, kainate application does not affect the frequency of miniature EPSCs recorded in CA3 pyramidal neurons (see below and Ref. 24) even if a selective modulation by kainate of mossy fiber miniature EPSCs, which are large amplitude events occurring at a low frequency[28], cannot be excluded.

Several observations suggest that different concentrations of kainate can have opposite effects. Thus, in the elegant study of Kamiya and Ozawa[35], low concentrations of kainate (200 nM) that generate seizures in CA3 (Ref. 36), increase the mossy fiber afferent volley but reduce the mossy fiber EPSP. Higher concentrations of kainate (3 μM) reduce both the afferent volley

Box 2. The fate of inhibition in temporal lobe epilepsy

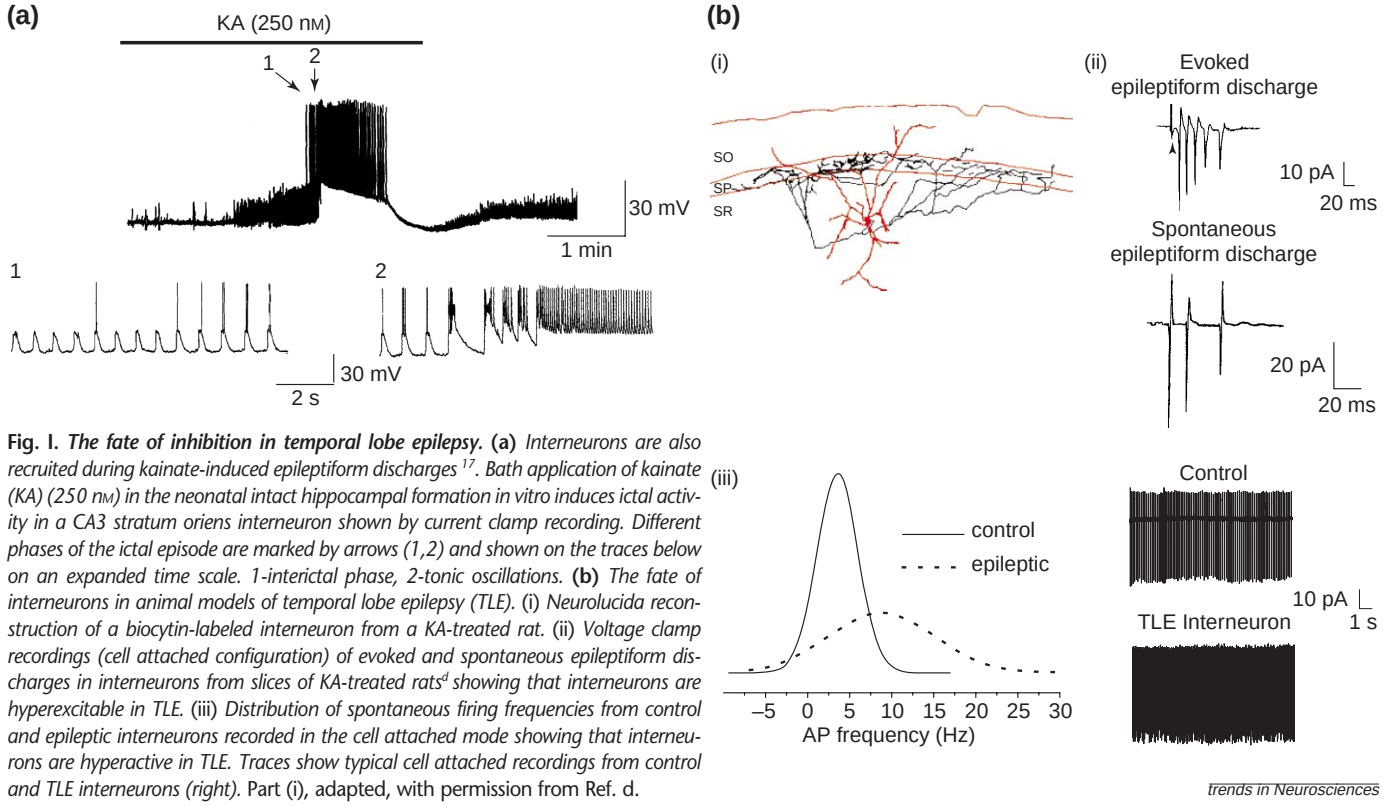

Fig. I. ***The fate of inhibition in temporal lobe epilepsy.*** **(a)** *Interneurons are also recruited during kainate-induced epileptiform discharges [17]. Bath application of kainate (KA) (250 nM) in the neonatal intact hippocampal formation in vitro induces ictal activity in a CA3 stratum oriens interneuron shown by current clamp recording. Different phases of the ictal episode are marked by arrows (1,2) and shown on the traces below on an expanded time scale. 1-interictal phase, 2-tonic oscillations.* **(b)** *The fate of interneurons in animal models of temporal lobe epilepsy (TLE).* (i) *Neurolucida reconstruction of a biocytin-labeled interneuron from a KA-treated rat.* (ii) *Voltage clamp recordings (cell attached configuration) of evoked and spontaneous epileptiform discharges in interneurons from slices of KA-treated rats[d] showing that interneurons are hyperexcitable in TLE.* (iii) *Distribution of spontaneous firing frequencies from control and epileptic interneurons recorded in the cell attached mode showing that interneurons are hyperactive in TLE. Traces show typical cell attached recordings from control and TLE interneurons (right).* Part (i), adapted, with permission from Ref. d.

$GABA_A$ receptor-mediated inhibition is a concept that encompasses a constellation of variables, including the tonic activity of GABAergic interneurons, the properties of the presynaptic GABAergic terminals impinging upon their target and the properties of postsynaptic receptors. The multiplicity of inhibitory interneurons types, each defining morphologically, physiologically and functionally distinct classes[a,b] adds to the difficulty in measuring inhibition. In the kainic acid model of temporal lobe epilepsy (TLE), several parameters characterizing inhibition have been investigated in two morphologically and functionally different inhibitory pathways in the CA1 region of the hippocampus: the pathway comprising the class of interneurons that specifically project to the perisomatic region of CA1 pyramidal cells and which tightly control their output and the pathway comprising the class that project to the dendrites and which control excitatory inputs and dendritic firing[c]. According to the parameter being measured, inhibition can appear unchanged[d], decreased[e–g] or increased[g–i] in TLE. The following alterations have been reported in brain slices from epileptic animals:

(1) A reduction of synaptic and extrasynaptic $GABA_A$ receptor-mediated currents following a probable modification of the composition of $GABA_A$ receptors subunits[d,j].

(2) A deficit of GABA quantal release at perisomatic synapses and a depletion of the reserve pool of GABA-containing vesicles consistent with the suggestion of an impairment of vesicular release, although the number of perisomatic GABAergic terminals on pyramidal neurons is not modified in TLE (Ref. f).

(3) A reduction of the frequency of spontaneous IPSCs in dendritic but not somatic recordings that is probably caused by the loss of dendritic projecting interneurons[k].

(4) An increase of the excitability of various populations of interneurons, i.e. both the number and the firing frequency of spontaneously firing interneurons are increased by 50% in TLE (Ref. l).

These observations clearly show a multiplicity of modifications, which can go in opposite directions even within a given inhibitory pathway. However, it is possible to get an overall view of 'inhibition' in TLE by looking at the spontaneous GABAergic currents received by the soma and dendrites of pyramidal cells during steady state. This measurement reveals that the net flux of Cl^- through $GABA_A$ receptors is increased by 50% in somata in TLE, i.e. the hyperactivity of perisomatic projecting interneurons more than compensates for the pre- and postsynaptic deficits. By contrast, the inhibitory drive is decreased in the dendrites, i.e. the hyperactivity of the surviving dendritic projecting interneurons does not compensate totally for the loss of other populations of dendritic projecting interneurons and for the postsynaptic deficit. This example demonstrates that the assessment of the fate of inhibition necessitates the evaluation of each of the parameters that define inhibition and for each subspecific pathway.

References

a Freund, T.F. and Buzsaki, G. (1996) Interneurons of the hippocampus. *Hippocampus*. 6, 347–470

b Parra, P. *et al.* (1998) How many subtypes of inhibitory cells in the hippocampus? *Neuron* 20, 983–993

c Miles, R. *et al.* (1996) Differences between somatic and dendritic inhibition in the hippocampus. *Neuron* 16, 815–823

d Esclapez, M. *et al.* (1997) Operative GABAergic inhibition in hippocampal CA1 pyramidal neurons in experimental epilepsy. *Proc. Natl. Acad. Sci. U. S. A.* 94, 12151–12156

e Buhl, E.H. *et al.* (1996) Zinc-induced collapse of augmented inhibition by GABA in a temporal lobe epilepsy model. *Science* 271, 369–373

f Hirsch, J.C. *et al.* (1999) Deficit of quantal release of GABA in experimental models of temporal lobe epilepsy. *Nat. Neurosci.* 2, 499–500

g Gibbs, J.W. *et al.* (1997) Differential epilepsy-associated alterations in postsynaptic GABA(A) receptor function in dentate granule and CA1 neurons. *J. Neurophysiol.* 77, 1924–1938

h Nusser, Z. *et al.* (1998) Increased number of synaptic GABA(A) receptors underlies potentiation at hippocampal inhibitory synapses. *Nature* 395, 172–177

i Brooks-Kayal, A.R. (1998) Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. *Nat. Med.* 4, 1166–1172

j Gibb, J.W. *et al.* (1996) Characterization of GABAA receptor function in human temporal cortical neurons. *J. Neurophysiol.* 75, 1458–1471

k Bernard, C. *et al.* (1997) Selective loss of GABAergic inhibition in the apical dendrites of CA1 pyramidal neurons in temporal lobe epilepsy. *Soc. Neurosci. Abstr.* 838.10

l Bernard, C. *et al.* (1999) Increased inhibitory GABAergic drive in the soma of CA1 pyramidal cells in experimental epilepsy. *Soc. Neurosci. Abstr.* 340.10

and the EPSP probably because of a conduction block as a result of axonal membrane depolarization. This study not only reflects the importance of the dose of kainate used but also the lack of direct relationship between epileptogenesis and reduction of the evoked EPSP. In physiological conditions, i.e. no glutamate antagonists and intact preparations[15], low concentrations of kainate are sufficient to generate seizures, whereas larger concentrations (micromolar doses) usually produce an irreversible loss of synaptic activity, presumably as a result of cell swelling and cell death.

Kainate has also been suggested to modulate glutamate release in the CA1 region. Indeed, kainate reduces the release of [^{3}H]L-glutamate from synaptosomes and the amplitude of evoked NMDA receptor-mediated EPSCs (Ref. 21). However, because this effect requires particularly large concentrations (1–100 μM) and prolonged applications (10–30 min), its physiological relevance remains to be established.

Therefore, a presynaptic action of kainate that can reduce glutamate release from mossy fibers probably plays an important role in mediating some effects of kainate, but it is presently unclear how this participates in the epileptogenic actions of the toxin.

Other non-direct effects of kainate

Kainate has additional effects that might enhance or reduce the excitability of pyramidal neurons. Although these early studies were carried out before the availability of selective AMPA receptor antagonists, the effects observed with low concentrations of kainate were mediated by kainate and not AMPA receptors because only kainate receptors are activated with submicromolar concentrations[13]. The following effects deserve emphasis.

(1) Low concentrations of kainate (100–200 nM) facilitate the repetitive firing of CA1 pyramidal neurons[37]. This effect is mediated by the attenuation of the Ca^{2+}-dependent K^+ current (I_{AHP}), which is responsible for the afterhyperpolarization following Na^+ spikes and by the reduction of the inward rectifier K^+ current (I_Q) (Ref. 19).

(2) In the elegant study of Nistri and Cherubini[20], kainate (50–400 nM) depressed the L-type Ca^{2+} current. This effect was prevented by intracellular dialysis with BAPTA, suggesting that it is mediated by an increase in the inactivation of Ca^{2+} currents via a rise in free intracellular Ca^{2+}. It is possible that such a rise of intracellular Ca^{2+} mediates other effects of kainate.

(3) Kainate reduces postsynaptic $GABA_B$ receptor-activated K^+ currents[38], further stressing possible second-messenger cascades-mediated effects.

Concluding remarks

Kainate acts as a 'double-agent' controlling the hippocampal network activity via the activation of an heterogeneous network of kainate receptors differentially distributed among inhibitory interneurons and excitatory pyramidal cells. Hence, kainate in the nanomolar range generates seizures in CA3 at least in part through the activation of GluR6-containing receptors localized postsynaptically at mossy fiber synapses on pyramidal cells. Kainate, at similar low concentrations, massively increases tonic inhibition via the activation of GluR5-containing receptors localized at glutamatergic synapses on GABAergic inter-neurons. This differential expression of kainate receptors between neuronal subtypes is reminiscent of other pathways, for example of glutamate acting on metabotropic receptors[39] or ACh acting on nicotinic receptors[40].

The multiple facets of inhibition (i.e. evoked, spontaneous or miniature) and the extremely diversified network of interneurons provide a rich repertoire of excitability modulations that cannot be classified simply as an increase or decrease of 'inhibition'. Thus, there is now direct evidence for two distinct forms of inhibition on pyramidal cells[41] originating from two broad classes of interneurons: those that innervate the dendrites, control the input of the hippocampal network and the propagation to the soma of large calcium currents, and those that innervate the soma, control the generation of Na^+ action potential and hence the output of the hippocampal network. Furthermore, a population of interneurons is specialized to innervate other interneurons[42], thereby enabling a fine control of the excitability of these cells. As the distribution of kainate receptor subtypes appears to be age-, subunit- and target-selective, the net consequence of the activation of kainate receptors will vary: an increase of the interneuronal activity by kainate might even result in a paradoxical reduction of its epileptogenic effects.

However, because only evoked kainate responses have been observed, the physiological conditions under which kainate receptors are activated are not presently clear. Interestingly, it has been recently reported[43] that pure kainate receptor-mediated spontaneous PSCs (not associated to AMPA receptor-mediated PSCs) are observed at early stages of maturation. This suggests a differential activation of AMPA and kainate receptors that can be regulated by the neuronal activity level or conditioned by a concomitant activation of other transmitters or synaptic pathways. All these possibilities point to a wider repertoire of modulation of ionotropic glutamatergic synapses than previously envisaged. Interestingly, activation of kainate receptor-mediated PSCs in CA3 pyramidal neurons requires brief tetani, whereas in interneurons a single stimulus is sufficient suggesting that under physiological conditions postsynaptic kainate receptors on interneurons might be more frequently recruited than those on pyramidal cells at mossy fiber synapses. Furthermore, there are some examples where the efficacy of EPSP-spike coupling is particularly strong and precise in interneurons resulting in an efficient feed-forward recruitment of interneur-ons[44]. If this is the case, kainate receptor-mediated EPSPs might play an important role in resetting endogenous rhythmic activities that are controlled by interneurons during the generation of seizures in addition to normal physiological conditions. It remains to determine the mechanisms responsible for the shift of the inhibitory/excitatory balance in the somato-dendritic compartments that will ultimately lead to epileptogenesis.

Selected references

1 Ben-Ari, Y. (1985) Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience* 14, 375–403

2 Nadler, J.V. (1981) Minireview. Kainic acid as a tool for the study of temporal lobe epilepsy. *Life Sci.* 29, 2031–2042

3 Sloviter, R.S. (1996) Hippocampal pathology and pathophysiology in temporal lobe epilepsy. *Neurologia* 11, 29–32

4 Miles, R. and Wong, R.K (1983) Single neurones can initiate synchronized population discharge in the hippocampus. *Nature* 306, 371–373

5 Monaghan, D.T. and Cotman, C.W. (1982) The distribution of [3H]kainic acid binding sites in rat CNS as determined by autoradiography. *Brain Res.* 252, 91–100

6 Tremblay, E. *et al.* (1985) Autoradiographic localization of kainic acid binding sites in the human hippocampus. *Brain Res.* 343, 378–382
7 Berger, M.L. *et al.* (1986) Limbic seizures induced by systemically applied kainic acid: how much kainic acid reaches the brain? *Adv. Exp. Med. Biol.* 203, 199–209
8 Gaiarsa, J.L. *et al.* (1994) Neonatal irradiation prevents the formation of hippocampal mossy fibers and the epileptic action of kainate on rat CA3 pyramidal neurons. *J. Neurophysiol.* 71, 204–215
9 Debonnel, G. *et al.* (1990) Neurotoxic effect of domoic acid: mediation by kainate receptor electrophysiological studies in the rat. *Can. Dis. Wkly Rep.* 16, 59–68
10 Ben-Ari, Y. and Gho, M. (1988) Long-lasting modification of the synaptic properties of rat CA3 hippocampal neurones induced by kainic acid. *J. Physiol.* 404, 365–384
11 Fisher, R.S. and Alger, B.E. (1984) Electrophysiological mechanisms of kainic acid-induced epileptiform activity in the rat hippocampal slice. *J. Neurosci.* 4, 1312–1323
12 Robinson, J.H. and Deadwyler, S.A. (1981) Kainic acid produces depolarization of CA3 pyramidal cells in the vitro hippocampal slice. *Brain Res.* 221, 117–127
13 Cossart, R. *et al.* (1998) GluR5 kainate receptor activation in interneurons increases tonic inhibition of pyramidal cells. *Nat. Neurosci.* 1, 470–478
14 Rodriguez-Moreno, A. *et al.* (1997) Kainate receptors presynaptically downregulate GABAergic inhibition in the rat hippocampus. *Neuron* 19, 893–901
15 Khalilov, I. *et al.* (1999) Maturation of kainate-induced epileptiform activities in interconnected intact neonatal limbic structures *in vitro*. *Eur. J. Neurosci.* 11, 3468–3480
16 Cronin, J. and Dudek, F.E. (1988) Chronic seizures and collateral sprouting of dentate mossy fibers after kainic acid treatment in rats. *Brain Res.* 474, 181–184
17 Represa, A. *et al.* (1987) Kainate binding sites in the hippocampal mossy fibers: localization and plasticity. *Neuroscience* 20, 739–748
18 Nadler, J.V. *et al.* (1980) Loss and reacquisition of hippocampal synapses after selective destruction of CA3–CA4 afferents with kainic acid. *Brain Res.* 191, 387–403
19 Gho, M. *et al.* (1986) Kainate reduces two voltage-dependent potassium conductances in rat hippocampal neurons *in vitro*. *Brain Res.* 385, 411–414
20 Nistri, A. and Cherubini, E. (1991) Depression of a sustained calcium current by kainate in rat hippocampal neurones *in vitro*. *J. Physiol.* 435, 465–481
21 Chittajallu, R. *et al.* (1996) Regulation of glutamate release by presynaptic kainate receptors in the hippocampus. *Nature* 379, 78–81
22 Castillo, P.E. *et al.* (1997) Kainate receptors mediate a slow postsynaptic current in hippocampal CA3 neurons. *Nature* 388, 182–186
23 Vignes, M. and Collingridge, G.L. (1997) The synaptic activation of kainate receptors. *Nature* 388, 179–182
24 Frerking, M. *et al.* (1998) Synaptic activation of kainate receptors on hippocampal interneurons. *Nat. Neurosci.* 1, 479–486
25 Mulle, C. *et al.* (1998) Altered synaptic physiology and reduced susceptibility to kainate- induced seizures in GluR6-deficient mice. *Nature* 392, 601–605
26 Vignes, M. *et al.* (1998) The GluR5 subtype of kainate receptor regulates excitatory synaptic transmission in areas CA1 and CA3 of the rat hippocampus. *Neuropharmacology* 37, 1269–1277
27 Bahn, S. *et al.* (1998) Kainate receptor gene expression in the developing rat brain. *J. Neurosci.* 14, 5525–5545
28 Henze, D.A. *et al.* (1997) Large amplitude miniature excitatory postsynaptic currents in hippocampal CA3 pyramidal neurons are of mossy fiber origin. *J. Neurophysiol.* 77, 1075–1086
29 Bureau, I. and Mulle, C. (1998) Potentiation of GABAergic synaptic transmission by AMPA receptors in mouse cerebellar stellate cells: changes during development. *J. Physiol.* 509, 817–831
30 Clarke, V.R. *et al.* (1997) A hippocampal GluR5 kainate receptor regulating inhibitory synaptic transmission. *Nature* 389, 599–603
31 Min, M.Y. *et al.* (1999) Synaptically released glutamate reduces gamma-aminobutyric acid (GABA)ergic inhibition in the hippocampus via kainate receptors. *Proc. Natl. Acad. Sci. U. S. A.* 96, 9932–9937
32 Rodriguez-Moreno, A. *et al.* (2000) Two populations of kainate receptors with separate signaling mechanisms in hippocampal interneurons. *Proc. Natl. Acad. Sci. U. S. A.* 97, 1293–1298
33 Frerking, M. *et al.* (1999) Mechanisms underlying kainate receptor-mediated disinhibition in the hippocampus. *Proc. Natl. Acad. Sci. U. S. A.* 96, 12917–12922
34 Petralia, R.S. *et al.* (1994) Histological and ultrastructural localization of the kainate receptor subunits, KA2 and GluR6/7, in the rat nervous system using selective antipeptide antibodies. *J. Comp. Neurol.* 349, 85–110
35 Kamiya, H. and Ozawa, S. (2000) Kainate receptor-mediated presynaptic inhibition at the mouse hippocampal mossy fibre synapse. *J. Physiol.* 523, 653–665
36 Westbrook, G.L. and Lothman, E.W. (1983) Cellular and synaptic basis of kainic acid-induced hippocampal epileptiform activity. *Brain Res.* 273, 97–109
37 Cherubini, E. *et al.* (1990) Effects of kainate on the excitability of rat hippocampal neurones. *Epilepsy Res.* 5, 18–27
38 Rovira, C. *et al.* (1990) Block of GABAb-activated K1 conductance by kainate and quisqualate in rat CA3 hippocampal pyramidal neurones. *Pflügers Arch.* 415, 471–478
39 McBain, C.J. *et al.* (1994) Activation of metabotropic glutamate receptors differentially affects two classes of hippocampal interneurons and potentiates excitatory synaptic transmission. *J. Neurosci.* 14, 4433–4445
40 Frazier, C.J. *et al.* (1998) Acetylcholine activates an alpha-bungarotoxin-sensitive nicotinic current in rat hippocampal interneurons, but not pyramidal cells. *J. Neurosci.* 18, 1187–1195
41 Miles, R. *et al.* (1996) Differences between somatic and dendritic inhibition in the hippocampus. *Neuron* 16, 815–823
42 Gulyas, A.I. *et al.* (1996) Interneurons containing calretinin are specialized to control other interneurons in the rat hippocampus. *J. Neurosci.* 16, 3397–3411
43 Kidd, F.L. and Isaac, J.T. (1999) Developmental and activity-dependent regulation of kainate receptors at thalamocortical synapses. *Nature* 400, 569–573
44 Csicsvari, J. *et al.* (1998) Reliability and state dependence of pyramidal cell-interneuron synapses in the hippocampus: an ensemble approach in the behaving rat. *Neuron* 21, 179–189
45 Represa and Ben-Ari, Y. (1992) Kindling is associated with the formation of novel mossy fibre synapses in the CA3 region. *Exp. Brain Res.* 92, 69–78

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BOOK REVIEWS

Psychological Mechanisms of Pain and Analgesia

by Donald D. Price, IASP Press, 1999. $69.00 (xiii + 248 pages) ISBN 0 931092 29 9

Most theoretical articles and books on pain begin nowadays with the official IASP definition of pain: 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage'[1]. They then go on to agree with the definition and proceed with their discussion. Donald Price in his book *Psychological Mechanisms of Pain and Analgesia* follows a different tack. He disagrees with the definition, arguing that it does not emphasize the experiential nature of pain. He proposes a new definition: pain is *'a somatic perception containing (1) a bodily sensation with qualities like those reported during tissue-damaging stimulation, (2) an experienced threat associated with this sensation, (3) a feeling of unpleasantness or other negative emotion based on this experienced threat'*.

Notice how much Price packs into his definition of pain. Each unit of pain contains the sensation of pain itself, the feeling that one is somehow being threatened and a negative affective reaction to the sensation and feeling. However, this is too much, for two reasons. First, we can dissociate the negative affective reactions from pain sensations, either pharmacologically using, for example, fentanyl, or biologically using, for example, a frontal lobotomy. Understanding and treating the concomitant emotional reactions to pain sensations are important and Price is correct in stating that scientists and clinicians do not pay enough attention to this aspect of pain patients. However, acknowledging these facts does not make reactions to pain part of pain itself.