COMMENTARY

LIMBIC SEIZURE AND BRAIN DAMAGE PRODUCED BY KAINIC ACID: MECHANISMS AND RELEVANCE TO HUMAN TEMPORAL LOBE EPILEPSY

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"Perhaps the word kainic comes from Cain"
U.S. Von Euler at a meeting

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Kainic acid (KA—in Japanese literally the ghost of the sea) was isolated three decades ago from the seaweed Digenea simplex which has been extensively used in post-war Japan to eradicate ascariasis. The active principle of other biological extracts used for the same purpose has also been identified (notably domoic and quisqualic acids, ibid.). The anti-helminthic action is shared by other analogues of glutamate in particular those having carboxyl (and carboxymethyl) substituents in the 2 and 3 positions. The next important step was made by Shinozaki and Konishi who showed that when microiontophoretically ejected upon cortical neurons these agents have a potent excitatory action, consisting of prolonged spike discharge. Kainate was the most potent analogue.

It had been known for some time that parenterally administered monosodium glutamate destroys retinal ganglion cells in developing mice. Olney showed that glutamate also destroys neurons of various brain regions lying outside the blood–brain barrier, notably the arcuate nucleus. The neurotoxicity of KA was first shown by Olney et al. using the same experimental paradigm. Parenteral KA in immature mice caused rapid necrotic changes in the somata and dendrites of arcuate neurons; this was associated with hyperplasia of glial elements and followed by phagocytosis which could be manifest as early as 3–4 h after administration. Afferent fibers and axons in the necrotic area appeared normal (also see Ref. 134).

In subsequent reports, the relationship between the structure of a series of glutamate analogues, their excitatory potency (as suggested from microiontophoretic experiments) and their toxicity toward the arcuate was studied. Since every analogue of glutamate which produced neuronal excitation also caused dendrosomatotoxic activity, the term "excitotoxic" was proposed by Olney and coworkers which implies that neuronal cell death is due to excessive depolarization produced by the excitatory substances. It was proposed that the sensitivity of a given neuron to KA is determined by the percentage of its dendritic surface occupied by glutamatergic receptors. The resistance of axons was interpreted as resulting from the lack of amino acid receptors on fiber tracts.

Intracerebral iontophoretic ejection of glutamate produces a lesion in the rat cerebral cortex. The demonstration that direct intracerebral injection of KA also produces an “axon-sparing lesion” prompted the use of KA as a tool in neurobiology. The rational for this use stems primarily from the
possibility to destroy selectively the neurons of a given brain region and thus to circumvent the problem of the contribution of fibres de passage, which are also destroyed by conventional (electrolytic) lesions. Such studies have provided several experimental models of human diseases such as a hemiballism64 or Huntington's chorea (e.g. Coyle et al.,37 McGee et al.102 and Refs therein).

Other more recent studies indicate, however, that both the use of KA as an axon-sparing device and the postulated mechanisms to explain KA neurotoxicity must be reassessed.

(a) The concept that KA acts through glutamate receptors has been eroded by extensive biochemical and pharmacological studies.6,37,93,129,130,156 KA does not act on most glutamatergic receptors but it may involve a special class of glutamatergic receptors with unique pharmacological profile and regional distribution in the brain.115

(b) There is a clear-cut gradient of vulnerability of neurons to kainate; thus the pyramidal neurons of the CA3 region of the hippocampus are the most vulnerable neurons in the brain KA123 whereas neurons of the diagonal band of Broca or the mesencephalic trigeminal nucleus are resistant to its toxic effects.34,88 Even within the same region striking differences are noted, viz. the CA3 region and the granular layer of the hippocampal formation.121,123 Several observations, notably those made by Nadler and coworkers120,122 suggest that this gradient is not due to a differential distribution or density of glutamatergic receptors (also see Refs 89 and 157). Other potent excitatory analogues do not reproduce this gradient but destroy the neurons as a function of their proximity to the site of injection.2,88,96,121

(c) Other observations suggest a dissociation between the excitatory and toxic properties of KA, for instance lesion of the corticostriatal pathway confers a protection of striatal neurons from the toxic action of KA100 without altering the excitatory effect of KA on striatal neurons.105

(d) The axon-sparing characteristic of KA injections has been disputed100,107,123 and, at least in some brain regions, there is little doubt that fibre tracts are destroyed rapidly by local injection of KA.171

(e) Intracerebral injections of KA also produces recurrent epileptiform discharge,11,61 motor convulsions11,61,161 and subsequently damage at the site of injection and damage in structures distant from the injected site.11,61,135,161,198 The distant damage which is not caused by other major excitatory analogues (notably ibotenic acid48,121) was initially attributed to diffusion of the toxin combined with a greater vulnerability of the distant neurons to KA,135,198 we have however discovered the role of the limbic status epilepticus and paroxysmal discharge generated by KA as a cause of remote brain damage.13 Since the regional distribution of this damage is reminiscent of that associated with human temporal lobe epilepsy13,34,64,65,97,151,155,172,175 a better understanding of the epileptogenic properties of KA is of more than theoretical interest.

The preferential effect of KA on limbic structures is the main subject of the present commentary. The reader is referred to other monographs for more general reviews of the action of KA and other excitotoxins.65,77,107

SPECIFIC BINDING SITES FOR KA

High and low affinity sites

Following the initial observations of Simon et al.,106 the presence in the brain of saturable, specific binding sites for KA has been confirmed and extended by several groups.6,93,156,192 Whereas a single site was described in the earlier report, two sites were found in later studies. In the comprehensive work of London and Coyle,10 the high and low affinity sites \(K_a\) of 4–16 and 27–66 nM respectively were distinguished on the basis of their different association and dissociation constants. Kainate dissociates within seconds from the low affinity site, in contrast the dissociation from high affinity sites occurs in 1 h or more (M. L. Berger, in preparation). The affinity of various analogues for the two sites differs as well as the regional distribution in the brain.13 The stratum, hippocampus and forebrain regions are particularly enriched with high affinity sites whereas more than 90% of the total specific binding in the cerebellum or pons–medulla are to low affinity sites. The distribution of high affinity binding sites shows some correlation with the regional vulnerability to KA (see below).

Regional distribution

To gain a better understanding of the possible physiological significance of KA binding sites, it is essential to map carefully their distribution in the brain. In spite of the low specific activity of currently available radioactive KA, the distribution of KA binding sites has been described recently with autoradiographic techniques.13,104,108 The highest level of specific binding sites in the brain is located in the stratum lucidum of CA3 in the hippocampus (see Fig. 1C) i.e. the region innervated by mossy fibers originating from the granular layer of the fascia dentata. This region is also the most vulnerable to the excitatory and toxic properties of KA (see below). Quantitative densitometry shows that the density of silver grains (representing bound kainate) in the mossy fiber region is ten times higher than in adjacent layers.15 This labelling is due to binding to the high affinity (slowly displaceable) sites (ibid.), as confirmed by a more recent study (M. L. Berger, G. Chariton and Y. Ben-Ari, in preparation) in which the CA3 and CA1 plus fascia dentata were microdissected separately; in contrast to CA3, the CA1 and fascia dentata contained lower affinity sites. The autoradiographs also revealed that other forebrain regions are enriched in KA binding notably the amyg-
Kainate-glutamate interactions

Electrophysiological studies made primarily in the spinal cord suggest that there are various classes of excitatory amino acids including: (a) a N-methyl-D-aspartate-prefering site also activated by ibotenate, p-glutamate and antagonized by low concentrations of divalent metal cations and a series of D-amino acids; (b) a second class comprising quisqualate and kainate subpopulations (also see Ref. 73). However, since these studies rely on extracellular comparison of spike-inducing activity of various compounds, it is difficult to meet strict pharmacological criteria for receptor classification (e.g. reviews and discussion in Refs 129 and 130).

Using autoradiographic techniques, Monaghan et al. have recently shown that KA sites in stratum lucidum of CA3 also bind [3H]glutamate although with ionic dependence and antagonist sensitivity which differs from the major glutamate site observed in biochemical studies. These authors suggest the presence in the hippocampus of four pharmacologically and anatomically distinct glutamate binding sites. Because of the particular vulnerability of the CA3 region to KA and the fact that this region is particularly enriched in KA high affinity binding sites, the pharmacological profile of these sites has been extensively studied on membrane preparations. Transmitters such as acetylcholine or centrally active agents (diazepam, cardiazol or morphine, but also flurazepam or [Leugenkephalin) do not displace KA (M. L. Berger and Y. Ben-Ari, unpublished). The mossy fibers are also particularly enriched in dynorphin and Zn2+ (e.g. Frederickson and Refs therein) but neither the former nor the latter (in millimolar concentrations) influence the binding of KA (M. L. Berger and Y. Ben-Ari, unpublished); we have also found no displacement of KA by nerve growth factor even if there are suggestions that this substance may be released in this region. Putative endogenous kainate-like substances, notably folic acid or quinolinic acid (see chapter below on Kainate-like endotoxins) also produced negligible displacement of KA from the CA3 sites (ibid.). These observations suggest that the specific high affinity KA sites in CA3 have rather restricted conformational requirements. It bears stressing that the transmitter released by the mossy fibers has not been fully characterized; the evidence in favor of glutamate is for instance less compelling than it is for the Schaffer collaterals or the perforant path.

POTENT EXCITATORY EFFECTS OF KAINATE

The particularly powerful excitatory action of KA has been described in several brain areas. This effect has a slow onset and prolonged duration; in several preparations recovery is not obtained. The principal actions of KA on invertebrate preparations is to potentiate glutamate-induced depolarization. In vertebrate spinal cord, KA directly produces a large and often irreversible depolarization which is associated with a reduction in input resistance and depends on the presence of extracellular sodium. As stressed by Nistri, it is however possible that the activity of KA is due to an increase in Ca2+ permeability. This would readily explain the strong conductance increase and
Fig. 1. Preferential metabolic alteration of the CA3 region. Following parenteral administration of KA, a rise in 2-deoxyglucose autoradiographic staining is noticeable in the CA3 region in immature (A—12 days of age) or adult animals (arrow, D) (B). In immature animals, this is the only brain region in which we have detected a rise in 2-DG. In adult, at doses which produce limbic motor seizures other structures of the limbic system are labelled (D). Furthermore in both immature (not shown) and adult (C) specific high affinity KA+ binding sites are concentrated in the CA3 region. The 2-deoxyglucose pictures in (A) and (B) were superimposed in the stained sections from which the autoradiography had been obtained (e.g. Ben-Ari et al.,14 Berger et al.,16 Tremblay et al.'86).

Abbreviations: cing, cingulate gyrus; Fi, fimbria; g, granular layer; h, hilus; mol, molecular layer; p, pyramidal layer; SR, stratum radiatum.

Fig. 2. Selective and non-selective seizure-related damage produced by kainate. In (A) and (C), damage restricted to parts of CA3 by intra-amygdaloid injection of KA. (A) Neuronal cell loss with Nissl stain after survival period of 5 days; (B) extensive necrosis caused by intracerebroventricular (or also parenteral) KA. (C) Argyrophilia seen also after intra-amygdaloid injection of KA with short survival periods with a Fink–Heimer stain. Nissl stain.

Fig. 3. Long-term sequelae of parenteral KA in adult animals. Two months after administration of KA, the animal displayed spontaneous seizures (A) with paroxysmal discharge in both the hippocampus (H) and amygdala (A). These seizures were elicited often by handling. (B) and (C) depict in the same animal the extent of neuronal cell loss in the hilar region (C) and dark degenerating cells in (B); clear-cut evidence of phagocytosis was also often observed suggesting a continuous process of neuronal degeneration (e.g. Tremblay and Ben-Ari'84). (A) Scale in microvolts; LMS, limbic motor seizures.

Fig. 5. Parallelism between the distribution of neurons vulnerable to KA in the hilar region and GABAergic neurons revealed by glutamate decarboxylase (GAD) immunocytochemistry. With the Fink–Heimer technique following parenteral KA and short survival periods argyrophilic cells are particularly frequent in stratum oriens and pyramidale of CA1 and in the hilar zone (medial part of CA3 C and polymorph zone B). In (C)–(F), GAD immunocytochemistry was performed in control animals following injections of colchicine in the cortex (L. Nitecka, M. Tappaz, E. Tremblay and Y. Ben-Ari, unpublished observations); (e.g. also Riback et al.'46 and Somogyi et al.'91). The boxed area in (D) is shown at a higher magnification in (F). Arrows in (E) and (F) point to GABAergic neurons. Abbreviations: a, alveus; f, fimbria; g, granular layer; m, molecular layer; o, stratum oriens; p, stratum pyramidale; r, stratum radiatum.

Fig. 7. Repetitive electrical stimulation of the perforant path produces a reduction of the Timm stain in the mossy fibers. (A), (C), (E) Control; (B), (D), (F) after electrical stimulation, from Sloviter.'86.

Fig. 9. Bleaching of Zn2+ stain produced a K+ pulse. Push–pull cannulae were stereotaxically introduced in the mossy fiber zone of each hemisphere. A K+ pulse (10 min, 30 mM) was applied to the right hemisphere and the animal perfused immediately at the end of the pulse. Note the reduction of the intensity of Zn2+ stain as compared to the contralateral (control) side. Timm stain (e.g. Ref. 168) counterstained with cresyl violet. Abbreviations: g, granular layer; h, hilus; IPSI, ipsilateral; p, pyramidal layer.
Fig. 1.
Fig. 9.
the powerful depolarization, since Ca$^{2+}$ carries more current than Na$^+$ and has a very positive reversal potential because of its very low intracellular free concentration (e.g. Puil).46

Because of the particularly potent seizure-inducing effect of KA in the hippocampus, a number of studies have examined its electrophysiological effects in this region. Microiontophoretic applications of KA to the hippocampus in situ produce a prolonged increase in firing rate of pyramidal cells. The effect is particularly prominent in CA3 where removal of the iontophoretic retaining current is often sufficient to produce an intense activation of neurons.44 CA1 and CA3 pyramidal cells have a similar sensibility to glutamate or ibotenic acid, thereby further stressing the unique features of the actions of KA in CA3. De Montigny et al.44 have also suggested that the GABAergic inhibition is not involved in this neuronal activation since (a) GABA does not exert any selective effect on the action of KA vs that of glutamate; (b) benzodiazepines selectively block the actions of KA in CA1—but not in CA3; this antagonism is not associated with enhancement of the inhibition produced by microiontophoretic applications of GABA (ibid.). Other studies have however suggested that the action of kainate is at least partly due to a reduction of the GABAergic inhibitory drive. Thus, following both parenteral administration of KA in the intact animal69 or bath applications of KA into slice preparation,106 there is a reduction of the recurrent inhibition measured with the paired pulse inhibition technique. A recent intracellular study58 has indeed shown that the principal action of KA on CA3 and CA1 pyramidal neurons appears to be a synaptic depression, which first influence the inhibitory postsynaptic potentials and (with higher doses) the excitatory postsynaptic potentials. This depression of inhibition is associated with minimal effects on membrane properties, action potentials, fiber conduction, etc. It is not associated with alterations in the response produced by pulses of GABA, and it is best explained by a reduction of presynaptic GABA release although a postsynaptic effect is not completely ruled out. The excitatory phenomena which occur following limited applications of KA were thus interpreted as being secondary to decreased inhibition, in keeping with the hypothesis that the removal of GABAergic inhibition plays an important role in epileptogenesis in this region.9 The potent excitatory action of other agents—notably acetylcholine10,189—also involves a removal of the inhibition. Interestingly, another study148 has shown that there is a large difference between the concentration of KA required to induce a reversible acceleration of spontaneous discharge (10 nM) and the one required to produce depolarization with apparently irreversible spike blockade (10 μM). This difference must be kept in mind when the mechanisms underlying the local and distant actions of KA are examined (see below).

## Preferential Actions of Kainate on Limbic Structures

### Parenteral Administration of Kainate: A Limbic Status Epilepticus

In the adult rat parenteral administration of the toxin produces a limbic seizure and brain damage syndrome which has not been reproduced by other excitatory amino acids. Since this illustrates, in a remarkable manner, the central role of limbic circuitry in the actions of KA, it will be described with some detail.

**Clinical signs.** The clinical signs produced by i.v.44 or i.p.14 injections can be divided in several distinct phases. During the first 20–30 min, the animals have “staring” spells. This is followed by head nodding and numerous wet-dog shakes for approximately 30 min. The next phase which starts 1 h after KA is characterized by the occurrence of individual recurrent limbic motor seizures. These are identical to the seizures produced by repeated daily electrical stimulation of the amygdala or other limbic structures69 and include masticatory and facial movements, forepaws tremor, rearing and loss of postural control. The seizures then become progressively more complex and prolonged, with a reduction in the interictal pause. In the following phase (1–2 h), the animal displays a full status epilepticus i.e. continuous convulsions and abnormal electro encephalogram. We have referred to this syndrome as a limbic motor syndrome:14 tonico–clonic generalized convulsions (i.e. of the bicuculline or picrotoxin type) are not produced by kainate in adult animals, except with massive doses about four or five times the lethal dose (E. Tremblay and Y. Ben-Ari, unpublished). Therefore the latter terminology which has often been used in the literature for KA-induced seizures is inappropriate and should be reserved to describe procedures causing tonico–clonic convulsions and electrographic seizures generalized to the entire cortex from their onset.

**Electric activity.** Electrographic records from cortical and subcortical structures have confirmed that the three main phases of behavioral abnormalities (staring, individual limbic seizures, status epilepticus) correspond respectively to (a) localized paroxysmal discharges in the hippocampus, (b) involvement of other limbic structures—notably the amygdala—and then (c) generalization to a number of other (non-limbic) structures. The hippocampus has a particularly low threshold to parenteral KA.143,144 This is in keeping with electrophysiological studies showing that in the slice preparation, seizure activity is produced by particularly low concentrations of KA.148

**The 2-deoxyglucose autoradiographic method.** This method also illustrates the central role played by the hippocampus in the initial stage which follows parenteral administration of KA (Fig. 1B). Administration of lower doses of KA44 or repeated injections of diazepam at doses which block the seizures except...
at the most susceptible sites (Y. Ben-Ari and E. Tremblay, unpublished) suggest that the temporal pole of the hippocampal formation is preferentially involved. A developmental study has also shown that before the end of the third week of age (in rats), the Ammon’s horn is the only brain region in which a rise in 2-deoxyglucose (2DG) labelling is observed following parenteral KA (Fig. 1A and Tremblay et al.184). The lateral septum—which receives a direct projection from the Ammon’s horn—is also involved at this early stage. During the second stage when overt limbic motor seizures are produced, the 2DG maps reveal an intense activation of the entire limbic system (Fig. 1D). With almost no exception, the structures in which there is a metabolic rise have massive direct axonal connections with one or more of the following areas: the hippocampal formation, the amygdaloid complex and the mediodorsal thalamic complex (e.g. Refs 14 and 94, and Refs therein). Thus a rise in glucose consumption is noted in the target structures of the fornix pre- and post-commissural efferent projections of the hippocampal formation (i.e. the accumbens, ventral pallidum and claustrum, anterior thalamic nuclei and infra-limbic cortex). The amygdaloid complex—which is also connected with the entorhinal cortex and other components of the hippocampal formation—is also highly labelled as well as structures to which the amygdala projects (notably the stria terminals and its bed nucleus, Fig. 1D). Finally, the labelling in the cortical mantle is restricted to the “limbic” cortex i.e. the infra- and pre-limbic cortices as well as the agranular insular, retrosplenial and perirhinal cortices, several of these areas are projected upon by the mediodorsal nucleus and the amygdaloid complex. Although the 2DG method does not allow conclusions to be reached concerning the labelling at the cellular level, there is little doubt that the rise in metabolism occurs in relation to the propagation of paroxysmal discharges to neuronal populations of total or subtotal necrosis, in addition to the neuronal cell loss, there is a loss of oligodendrocytes, demyelination, formation of astroglial scars and perivenous hemorrhages as well as vascular sprouting. Long term sequelae. The long-term sequelae (1–3 months) of parenteral KA further depict the involvement of limbic structures. Firstly, in keeping with observations made following intracerebral injections of KA,37 and animals display “spontaneous seizures” notably when handled. These seizures are of a limbic type and are accompanied with paroxysmal seizure discharges in the amygdala and hippocampus (Fig. 3A). Furthermore, examination of the brain (Fig. 3B) reveals a satellitosis in the Ammon’s horn and phagocytosis of neurons in strata oriens, radiatum and pyramidalis several weeks after the parenteral injection of KA i.e. at a time when the toxin has been removed from the brain. This reflects the on-going nature of the seizure and brain damage. In the pyramidal layer this late damage is more conspicuous in CA3 than in CA1.

Blood flow and oxygen consumption. In order to test directly the possible contribution to nerve tissue damage by a mismatch between increased local metabolic consumption and oxygen supply, we have measured blood flow, as well as partial pressure of oxygen and carbon dioxide in the vulnerable CA3 region of unanaesthetized freely breathing rats following parenteral administration of KA. It was found that seizure (as noticed by means of hippocampal records as well as the motor signs) are associated with a rise in the local blood flow which more than compensates the increased oxygen consumption (Fig. 4). Thus the PO2 and PCO2 remains within normal limits during the observation period (2 h), at a time when damage is caused. Severe seizures are actually associated with hyperoxia (and not hypoxia). This provides compelling evidence that at least in this region the damage caused by parenteral kainate is not due to anoxic ischemic episodes.
Limbic seizure and brain damage produced by kainic acid

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Fig. 4. The damage in CA3 produced by kainate is not caused by a mismatch between local blood flow and metabolism. The local partial pressure in CO₂ (PCO₂), oxygen (PO₂), the blood flow (CBF) and hippocampal electroencephalogram (EA Hipp) were continuously recorded in a chronically implanted, unanaesthetized freely breathing rat after parenteral administration of kainate. The PO₂ and PCO₂ were measured by gas spectrometry, the gases are drawn via a special cannula into the analysis chamber by an ultra high vacuum; the local blood flow was measured by the thermal clearance method. The cannulae and electrodes (for the hippocampal electroencephalogram) were chronically implanted in the CA3 region 2 weeks before the experiment. At the day of the experiment the animal was placed in a hammock, left to habituate for 1 h and the variables measured continuously and quantitatively before and (up to 3 h) after the administration of KA. The rat was anaesthetized and perfused Immediately after the recording sessions. Note that the seizures are associated with a rise in a local blood flow which more than compensates the increased metabolism. Damage in CA3 was found even though there were no hypoxia and hypercapnia in the CA3 region during the entire recording session. See e.g. Pinard et al. and Refs therein for techniques used.

Neurochemistry. Neurochemical changes after parenteral KA include a highly significant reduction in the activity of glutamate decarboxylase, a marker for GABAergic synapses. This reduction is found in the hippocampus and amygdala but not in striatum or frontal cortex; the effect starts after 24 h and reaches a maximum 2 days later. This is in keeping with the electrophysiological experiments described earlier, and further reflects the preferential involvement of these structures in the KA syndrome. Since both in the hippocampus and amygdala GABAergic neurons are intrinsic, these observations reflect a direct damaging effect of the procedure on these structures. Neurons that are GABAergic are also particularly vulnerable to KA in other structures (ibid. and Ref. 191) and in epilepticogenic foci.

Electrophysiological studies on hippocampal slices from animals which received KA 2 weeks earlier also indicate a consistent reduction in the inhibitory mechanisms. In keeping with these observations, the distribution of hippocampal neurons vulnerable to parenteral KA (as revealed with argyrophilic stains) is similar to the distribution of GABAergic interneurons as revealed by glutamate decarboxylase immunocytochemistry (Fig. 5, see also Ribak et al. and Somogyi et al.). In contrast, the cholinergic septohippocampal system is not sensitive to parenteral KA (see also note added in the proof).

Ontogenesis of the effects of parenteral kainate. In 3 week old animals, parenteral KA induces tonic or tonico-clonic convulsions, but not limbic motor signs. Limbic motor seizures are in fact first observed starting from the end of the third week; the shift from one type of syndrome to the other is sudden with almost no intermediary stage. This prompted us to study its anatomical, metabolic and biochemical correlates. In young animals the rise in 2DG labelling induced by KA is exclusively restricted to the hippocampus (more precisely the CA3 region and hilus) (e.g. Fig. 1A) and to the lateral septum, thus confirming the unique susceptibility of these regions to KA. The rise in 2DG in this region is already observed in 3 day-old animals which have immature mossy fibers when studied anatomically and electrophysiologically. Furthermore, in spite of the severe tonico-clonic type convulsions, there is no brain damage before the end of the third week of life. It is only after, that the toxin produces limbic motor seizures associated with metabolic rise in hippocampus, amygdala, limbic cortex, medial thalamus, and with typical pathological changes. Therefore the occurrence of limbic seizures and the presence of a fully mature mossy fiber system is necessary to cause damage in the Ammon’s horn.

In the light of these results, the developing limbic system seemed an interesting subject for testing the
ontogeny and role of KA binding sites. Both Scatchard analysis and autoradiographs revealed high affinity sites in the CA3 region at an early age but only low affinity sites in the amygdala. The high affinity sites appear in the amygdala only at the end of the third week of life, i.e. at a time when the KA convulsions have a limbic aetiology (also see Ref. 25).

Suggested sequence of events following parenteral kainate. To evaluate fully the sequence of events produced by parenteral KA, it is essential first of all to determine the amount of toxin reaching the brain, in particular the vulnerable regions of the limbic system. Earlier authors have underestimated the problem of the blood–brain barrier to KA and have interpreted the effects of KA as due to a direct action. This barrier is, however, highly impermeable to KA as much as to other acidic amino acids. Using the method described by Oldendorf and we found a brain uptake index for KA in the hippocampus and other limbic regions of 2–4×, this is close to the values found with the impermeant tracer saccharose (M. L. Berger, le Fauconnier, E. Tremblay and Y. Ben-Ari, in preparation). After systemic injection of [3H]KA, about 250 fmol KA/mg wet tissue remained in brain tissue unremovable by perfusion (ibid.). It is estimated that the actual brain concentration of KA is sufficient to produce paroxysmal discharge in CA3, but two orders of magnitude lower than the concentrations required to directly produce damage by in situ injections. These observations cannot be reconciled with the suggestions that the damage in the hippocampus following parental KA is due to a direct interaction of KA with (glutamatergic) receptors.

The progressive sequence of activation of the limbic circuitry and the lack of damage (even with lethal doses) to structures such as the striatum (rich in KA receptors) and readily destroyed by direct injection of the toxin) argue against the hypothesis of a direct “toxic” action of parenterally injected KA.

A more likely explanation is that seizures are first elicited directly by KA in CA3. The disinhibition produced by removal of the powerful GABAergic inhibition will contribute to enhance the synchronous paroxysmal discharge. If the discharge is sustained, it will propagate to the entorhinal cortex and the lateral septum. Subsequently, it will recruit a limbic circuitry in which the amygdala has a key role. I suggest that recurrent activation of the amygdalo-hippocampal axis leads to the epileptogenic damage. Other studies in which the toxin was directly injected in vulnerable regions have also provided compelling evidence for a seizure-related damage produced by KA. These will be described prior to a discussion on the mechanisms of the damage.

Intra-amygdaloid injection of kainate: dissociation between local and “distant” seizure-related damage

Intra-amygdaloid injection of KA produces initially limbic motor seizures. Later, the symptomatology in the rat is more complex and includes barrel rotation, circling behavior etc., reflecting the secondary involvement of extra-limbic structures. This is a model of limbic seizures with a focal origin. In the cat and monkey, the symptomatology is also of an amygdaloid type (chewing, salivation, ipsilateral clonie of the face in the cat and oral automatism in the monkey). There is a secondary generalization to other structures particularly in the cat but not so in the monkey (ibid.).

The epileptiform activity which is first apparent in the injected amygdala rapidly propagates to the ipsilateral Ammon’s horn—notably the CA3 region—and the contralateral amygdala. 2-Deoxyglucose autoradiographs reveal an ipsilateral regional rise in labelling reminiscent of that obtained after i.p. injection of the toxin. At the site of injection—in keeping with the local effect of KA in a large number of structures (also see Fig. 2A,C). Patches of pyramidal neurons in CA3a and their distant apical dendrites are densely argyrophilic. The stratum lucidum where the mossy fibers terminate is in contrast vacuolated. Other parts of the ipsilateral Ammon’s horn are progressively affected: the CA3 area and hilus, then the CA1 area whereas CA2 and the granular layer of the fascia dentata are spared. In addition to the hippocampus, other “distant” areas show degeneration after intramygdaloid KA, these are part of the limbic system.

The simplest explanation for the distant lesions is that KA diffuses (either directly or through the ventricle) and that a sufficient concentration of the toxin reaches vulnerable neurons (such as the CA3 pyramidal neurons) to produce directly the damage. However we have shown that administration of diazepam in doses sufficient to antagonize the limbic seizures blocked the distant damage in the CA3 region without affecting the damage at the site of injection. It was therefore suggested that the KA brain damage following intra-amygdaloid injections had a dual aetiology: (a) local damage due to the direct toxic action of KA and (b) “distant” action mediated by the paroxysmal discharge and its associated convulsions. Other lines of supporting evidence for a major role of the epileptiform discharge in mediating the “distant” damage in the hippocampus include (e.g. Refs 8 and 12):

(a) Similar metabolic and hippocampal changes can be produced following microiontophoretic applications of KA in the amygdala at doses which are not compatible with a significant diffusion to the hippo-
Also 2–5 nmol KA in the amygdala are enough to produce destruction of the vulnerable CA3 region: this concentration is similar to that found neurotoxic when applied directly into the ventricle and only twice the one which must be applied directly in the hippocampus to produce a similar damage.

(b) With a lateral or an oblique approach to inject the toxin in the amygdala (i.e. conditions in which diffusion to the ventricle is unlikely) there is a similar hippocampal damage whereas injections of KA in the vicinity of the ventricle (i.e. in the septum–preoptic area or in the nucleus of the diagonal band) do not readily produce this damage, in keeping with the fact that the symptomatology of the seizures produced by injections in the septum is not of the limbic type, at least with brief survival periods.

(c) The distribution of [3H]kainate following direct intracerebral injections in the striatum is not consistent with the hypothesis that the necrotizing action of KA in distant regions is due to diffusion.

(d) The temporal pole of the hippocampus adjacent to the injected amygdala is less readily lesioned than the (distant) septal pole.

(e) There is an excellent correlation between the severity of epileptiform activity recorded in situ and the damage in the CA3 region. In an earlier study, every second of the hippocampal electrographic activity was classified in five categories (normal, post-ictal depression, paroxysmal discharge with or without concomitant motor events and record with regular or irregular spikes); the hippocampal damage correlated significantly with the total duration of post-ictal depressions and paroxysmal discharges associated with motor events but not with other abnormalities.

(f) Damage in the medial thalamus is occasionally more severe contralaterally to the injected site.

(g) Transection of the perforant path (through which the paroxysmal discharge probably propagates to the Ammon’s horn, see below) reduces post-ictal depressions and the distant but not the local damage. In contrast, this procedure does not protect the hippocampal damage following intracerebroventricular injection of KA.

(h) The correlations between the electrographic records, the metabolic maps and the regional distribution of neuronal damage suggest that axonal connections with the amygdala rather than proximity to the injection site play an important role. Several examples of this important feature of KA action have been provided in the literature following application of the toxin at various sites.

(i) There are major differences between the characteristics of local and distant damage, the former and not the latter is associated with a massive, early microglial proliferation perhaps of vascular origin. In contrast, glial proliferation in the hippocampus after injection of kainate into the amygdala is manifested 4–6 days after the injection, i.e. subsequent to the onset of neuronal damage. The direct action produced by KA on glial cells is indeed one of the most impressive and consistent effects of local damage.

(j) Repetitive electrical stimulation of the perforant path, which plays a central role in mediating the damage caused in the hippocampus by intramygdaloid KA, reproduces a similar pattern of damage.

The possibility that the distant damage is due to orthograde or retrograde transport of the toxin can be also excluded since it is not compatible with the time course of events especially in the CA3 region (e.g. Ref. 8).

All of these observations therefore strongly suggest that following intra-amygdaloid KA, the paroxysmal discharge is generated in the entorhinal cortex where the amygdala heavily projects. This discharge will exert a powerful excitatory action on CA3 pyramidal neurons primarily via the granule cells and their mossy fibers.

KAINATE-LIKE ENDOTOXINS

The observations that KA does not act through the predominant type of glutamic acid receptors raise the possibility that these effects could be mediated by another endogenous substance. The regional distribution of KA receptors as revealed by autoradiography in the Ammon’s horn reinforces this hypothesis. At present, the following candidates have been proposed as endogenous toxins since they reproduce some of the features of KA.

Folates

Ruck et al. reported that a folate derivative bound strongly to [3H]KA binding sites on rat cerebellar membranes and suggested that folates could be KA-like substances. This idea was reinforced by studies by Olney and coworkers who showed that intra-amygdaloid injections of folates reproduce the seizures and distant brain damage caused by KA but not the damage at the site of injection. Other observations, however, cannot be reconciled with this scheme. In fact, recent studies have found that folates bind very weakly to [3H]KA binding sites in cerebellum, striatum, frontoparietal cortex and also major limbic structures including the hippocampus and amygdala (IC50 of about 2 mM to displace 20 nM KA). In addition, injections of folates into the
hippocampus produce little or no motor abnormalities or pathological sequelae in contrast to KA. A detailed comparison of the electrographic, clinical, metabolic and histopathological sequelae of intramygdaloïd injections of folic acid and KA reveals major differences between the two syndromes and cannot support the observations of Olney et al. Thus, following KA injections, the 2DG maps reveal an enhanced metabolism in the injected amygdala and adjacent piriform lobe, but the most conspicuous rise in labelling was noted in the entire frontoparietal cortex up to the cingulate region, and in the ventral thalamic complex and globus pallidus, i.e., structures not labelled following KA treatment. In keeping with this, we found a complete necrosis of the piriform lobe and a massive anoxic-ischemic type of damage in the frontoparietal cortex including status spongiosus, ischemic cell changes with or without incrustations in the superficial layers of the cortex, etc. Interestingly, several features of the damage caused by KA—in particular in the boundary zones of the frontoparietal cortex—are reminiscent of the pathological alterations induced in primates and subprimates species by anoxic-ischemic episodes, notably atmospheric decompression or intracarotid air embolism, conditions which are associated with convulsions. We suggest that folic acid produces those toxic actions of KA which are related to anoxic-ischemic changes caused by the convulsion but not the neuronal cell loss which is selectively and directly produced by the paroxysmal discharge in vulnerable regions (such as CA3, see below).

Cholinomimetics

Olney and coworkers also reported that cholinomimetics injected in the amygdala caused a seizure and brain damage syndrome which is indistinguishable from KA except that, like folates, these agents do not cause damage at the site of injection but only the distant damage. From the data presently available it would seem that the distant damage is also of the non-selective seizure-related type (see below).

Quinolinic acid

Schwarcz and coworkers have recently reported that nanomolar amounts of the tryptophan metabolite quinolinic acid, injected intracerebrally, produce axon-sparing neuronal damage. Neurochemical, clinical and pathological observations show that quinolinic acid mimics the effects of excitotoxins. Thus, the agent shares with ibotenic acid (but not KA) the ability to produce circumscribed damage with few convulsions and no distant seizure-related damage. Other observations derived from tissue culture experiments indicate that, like for the case of KA, the actions of quinolinic acid depend on the presence of normal synaptic patterns and appropriate synaptogenesis in regions where it produces a toxic effect. Further studies must be performed with this agent in order to characterize it in the context of other exogenous excitotoxins.

MECHANISMS OF LOCAL DAMAGE

Local damage includes that one produced at the site of injection or structures poorly protected by the blood–brain barrier following intracerebral or parenteral KA respectively. According to the excitotoxic hypothesis of neuronal death, interaction of KA or other excitatory amino acids with appropriate receptors (which in the initial version of the concept were assumed to be glutamatergic and located postsynaptically) produces intense depolarization. It was postulated that this depolarization will cause "energy-dependent homeostatic mechanisms to draw heavily on the cell's energy stores in an effort to restore ionic balance, with cell death ensuing when energy stores are exhausted or lethal alternations in the composition of the cells internal milieu have occurred". In addition, glia at the site of injection are grossly oedematous and swollen, presumably because of the large increase in extracellular K associated with the seizure. This may impair glial uptake, produce a reduction in extracellular space and contribute to neuronal damage.

In keeping with the suggested sequence of events, electrophysiological studies have indeed shown that in hippocampal slices, high concentrations of KA produce intense depolarization associated with an irreversible loss of spike discharge. This effect appears to depend neither on synaptic transmission nor on the presence of presynaptic terminals. Extensive structure activity studies made primarily in the striatum, retina or the arcuate region suggest a relation between neuroexcitation and neurotoxicity, although the latter has been usually assessed from extracellular studies made in different brain sites. For instance the dimethyl ester derivative of kainate or its N-acetyl derivative, both of which are electrophysiologically inactive, are devoid of neurotoxic effects at doses 40–100 fold higher than KA itself. Strong support for the excitotoxic hypothesis comes from studies showing that KA also does not kill neurons insensitive to its excitatory actions (e.g., neurons of the mesencephalic trigeminal nucleus) whereas, neurons which are readily excited by KA such as CA3 pyramidal neurons are also very vulnerable to its toxic effects.

Other studies however cannot be reconciled with this scheme and suggest a more complex aetiology. Thus blockade of the excitation produced by KA in cerebellar cultures does not prevent the damage. Other observations suggest that the damage caused locally by KA is not explicable by the seizure discharge generated by the toxin. Thus, administration of anticonvulsants at sufficient doses to prevent the development of paroxysmal discharges, fails to prevent the local neurotoxicity of KA. In contrast, anaesthesia with γ-butyrolactone or chloralhydrate-
pentobarbital protects hippocampal neurons, suggesting that the anaesthetic and not the anticonvulsant action of drugs account for local protections\(^{300}\) (also see Ref. 12). Furthermore, a comparison of seizure and damage produced in the hippocampus by a variety of excitatory amino acids shows that the neurotoxicity poorly correlates with the neuroexcitatory effects;\(^{12,199}\) for instance N-methyl-D-aspartate produces continuous electroencephalogram seizures for 2 h with little subsequent damage \(\text{ibid.},\) also see Ref. 61).

Lesion experiments suggest a dissociation between the excitatory (postsynaptic) and toxic actions (involving the presynaptic elements) of KA,\(^{18,101,105}\) and raise the possibility of different receptors for both effects.\(^{106}\) The toxicity of intrahippocampal KA depends on the presence of the septohippocampal (cholinergic) and perforant (presumably glutamatergic) pathways.\(^{120,122}\) Interestingly, transection of the perforant path protects the granules of the fascia dentata from kainate but not from ibotenate lesions.\(^{99,157}\)

Other observations also emphasize the unique features of KA neurotoxicity which are not shared by other potent excitatory analogues. The actions of KA but not that of other excitants depends on the presence of mature synaptic patterns; thus KA but not ibotenate fails to produce damage before the end of the third week of age in the rat.\(^{25,131,147}\) Also, neurons are differentially susceptible to KA but uniformly vulnerable to other excitants, the damage produced by the latter depending simply on their proximity to the injection site.\(^{2,88,96,121}\) Several neuronal populations resistant to local KA—notably the cholinergic neurons of the medial septum and the diagonal band—are readily destroyed by local ibotenate.\(^{88}\) This suggests that the two agents exert their toxic effects by different mechanisms.

To account for these observations, a number of alternative changes to the initial excitotoxic concept have been proposed. These include (a) accumulation of toxic concentrations of glutamate through inhibition of glutamate uptake by KA\(^{101}\) (e.g. also Johnston et al.\(^{85}\)), (b) cooperative interaction between KA and endogenous glutamate released from presynaptic terminals\(^{18}\) and (c) presynaptic localization of KA receptors which would release toxic doses of glutamate and/or aspartate.\(^{86}\) There is however no compelling evidence in support of these mechanisms which have been challenged by other experimental observations. For instance, (a) dihydrokainate which is a more potent inhibitor of glutamate uptake is devoid of neurotoxic action,\(^{260}\) (b) the toxicity of KA is unaltered by coadministration of glutamate in the cerebellum\(^{63}\) and (c) the concentrations of KA required to release glutamate from cerebellar parallel fibers are 100 fold higher than the concentrations sufficient to kill the cells innervated by these fibers.\(^{63}\)

Also the hypothesis that excitotoxicity is due to a presynaptic release of toxic concentrations of glutamate (or aspartate) is also handicapped by the poor efficacy of these amino acids in causing damage when directly administered intracerebrally. Thus, to cause necrotic changes in the striatum, glutamate must be chronically infused for a week or more at particularly high concentrations.\(^{96,99}\) These high doses are assumed to be necessary in order to overcome the combined capacity of high and low affinity uptake systems. It is however not clear in this perspective how the damage would be so rapidly caused (1–2 h) following electrical stimulation or kainate administration in vulnerable regions \(\text{vide supra}\).

Summing up, the mechanisms of the "local" damage remains to be clarified. In the opinion of the present reviewer, the local toxic action of KA is caused by a multiple and complex series of effects which are not necessarily due to a single mechanism and cannot be categorized in a single framework. The mechanism of the toxicity would differ according to the locus of injection and epileptogenicity of the injected structure, the experimental conditions (anaesthesia, concentrations, the doses used etc.). A possible direct effect of KA on the vasculature of the injected locus also deserves investigation.

**MECHANISMS OF "DISTANT" DAMAGE**

This includes the damage produced in structures distant from the injection site or in structures protected by the blood–brain barrier following intracerebral or parenteral KA respectively. Two types of distant damage should be discussed separately (e.g. Fig. 2).

**Selective seizure-related damage**

This is causally related to the propagation of seizure discharge through a well-characterized synaptic connection and without the involvement of deleterious effects associated with the convulsions. The evidence in favor of this type of damage is compelling for the CA3 region. Indeed, as already indicated above, the damage produced by parenteral KA in this region is not explicable by hypoxia or a mismatch between oxygen demand and blood supply.\(^{142}\) However a number of other structures may share with CA3 this feature, notably the claustrum, deepest layers of insular cortex (presumably claustrum insulare), lateral part of the amygdaloid complex etc. (see below).

The evidence that damage to CA3 produced by parenteral or intra-amygdaloid KA is caused by the seizure has been discussed above and does not need further elaboration here. There is also evidence suggesting that seizure activity contributes to the hippocampal damage by intracerebroventricular KA.\(^{120,123}\) Nadler and coworkers have stressed the crucial role of the mossy fibers for the damage produced in CA3; in fact destruction of the mossy fibres provides immediate protection of the CA3 region against intracerebroventricular KA but not against direct intrahippocampal KA.\(^{120,122}\)
Fig. 6. Delayed changes in extracellular and intracellular free Ca\(^{2+}\) concentration \([\text{Ca}^{2+}]\) in CA2-3 region following repetitive stimulation of the fimbria. Upper \([\text{Ca}^{2+}]\) trace: extracellular recording with \(\text{Ca}^{2+}\)-sensitive microelectrode. Lower \([\text{Ca}^{2+}]\) trace: intracellular recording with same electrode: note large fall in \([\text{Ca}]\) when electrode penetrated cell. Oscilloscope traces (at bottom) were recorded at times indicated by broken arrows. Initially the fimbrial stimulation evoked an antidromic spike and a partly reversed inhibitory postsynaptic potential. During the 10 Hz tetanus (between arrows), there were only small fluctuations in \([\text{Ca}^{2+}]\) (reference barrel contained 3 M KCl), but there followed a period of post-tetanic depression, characterized by an absence of responses (including inhibitory postsynaptic potentials) to the same fimbrial stimulation. About 30 s after the end of the tetanus, paroxysmal responses suddenly appeared, in the form of bursts of population spikes. This was accompanied by a huge increase in \([\text{Ca}]\) (to a peak of about 700 nM) that lasted about 1 min. The return of \([\text{Ca}]\) to the intracellular base line level (close to 1 nM) was followed by gradual return to the inhibitory postsynaptic potential (now fully reversed).

The upper \([\text{Ca}]\) trace shows a comparably delayed large fall in extracellular \([\text{Ca}]\) evoked in a second run by the same stimulation shortly after the intracellular recording. Data were obtained in situ, from a rat under urethane anaesthesia (K. Krnjević, M. E. Morris and N. Ropert, unpublished observations).

Two types of mechanisms can be proposed to underline the selective seizure-related damage. The first one implies large increase in intracellular \(\text{Ca}^{2+}\) associated with the seizures; this will exceed the cell capacity to buffer the \(\text{Ca}^{2+}\) thus leading to an increase in intracellular free \(\text{Ca}^{2+}\) which, by means of proteolysis or blockade of the \(\text{Ca}^{2+}\) proton exchange across the mitochondrial membrane may cause neuronal damage.\(^{152}\) In support of this view, histochemical data indicate that the CA3 pyramidal cells have a limited \(\text{Ca}^{2+}\) binding capacity whereas the granules of the fascia dentata and their mossy fibers are rich in \(\text{Ca}^{2+}\) binding proteins\(^{153}\) (also see Ben-Ari\(^{9}\)). Direct evidence in favor of this hypothesis has been recently obtained by Krnjević and coworkers\(^{10,11,16}\) who have determined the changes in extracellular and intracellular \(\text{Ca}^{2+}\) in the hippocampus during seizures with ion-sensitive electrodes. It was found that a particularly important decrease in extracellular \(\text{Ca}^{2+}\) occurs in the pyramidal layer of CA3 during seizures, and that this is associated with an increase in intracellular \(\text{Ca}^{2+}\) (Fig. 6). Other experiments suggesting the involvement of \(\text{Ca}^{2+}\) in excitotoxic damage have been reported.\(^{47,108}\)

Other observations also indicate that factor(s) perhaps exclusively localized to the mossy fibers, may be released and produce damage during the seizures. A number of substances found in the mossy fibers deserve some discussion. For instance, the mossy fibers are rich in dynorphin\(^{109}\) which conceivably could play a role in this event. Perhaps more likely is the possibility of an endogenous kainate-like compound since this region is amongst the richest in the brain in high affinity KA receptors.\(^{15,114,188}\) Interestingly, KA binding in the stratum lucidum (in the mossy fiber zone) and not in other hippocampal regions is decreased by \(\text{Ca}^{2+}\);\(^{113}\) this is consistent with the observation of Beaumont et al.\(^{6}\) that \(\text{Ca}^{2+}\) is a potent inhibitor of KA binding. Since a significant reduction in extracellular \(\text{Ca}^{2+}\) is readily observed during seizures,\(^{90}\) this will further contribute to the toxicity by increasing the effectiveness of KA on its receptor sites.

Nevertheless, it is possible that the toxic factor is not the transmitter itself but another agent released with the transmitter during severe seizure discharge. \(\text{Zn}^{2+}\) is a likely agent. Hence, (a) \(\text{Zn}^{2+}\) is particularly concentrated in the mossy fibers\(^{9}\) where it is found in synaptic vesicles\(^{15}\) (the actual concentration of \(\text{Zn}^{2+}\) in this region is amongst the highest in the brain\(^{90,166}\)), (b) \(\text{Zn}^{2+}\) is accumulated by the mossy fibers following parenteral administration of the...
Limbic seizure and brain damage produced by kainic acid

Artificial CSF
Spontaneous release
K+ (30 mM) 2 min

Fig. 8. In situ release of endogenous zinc in the Ammon's horn. A push–pull cannula was stereotaxically implanted in the CA3 zone in urethane anaesthetized animals. Zinc was measured by atomic adsorption. A spontaneous release of zinc was observed approximately twice the concentration of zinc was found in the artificial cerebrospinal fluid (CSF). A pulse of K+ (arrow, 30 mM, 2 min) caused a five-fold increase in the amount of zinc released followed by a prolonged decrease of zinc with levels close to the blank; 15–20 min elapsed before the recovery (Ben-Ari et al. G. Charton, C. Rovira, Y. Ben-Ari and V. Leviel, submitted for publication).}

radioactive metal. There is some evidence that the population spike produced in CA3 by the activation of the mossy fibers is reduced by chelating agents or diet conditions in which zinc levels are reduced. (d) During repetitive electrical stimulation of the fascia dentata (when CA3 damage is produced) there is a loss of zinc stain in the mossy fibers (Fig. 7, and Sloviter); this might be due to release of Zn²⁺ during the paroxysmal discharge. In a recent study, we have measured by atomic adsorption the spontaneous and evoked release of endogenous Zn²⁺ in the Ammon’s horn of unanaesthetized rats using push–pull cannulae stereotaxically implanted. It was found that when the cannula is located in the vicinity of the mossy fibers there is a significant spontaneous release of Zn²⁺ (two to four times the blank levels). A brief pulse of K⁺ (1 min) produces an evoked release of Zn²⁺ to (maximal) levels which are two orders of magnitude above the blank. When the cannula was located in the fimbria or other parts of the Ammon’s horn there was neither spontaneous nor evoked release (e.g. Fig. 8, and G. Charton, C. Rovira, Y. Ben-Ari and V. Leviel, submitted for publication). Also, a K⁺ pulse applied unilaterally just prior to perfusion-fixation produced a bleaching of histologically demonstrable Zn²⁺ (Fig. 9 and Ref. 8a). Release of Zn²⁺ has also been found in slice preparations. (e) Earlier studies have also shown that intracerebral application of Zn²⁺ also produce seizures. The possible mechanisms through which Zn²⁺ might contribute to the damage are not yet clear in view of the large spectrum of cellular activities in which Zn²⁺ is involved. It is however of particular interest to note that zinc blocks very efficiently sodium–potassium adenosine triphosphatase and glutamate decarboxylase. Summing up, several features of the mossy fibers–CA3 synapse might cause the selective seizure-related damage of the CA3 pyramidal neurons. Perhaps a likely sequence of events includes orthodromic generation of paroxysmal discharge in the granular layer of the fascia dentata by intra-amygdaloid KA; this causes a removal of the GABAergic inhibition in the hilar zone and thus a disinhibition of the mossy fiber input to CA3. Since the granular cells are resistant to epileptogenic procedures, they will discharge for prolonged periods. The release of an endokain will excite the CA3 pyramidal neurons by means of the postsynaptic KA receptors which might be directly involved in the control of Ca²⁺ channels. This is associated with a decrease in the extracellular Ca²⁺—and presumably an increased effectiveness of the endokain—and an increase in intracellular Ca²⁺ directly producing the damage. In addition, large shifts in the concentrations of zinc will contribute to kill the CA3 pyramidal neurons by unclarified mechanisms. It is probably the convergence of all these factors which underlines the particular vulnerability of the CA3 pyramidal neurons which can be destroyed by rather brief seizure episodes without an involvement of vascular or other deleterious conditions.

Non-selective seizure-related damage

This is also related to the seizures and, as such, blocked by administration of anticonvulsants. How-
ever, in contrast to the selective seizure-related damage, this is not associated with a specific synaptic connection, releasing a toxic factor but rather with more general deleterious conditions caused by the convulsions in some brain structures. This is best illustrated by the necrosis seen in the piriform region (e.g. Fig. 2) after parenteral or intracerebroventricular KA, but also intramygdaloïd folic acid. The necrosis involves the entire frontobasal and temporobasal portions of the brain separated from normal cortex by the fissura rhinalis dorsally, reaching rostrally the olfactory bulb and occipital cortex. With survival times of 1–3 days, the damage includes in addition to neuronal cell loss, demyelination, astroglial scaring periventricular hemorrhages and extensive vascular sprouting. As stressed recently by Sperk et al., this incomplete parenchymal necrosis is a lesion typical of anoxic-ischemic damage, although the topography of the lesions differ from that produced by generalized hypoxia or ischemia. The authors therefore suggested that the overactivity caused by KA in this region leads to release of water and metabolites and subsequently oedema; this by compressing drainage vessels against the skull in the frontobasal region result in local disturbance of blood flow and anoxic-ischemic changes. This non-selective seizure-related damage is probably also produced in a number of other brain regions including the lateral septum, the CA1 field of the Ammon’s horn and perhaps also medial thalamic nuclei. A severe parenchymal necrosis is also produced in these regions notably in CA1 which is more vulnerable to anoxic-ischemic episodes than CA3. Interestingly, these regions have little or none of the features associated with the selective seizure-related damage in CA3; for instance the lateral septum and medial thalamus are virtually devoid of high affinity specific KA receptors and zinc.

RELEVANCE TO HUMAN EPILEPSY

Clinical considerations

Psychomotor or temporal lobe epilepsy or complex partial seizures as defined in the international classification is seen mainly in adults. Although seizure disorders frequently have their onset early in life, the seizures are more frequently of the generalized type in children younger than 15 years whereas temporal lobe epilepsy is much less frequent (21% as compared to 56% in adults). Furthermore since temporal lobe epilepsy is amongst the most difficult type to treat medically, surgery has become a useful form of therapy. The symptomatology of temporal lobe epilepsy, its electrographic, metabolic and histopathological sequelae have been reviewed in earlier monographs to which the reader is referred. This disorder is complex, has a large number of different aetiologies, includes a symptomatology which is not readily defined in experimental animals, and is often a life time process with a complex interaction occurring between an initial event (occurring at birth) creating a scar; the latter favors the occurrence of seizures which may also contribute to further brain damage. Therefore even if species differences are put aside, an animal model cannot be expected to reproduce all the features of such a complex disorder. The researcher is faced with lifetime neurological disorders such as temporal lobe epilepsy with the obvious necessity of adopting a very pragmatic attitude and accepting the fact that the model will at best reproduce circumscribed features of the disease. Hence, I shall briefly present the aspects of human temporal lobe epilepsy which deserve particular attention as they can be partly interpreted in the framework of experimental data.

The hippocampal formation and amygdala occupy a central position in temporal lobe of epilepsy. Initially described by Jackson, temporal lobe epilepsy is associated with a symptomatology which includes: (1) sensory symptoms (visual, olfactory, gustatory, sensations, etc.); (2) mental symptoms (clouded consciousness, “forced thinking”, déjà vu, hallucinations, etc.); (3) visceral symptoms (epigastric sensations, chewing with salivation, oral automatisms, etc.); (4) somatomotor symptoms (tonico-clonic movements).

There is a general consensus that the temporal lobe occupies a central position in this syndrome, hence the name. Thus, although initially controversial, electroencephalographic studies using superficial and deep electrodes have shown that the clinical symptomatology is associated with the presence of paroxysmal discharge in the temporal lobe region. More recently, the involvement of these regions is also suggested by positron emission tomography. Although the paroxysmal discharge is often present in the hippocampal formation—in keeping with its particularly low threshold to epileptogenic procedures—the amygdaloid complex plays a more direct role in the occurrence of several symptoms notably the oro-alimentary signs. Thus, in the case report made by Wieser, the episode began with recurring visceral symptoms. These were associated with hippocampal right discharges usually starting in the amygdala: bilateral hippocampal discharges were associated with an impairment of consciousness. Direct electrical stimulation of the amygdala—but not of the hippocampus— reproduces several of these signs which closely resemble the symptoms displayed by patients during “spontaneous” seizures. Furthermore, Buser et al. have shown that whereas all patients display hippocampal-evoked responses to stimulation of the amygdala, an amygdaloïd response to stimulation of the hippocampus is only observed in temporal lobe epilepsy. Raising the possibility that the facilitation of hippocampoamygdaloïd connections is instrumental for the aetiology of this disorder. This is particularly important in view of the
anatomical evidence for the involvement of the amygdala (and not the hippocampus) in several clinical signs associated with epilepsy of the temporal lobe. In fact, in both rats and primates, the central amygdala directly projects to motor structures coordinating facial and masticatory movements; the amygdala is also in a position to directly control vegetative and autonomic function through its massive projections to the hypothalamus and brain stem centers (e.g. Ben-Ari and Refs therein). Projections from the amygdala to the striatum are probably involved in some of the stereotypies associated with temporal lobe epilepsy. These anatomical observations are particularly relevant since they constitute, in the opinion of the present reviewer, a common ground for discussion between the clinician and the researcher.

The temporal lobe region harbors the main pathological substrate of psychomotor epilepsy. Five decades after the observations of Bouchet and Cazauvieil made in 1815, Sommer made a detailed description of the “Ammon’s horn sclerosis” which consisted in pyramidal cell loss with a typical gradient of vulnerability. The observation that a sclerosis of the hippocampus constitutes the most frequent damage in post-mortem examination of chronic epileptics has been repeatedly confirmed (it is on average found in 50–70% of the cases). In the work of Margerison and Corsellis and Sano and Malamud the patients were clinically and electroencephalographically studied and a significant correlation between the symptoms and the subsequent brain damage was noted. The brain damage involves in addition to the hippocampus—where the most frequent degeneration affects the Sommer sector, the endofium and in severe cases the granules of the fascia dentata—the amygdala, adjacent cortical regions, cerebellum, and midline thalamic structures. Several of these regions are vulnerable to a variety of deleterious conditions such as febrile convulsions but also anoxia and other conditions which are not associated with convulsions.

Temporal lobe seizures are particularly resistant to drug treatment and therefore surgery has been used for treatment of intractable epilepsy. With the advent of these procedures, the nature and characteristics of the lesions have been examined in more detail. Studies such as those of the Scheibels (also see Brown) have revealed the progressive time course of pathological changes. Several reports have shown that when the pathological abnormalities are removed, the patients benefit substantially from the procedure, i.e. there is general consensus that the sclerotic area contributes to the temporal lobe seizures.

In contrast, there is less consensus amongst the clinicians on the aetiopathology of the brain damage. Initially Sommer suggested that the sclerosis was the cause of epilepsy; the studies of German pathologists notably Spielmeyer reversed the hypothesis and interpreted the lesion as ictal image or more precisely as resulting from hypoxic–ischemic episodes associated with the seizures. However, since epilepsy is associated with vascular dilatation and not constriction (e.g. Ref. 141, other suggestions have been made including excessive increase in metabolism (i.e. a mismatch between metabolic demand and blood supply), increased permeability of the blood–brain barrier, or also increased brain volume (cerebral oedema which would lead to compression of vessels with herniation at the supratentorial region and compression of the posterior cerebral arteries). Other theories include early damage to the hippocampus due to birth or early infancy head trauma or febriles convulsions. Since, there is no clinical consensus for aetiology of the damage, animal models are useful to clarify this issue.

Relevance of animal models for human temporal lobe epilepsy

To bear some relevance to human temporal lobe epilepsy, the animal model must fulfill at least the following criteria: (a) the hippocampus, amygdala and other limbic structures must play a central role in the symptomatology; (b) it must reproduce a pattern of brain damage which is reminiscent of Ammon’s horn sclerosis; (c) it must reproduce the spontaneous and repetitive seizures of the temporal lobe type that are the hallmarks of epilepsy; (d) it must be relatively resistant to anticonvulsants in parallel to human temporal lobe epilepsy in order to constitute a useful test model for developing new drug treatment. Several animal models have been used to experimentally reproduce temporal lobe epilepsy.

Animal models which produce generalized tonico–clonic generalized convulsions. Parenteral administration of a number of potent convulsants such as bicuculline, allylglycine or pentetrazole, produce generalized convulsions (e.g. Refs 19, 108, 111 and 176, and Refs therein). These animal models however do not fulfil the above mentioned criteria.

(a) Electrographic and clinical data indicate that the hippocampus and other limbic structures are not preferentially involved in these convulsions as the seizures are generalized from the onset to the entire cortical mantle (ibid.). In immobilized artificially ventilated rats, Siesjö and Abdul-Rahman have shown by means of 2DG autoradiography a metabolic rise in the hippocampus; however in freely moving animals there is no rise in metabolism in hippocampus. A recent study, in which a quantitative determination of 2DG in several brain regions was made, has confirmed and extended this observation, the overall picture with bicuculline in freely moving animals is that of a marked regional metabolic depression, including in the hippocampus. This also reflects the difficulties in extrapolating from immobilized preparations—on which most of the
studies using bicuculline have been made—to the freely moving conditions.

(b) In a number of studies made in immobilized artificially ventilated animals, bicuculline and similar convulsants produce hippocampal damage; however the lesions are quite widespread and include several non-limbic structures (superficial layers of neocortex, basal ganglia, cerebellum, etc.). Other studies have failed to demonstrate significant hippocampal damage. In one study made in freely moving animals, damage to the basket cells of the cerebellum was the most consistent observation. Söderfeldt and coworkers have furthermore shown that after brief periods of recovery the oedema caused by the convulsants has vanished and most neurons look normal.

(c) To the best of the present reviewer’s knowledge, "spontaneous" seizures are not observed after such procedures (e.g. Meldrum). Unless such seizures are produced—and particularly seizures of the limbic type—the relevance of these procedures to study temporal lobe epilepsy can be questioned.

Using the bicuculline model, Meldrum and coworkers initially concluded that damage to the hippocampus is primarily due to anoxic–ischemic episodes. The vacuolization noted in several brain regions was interpreted as primarily due to anoxic–ischemic episodes. In a number of studies made in immobilized animals, damage to the basket cells of the cerebellum was the most consistent observation. Söderfeldt and coworkers have shown that the vacuolization seen by light microscopy reflected dilatation of the endoplasmic reticulum and cytoplasmic vacuoles, and not mitochondrial swelling. In subsequent studies, Meldrum et al. noted that the cerebellar damage was more reduced than the hippocampal one by paralyzing the animal, suggesting that the former may be causally related to the motor convulsions. Since in a later study hypoxia surprisingly reduced—rather than enhanced—as expected—the hippocampal damage, the authors suggested that the epileptic seizures per se could play an important role in the aetiology of the lesion. Subsequent studies in which the blood flow was measured provided further evidence that the damage was not solely due to an imbalance between metabolic demand and supply. These experiments have not been confirmed however by Söderfeldt et al. using a similar preparation, since it was shown (a) that hypoxia augments rather than reduces the severity of the damage and (b) the measurement of the ratio between the local cerebral blood flow and glucose consumption indicates that a mismatch in the hippocampus probably plays an important role in the damage seen with the bicuculline model.

To summarize, procedures which produce tonic–clonic generalized convulsions have provided controversial results concerning the nature and regional distribution of the damage and the aetiology of this damage. It is the present reviewer’s opinion that the pathological sequelae noted after severe tonic–clonic generalized convulsion are explicable by the general deleterious conditions associated with the convulsions i.e. these procedures do not produce the selective seizure-related damage. To obtain compelling evidence for a causal role of the paroxysmal discharge per se it is essential: (a) to directly measure the local PO$_2$, PCO$_2$ and blood flow in vulnerable regions in order to exclude local hypoxia as an important factor in the pathology; and (b) to define in detail the anatomical substrate of the propagation seizure activity and the possible preferential participation of a given excitatory input to the hippocampus or other vulnerable regions.

Animal models which produce focal seizures with a limbic symptomatology. To produce focal secondarily generalized seizures with a limbic symptomatology, several experimental approaches have been used, including local injection into the amygdala of aluminum cream or cothalt, into the lateral ventricle or of ouabain into the lateral ventricle or septum. Another method is based on continuous electrical stimulation of the amygdala in previously kindled rats. Some of these procedures fulfil a number of the aforementioned criteria including hippocampal damage and "spontaneous" seizures.

The kainate model—which has been more extensively studied than the aforementioned procedures—is particularly useful for the study of the evolution, propagation and pathological consequences of epileptic discharge in the limbic system. It clearly fulfils the criteria mentioned above with regard to human temporal lobe epilepsy since: (a) the hippocampus, amygdala and other limbic structures play a central role in its symptomatology; (e.g. Ben-Ari and Refs therein); (b) the pattern of brain damage is clearly reminiscent of Ammon’s horn sclerosis with a similar gradient of vulnerability. A number of differences have been noted, however, including the neuronal cell loss in the granular layer of the fascia dentata in humans whereas this is resistant in the animal models. However, cell counts were not made in these studies and in a more recent work, patches of necrosis were noted in this region after survival periods of 1–2 months (L. Nitecka, E. Tremblay and Y. Ben-Ari, in preparation); (c) spontaneous seizures with a limbic symptomatology are consistently noted following parenteral injection of KA (Fig. 3), or intracerebral KA. In keeping with this, electrophysiological studies in hippocampal slice suggest that the kainate-lesioned hippocampi become epileptogenic; (d) available anticonvulsants are weak against the seizures generated by KA.

The kainate model can shed some light on temporal lobe epilepsy in humans. For instance, the observation that selective seizure-related damage in CA3 can be produced without hypoxia or hypercapnia suggests that restricted paroxysmal discharge may produce a selective seizure-related localized damage in the human hippocampus. Conversely, the damage noted in the human temporal cortex might be due to general disturbances associated with the seizures. If the Ammon’s horn region to which the mossy fibers project is enriched in high affinity kainate receptors (present in human brains), this may indicate that the release of an endokain from mossy fibers plays a role in the aetiopathology of the sclerosis. Autoradiographic techniques and zinc stains on human
post-mortem material can be used to study the biochemical organization of this region in normal and epileptic brains. It will be of particular interest to see whether there is also an abnormal sprouting of mossy fibers in the affected Ammon's horn. In fact, Nadler and coworkers have recently shown that following destruction of the CA3 pyramidal layer, the mossy fibers deprived of their postsynaptic targets, establish new connections, with the granules of the fascia dentata. This new circuit increases the excitability of dentate granule cells which now become epileptogenic. Further animal studies should examine whether surgical removal of the necrotic region eliminates spontaneous seizures in temporal lobe epilepsy; there is some indication in the literature that this may be indeed the case.

CONCLUSIONS

Kainate is perhaps not as useful a tool as initially thought to produce axon-sparing lesions. On the other hand, studies made with kainate in the last few years reflect the considerable usefulness of KA and other excitotoxins to reproduce experimentally a number of models of interest to human pathology. As the emphasis of the present reviewer's own research suggests, it is the field of temporal lobe epilepsy which will particularly benefit from studies of the mechanisms of action of kainate. The use of KA undoubtedly will provide us with a better understanding of the normal and pathological functioning of the hippocampus and other limbic regions.

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REFERENCES

Limbic seizure and brain damage produced by kainic acid


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*Note added in proof.* In a recent study (L. Nitecka, D. Riche, G. Ghilini and Y. Ben-Ari, in preparation) using an antibody directed against GABA, we have observed a rapid destruction of GABA-containing neurons in the instar zone after parenteral administration of KA.