MATURATION OF KAINIC ACID SEIZURE-BRAIN DAMAGE SYNDROME IN THE RAT. I. CLINICAL, ELECTROGRAPHIC AND METABOLIC OBSERVATIONS

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Abstract—The maturation of the seizure/brain damage syndrome produced by parenteral administration of kainate was studied in the rat. The motor, electrographic and metabolic alterations are described in the present report, the maturation of the pathological abnormalities and of the specific kainate binding sites are described in the two following companion papers.

Parenteral kainate produces tonico-clonic seizures until the end of the third week of age when limbic motor signs (wet-dog shakes, facial myoclonia, paw tremor etc.) were first produced. Using the 2-deoxyglucose autoradiographic method, we found that in animals of 3 days of age and until the third week of age, kainate produced a rise in metabolism restricted to the hippocampus and lateral septum. This was paralleled by paroxysmal discharges which were recorded in the hippocampus.

Starting from the end of the third week of age approximately—i.e. when the toxin produced limbic motor seizures—there was a rise of labelling in other structures which are part of or closely associated to the limbic system i.e. the amygdaloid complex, the mediodorsal and adjacent thalamic nuclei, piriform, entorhinal and rostral limbic cortices and areas of projection of the fornix. These metabolic maps are thus similar to those seen in adults.

Two main conclusions can be drawn from these experiments: (1) kainate activates the hippocampus from a very early age probably by means of specific receptors present in this structure and (2) the limbic syndrome will only be produced by the toxin once the limbic circuitry—including in particular the amygdaloid complex—is activated by the procedure i.e. after the third week of age.

Parenteral administration of the potent neuroexcitatory-neurotoxic agent kainic acid (KA) in rats produces a limbic seizure/brain damage syndrome.6,59,62 Paroxysmal discharge is always noted first in the hippocampal formation—by far the most vulnerable structure to KA48—and electrophysiological studies made in the slice preparation have shown that seizure activity is produced with concentrations lower than 1 μM in the particularly vulnerable CA3 region48 which is also particularly rich in KA receptors.5,45,66 Metabolic studies, using the 2-deoxyglucose (2DG) autoradiographic method, depict with remarkable detail the onset of enhanced neuronal activity in the hippocampal formation and its propagation to other limbic structures along a circuitry which has already been analysed in detail.63 This is associated with limbic motor seizures which are reminiscent of those observed after repetitive electrical stimulation of limbic structures, notably the amygdala and hippocampus.35 Furthermore, the map of subsequent neuronal degeneration parallels to a large degree the 2DG maps;6 this suggests (in keeping with experiments using intracerebral injection of KA45,47,63) that the presence of cell damage is intimately related to an excessive increase in neuronal activity and metabolism.

Since this pattern of brain damage is remarkably reminiscent of that seen in hospitalized chronic epileptics,5,38,56,57 this procedure constitutes a useful means for studying the controversial and complex problem of the relationship between epilepsy and brain damage (also see Ben-Ari3). Furthermore, since the rats display spontaneously, individual limbic seizures 1–2 months after systemic or intracerebral injections of KA, this procedure may constitute a good model of temporal lobe epilepsy.

The present experiments were undertaken in this framework in order to comprehend better the particular vulnerability of limbic structures—in particular the hippocampal formation—to KA. We reasoned that since these structures undergo considerable postnatal development, the study of the maturation of KA pathogenesis would enable us to delineate the aetiological factors, notably the anatomical correlate of this vulnerability. In the present report we describe the maturation of the motor, electrographic and metabolic alterations (as revealed by the 2DG method) induced by KA. In the companion papers, we shall successively describe the maturation of brain damage and of specific KA binding sites.59,69 Our results have been presented in part elsewhere.39
EXPERIMENTAL PROCEDURES

Female Wistar rats with their litter of 2 days after birth were purchased from Iffa Credo. The mothers and their offspring were kept in individual cages in a 12 h light/dark lighting schedule and given access to unlimited water and laboratory chow. The effects of KA were tested at five developmental stages: 3, 9-12, 14-16, 19-24 and 35-36 days after birth (P3, P9-12 etc.). Kainic acid (Sigma; 1–9 mg/kg depending on the age) was injected (i.p.) in a constant volume of phosphate buffer (5 ml/kg). Control animals of the same age (n = 5 for each age) were injected with the vehicle. Each animal was used only once. Immediately after the injection, animals were placed individually in transparent boxes and the behaviour noted for periods varying from 2 to 3 h, by two independent observers, one of whom was unaware of the treatment.

Electrographical changes after KA were only examined in 10-12 day old rats (n = 4). They were anaesthetized with equithesin (Jensen Salisbury, 4 ml/kg) and implanted with a twisted electrode (50 µm wire) in the dorsal hippocampus. For implantation they were put in a mould in wax, prepared in the laboratory to maintain head and body in a well-defined position. Coordinates of the electrode (approximately A = +12; L = +2 and H = +17 according to the atlas of Sherwood and Timiras) for young rats were determined in a separate group of rats and controlled by histological procedures after the experiments. Also an epidural silver deposit was put in the frontal region for electrocorticogram. Kainic acid was given the same day, after recovery from anaesthesia.

Alterations of brain activity after KA injection were evaluated autoradiographically following the administration of 2-deoxyglucose. In a first experimental series, 2-deoxy-2-[14C]glucose (2DG, CEA Saclay, sp. act. 40–50 mCi/mmol) was given i.p. (100–300 µCi/kg) in rats pretreated with the vehicle solution or KA at P3, 7, 12 and 24 (two to five rats per group). In each KA-treated rat, 2DG was given 5–10 min after the beginning of the motor seizures. Forty-five minutes after the 2DG injection, the brains were rapidly removed after cervical dislocation, cooled in isopentane kept at −50°C and stored at −20°C until sectioning. Sections of the entire brain (usually 32 µm) were made using a cryostat (−22°C) and then dried and put on mammary 13 films (Agfa Gevaert) in an X-ray cassette, usually for 21 days for animals of 12 days (or more) of age: for younger animals (P3, P7), up to 4 weeks of exposure time was needed (see below). After development of the films, the sections were stained with cresyl violet; superposition of the autoradiographs on the stained sections from which they were obtained permitted a better recognition of brain structures with different 2DG labelling. In some cases, densitographs were taken from the autoradiographs by a computer-assisted Leitz microphotometer to quantify the differences in labelling of hippocampal subfields (Fig. 6, also see Berger and Ben-Ari).

Another group of control and KA-treated rats (n = 4 for each age group) similarly received 2DG (25 µCi/kg) and were sacrificed 45 min later; the brains were then rapidly dissected on an ice-cold plate. Each dissected brain area was immediately weighed and dissolved in Protosol (NEN). Radioactive material present in each structure was measured by liquid scintillation counting. Since there were some significant differences in the total radioactivity found in the brains between the various cases after peripheral injection of 2DG, the cpm/mg wet wt found in each structure was

<table>
<thead>
<tr>
<th>age</th>
<th>3d</th>
<th>9-12d</th>
<th>14-16d</th>
<th>19-24d</th>
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<tr>
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<tr>
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<td>(n)</td>
<td>(11)</td>
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![Fig. 1. Percentages of rats presenting tonico-clonic seizures, wet-dog shakes or limbic motor seizures after an i.p. injection of KA. The age of rats (in days: d), dose of KA and number of rats (n) are indicated for each group. Proportions of rats with continuous motor seizures during at least 1 h after their occurrence are indicated in the dotted area.](image-url)
Seizures after kainate in developing rats

Table 1. Influence of the age on the delays of occurrence of kainic acid-induced motor signs

<table>
<thead>
<tr>
<th>KA (mg/kg)</th>
<th>Motor signs</th>
<th>P3</th>
<th>P9–12</th>
<th>P14–16</th>
<th>P19–24</th>
<th>P35</th>
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<tbody>
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<td>15–25 min</td>
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<td></td>
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<tr>
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<td>15–20 min</td>
<td>30–35 min</td>
<td>20–30 min</td>
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<tr>
<td></td>
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<tr>
<td>4</td>
<td>Scratching or WDS</td>
<td>25–30 min</td>
<td>25–30 min</td>
<td>40–50 min</td>
<td>60–70 min</td>
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<td></td>
<td>motor seizures</td>
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<tr>
<td>9</td>
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<td>motor seizures</td>
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Latency of the motor signs produced by parenteral KA as a function of the animal's age. Note the increased latency with age, in particular during the first 16 days of life. Same cases as in Fig. 1. WDS, wet-dog shakes.

RESULTS

Behavioural observations

In 3–16 day old rats (P3–16), i.p. KA induced a regular pattern of clinical signs, not seen in vehicle-injected rats (n = 5 in each group). They started with scratching-like movements of the hindpaws; later, the animals lost postural control and turned on one side or on the back. At this stage, the scratching-like movements disappeared and were replaced by clonic (and sometimes tonico-clonic) seizures, involving movements of all paws, and associated with tremor of the head. However, several differences have been observed in the effects of KA injected during the first, second or beginning of the third week after birth. They consisted first in an increased latency of the signs with age (Table 1); the dose of KA clearly did not influence these delays. Furthermore, in older rats, higher doses of KA were necessary to induce motor seizures (Fig. 1). Thus, 1 mg/kg KA induced continuous clonic seizures in all rats of the first-week group, only in half of those of the second-week group. Three out of six rats tested during the first week died after 2 mg/kg KA (in less than 1 h; not illustrated), whereas the same dose was well tolerated in the second week of life, with a 100% seizure response (Fig. 1), being almost without effect in the third-week group (intermittent clonic seizures in only one out of nine rats tested, Fig. 1). The scratching-like movements seemed to be less dependent on age or dose of KA, since we observed them in all rats tested (i.e. from P3 to 35).

Pronounced changes in KA-induced behaviour occurred before the end of the third week of life (Fig. 1). After P19, the animals displayed periods of immobility during the first 25–35 min after the injection, followed by the simultaneous appearance of scratching-like movements and wet-dog shakes, which were repeatedly seen (1–2/min) until the end of the first hour. Thereafter, these signs were progressively replaced by facial clonic and masticatory movements, paw tremor and rearing, i.e. a stage 4 limbic motor seizure, accompanied by salivation. These clinical signs as well as their delay in occurrence (see Table 1) are similar to those seen in adult animals (250–300 g) after systemic injection of KA. Limbic motor seizures were seen in all 19–24 day old rats injected with 6 mg/kg KA (Fig. 1); starting from this age, clonic or tonico-clonic seizures were never observed, even after much greater doses of KA (9 mg/kg, n = 10, not illustrated).

In the fourth week of life, KA-induced limbic motor seizures lasted for several hours, interrupted by periods of hyperactivity (running, jumps, escape attempts). Only in the fifth week, (n = 9, not illustrated), did recurrent limbic motor seizures culminate in a true status epilepticus (e.g. uninterrupted seizures), similar to that described in adult rats.

Electrographic observations

The electrographic activity of dorsal hippocampus and the electroencephalogram have been recorded before and after KA injection in P10–12 rats. As mentioned above (see Experimental Procedures), KA was injected in rats after recovery from anaesthesia; this probably explains why a dose higher (4 mg/kg) than in rats not previously anaesthetized (see Fig. 1) was needed to produce clinical (and also electrographic) alterations. In all cases (n = 4), the electrographic changes appeared after a delay of at least 15 min following KA injection and preceded the occurrence of the first motor manifestations (see Fig. 2A). Then, and without any clear relation to the motor manifestations, epileptiform activity was progressively more frequent in both hippocampus and cortex; however the spiking activity of high frequency was more pronounced at the hippocampal level than at the cortical one (Fig. 2B–D), even at longer delays when motor seizures were continuously observed (Fig. 2D).

Regional metabolic changes

In the rat, several brain structures do not approach
Fig. 2. Illustration of seizure activity recorded in the right hippocampus (rH) and cortex (rCx) of a 10 day old rat after KA injection (2 mg/kg i.p.; arrow in A). (A) The electrographic changes occurred rapidly (17 min) after KA injection and preceded the first behavioral abnormalities (20 min). They became progressively more sustained in both structures (B and C) and were rather continuous 37 min after the injection (D), when the rat presented also continuous clonic or tonico-clonic seizures.
maturity in term of synaptic organization, neurotransmitter content and receptor distribution until the fourth week of age (see below); in keeping with this, the developmental pattern of 2DG consumption in the brain of young animals changed conspicuously during the period of maturation. The autoradiographic maps obtained from the control (i.e. saline-injected) cases are described first; special emphasis is put on the regional distribution of labelled material in the hippocampus and other limbic structures which are particularly involved in the kainate effects.

Control cases.

First week.

At P3 (n = 3), 2DG labelling was rather weak and showed limited variation between brain structures. Thus even the difference between white and grey matter which is readily apparent in more mature brains was not conspicuous (Fig. 3A-D). The most consistently labelled regions were the plexus choroides and the germinal subependymal layers of the ventricle (Fig. 3A, B and 7A) and a number of thalamic (notably in the ventrobasal complex, i.e. Fig. 3C-D), brainstem (notably the vestibular nuclei) and cerebellar nuclei (not illustrated). In the forebrain, the most conspicuous labelled structure was the anterior olfactory nucleus (not shown).

At P7 (n = 2), higher levels of labelled material were noted in dosolateral tegmental nucleus, olivary superior complex, and locus coeruleus (not shown), ventromedial thalamic nucleus (Fig. 3E), area pretectalis and substantia nigra, pars compacta (Fig. 3F). In the hippocampal formation, there was a gradient between the highly labelled main neuronal layers (i.e. the pyramidal and granular layers) and the weakly labelled neuropil in stratum radiatum, oriens and molecular (Figs 3F, F and 6A). This distinct gradient was apparent also at P3 (Figs 3B, D and 6A) but was inverted at a later age (see below). In contrast we did not observe a similar clear-cut gradient in the neocortical matrix.

Second week.

The pattern of 2DG consumption in that period is presented in detail in Fig. 4, obtained from two cases at P12. The distinct difference between the white and grey matter was now apparent as well as discrete sites of high labelling which enabled us to readily distinguish a number of brain structures. Thus, in the auditory system, the cochlear nuclei and superior olivary complex (Fig. 4E, F) were highly labelled. as well as the dorsolaterallemniscal nuclei (Fig. 4G), the inferior colliculi (Fig. 4G)—which have the highest glucose consumption in the resting adult brain—and the medial geniculate body and auditory cortex (not shown). Intense labelling was also conspicuous in optokinetic and motor systems including the pre-tectal area (not shown), vestibular nuclei and cranial nerves nuclei (Fig. 4E, F). Extrapyramidal structures such as pars compacta of the substantia nigra or subthalamicus nucleus (Fig. 3G, H) also show relatively high labelling. Structures belonging to the somatosensory system such as nucleus of trigeminal nerve (Fig. 4E, F), ventrobasal complex of the thalamus (Figs 3H and 4D) and somatosensory cortex (Figs 3G and 4C, D), also developed high levels of labelling. In the somatosensory cortex, the rise of labelling was particularly conspicuous in layer IV and the superficial part of layer VI (Fig. 4C, D), although all cortical layers have shown distinctly high levels of labelling. In the limbic cortex, the deep layers in the vicinity of the rhinal fissure were particularly dark. In the ventral forebrain, in addition to the anterior olfactory nucleus which was highly labelled in younger cases, the most conspicuously labelled area was the cholinergic basal forebrain system, in particular the vertical and horizontal limbs of the diagonal band of Broca (not shown) and its adjacent magnocellular preoptic nucleus (Fig. 4A). Interestingly, the ventrobasal complex of the thalamus was also strongly labelled, also stains heavily for acetycholinesterase at this developmental stage.

In the hippocampal formation, the basal pattern was now qualitatively comparable to that seen in adults. In the fascia dentata, in both the septal and temporal poles, the granular layer (Figs 3G, H and 6A) showed lighter labelling than the adjacent molecular layer, i.e. a reversed pattern to that seen in P3 and P7 cases. Interestingly, in both the septal and temporal poles, a high level of labelling was noticeable in a region corresponding to the boundary between CA3 and CA1 (Fig. 3G, H); this could reflect the CA2 subfield but this region is ill-defined in rats.

Age 74 days.

The pattern of 2DG labelling differs only in few details from that described above for the 2nd week. A particularly significant feature of the 2DG maps at this age is the presence of high labelling in the inferior thalamic peduncle region (Fig. 5C). The reasons for this labelling are not clear since fibre tracts have usually a low metabolism, this labelling could be due to the presence of neurons migrating along this bundle of fibres. The basolateral nucleus of the amygdala was also highly labelled (Fig. 5D); this is characteristic in adults and was not observed in the earlier periods of the postnatal life. In the hippocampal formation, the molecular layer of fascia dentata was highly labelled (Fig. 5E) as well as the stratum oriens and pyramidal layer of CA1/CA3 border regions.

The main observations concerning the hippocampus are described in Fig. 6 where, using a microphotometer device, optical densitographs were generated (see Experimental Procedures and legend to Fig. 6). At P3, the granular and pyramidal layers show heavier labelling, whereas already at P12 the molecular layer of the fascia dentata is darker than the main
cellular layers. Similar observations were made previously.41

Effects of parenteral administration of kainic acid.

First week.

The hippocampal formation, notably the CA3 field, was the only structure in which KA produced a clear-cut alteration in these animals (n = 5) as compared to control cases (Fig. 7). Within the hippocampal formation, there was a clear septotemporal as well as lateromedial gradient. Maximal rise in labelling was noted in pyramidal layer of CA3 (ibid.) but also to a lesser extent in strata radiatum and oriens of the lateral-most part of CA3 and partly in the adjacent fimbria–fornix (Fig. 7C). The dark area seemed to diverge to the border with CA1 (to a small area which is highly labelled in control animals, see Fig. 3) and to extend to the hilus medially. The temporal pole of the hippocampal formation including the entorhinal cortex was not labelled.

Second week.

The labelling in the hippocampal formation is depicted in detail in Figs 8 and 9. The extensive distribution of this labelling in two cases (with frontal and sagittal sections) is reflected in Fig. 8, and details of this distribution is illustrated in Fig. 9, using the superposition technique (see Experimental Procedures). The activated regions included: (1) in the septal portion: all layers of CA3, the hilus and the border (CA3–CA1) area; (2) in temporal portion: all layers of CA1 and CA3 as well as subiculum and the

Fig. 3. Regional glucose consumption in three control rats, 3 (case 1535, A–D), 7 (case 1590, E, F) and 12 days (case 1249, G, H) after birth. For this and following figures, the animals received [14C]2DG (200–300 µCi/kg i.p.) and the brains were removed after 45 min as described in the Experimental Procedures. The frontal (or sagittal, Fig. 8) sections were 32 µm thick, and were usually exposed for 21 days with the exception of P3 and P7 cases (A–F) where the exposure time was 4 weeks. Note the high labelling in the pyramidal and granular layers of the hippocampal formation at P3 and P7 whereas the molecular layer is more labelled at P12. Also note the strong labelling of the choroid plexus at P3 (A, B) and the labelling of the CA1/CA3 border at P12 (G and H, arrows). Asterisks in (D) indicates artefacts. Double arrows indicate the highly labelled parietal cortex (also see Figs 4 and 5). Abbreviations in this and the following pictures are given on a separate list.

Abbreviations used in figures

Ad, Abd basolateral nucleus of the amygdala
AC anteror commissure
AON anterior olfactory nucleus
CA1–4 fields of the Ammon's horn according to Lorente de Nó
CA3–S septal region of CA3
CA3–T temporal region of CA3
cc corpus callosum
CeN cerebellar nuclei
CEX external capsule
CIF inferior colliculus
CNG cingulate cortex
CL claustrum
CN cochlear nuclei
CP caudate-putamen
cx cortex
D LN dorsolateral lemniscal nucleus
EA entorhinal area
FD dentate gyrus
FH hippocampal fissure
Fi fimbria of the hippocampus
g granular layer of the dentate gyrus
h hilus of the dentate gyrus
HN habenular nuclei
IL infrahinal area
INS insular cortex
IP interpeduncular nucleus
ITP inferior thalamic peduncle
LL lateral lemniscus and nuclei
LS lateral septal nucleus
m molecular layer of the dentate gyrus
M, MB mamillary body
MD medial dorsal thalamic nucleus
MEA medial entorhinal area
MG medial geniculate body
ND cerebellar dentate nucleus
NDB(v) nucleus of the vertical limb of the diagonal band
NF cerebellar fastigial nucleus
NI cerebellar interpositus nucleus
NR red nucleus
NTS–V nucleus of the spinal tract of the trigeminal nerve
N–III motor nucleus of the oculomotor nerve
N–VII motor nucleus of the facial nerve
O superior olivary complex
p pyramidal layer of the hippocampus
PIR piriform cortex
PL prelimbic area
Poma magnocellular preoptic nucleus
Pt pretectal area
PV paraventricular thalamic nucleus
RE reuniens thalamic nucleus
S septum
SNc substantia nigra (pars compacta)
SR rhinal sulcus
St stria terminalis
STN subthalamic nucleus
TOL lateral olfactory tract
TT taenia tecta
TUS olfactory tubercle
V lateral ventricle
VB ventrobasal thalamic complex
VL ventrolateral thalamic complex
VM ventromedial thalamic complex
VN vestibular nuclei
I–VI cortical layers I–VI
Fig. 3.
Fig. 4. Distribution of 2DG labelling in 12 day old and control rats. Larger magnification than Fig. 3 to depict various details. In (A), (B) and (E), the autoradiographs were superposed on the Nissel-stained sections from which they had been obtained. This enables one to obtain a better anatomical resolution. Thus, note in (A) the magnocellular preoptic nucleus (Poma) which heavily stains (as compared for instance to the adjacent piriform cortex) and in (E) the vestibular (VN) and cochlear nuclei (CN) as well as the nucleus of the trigeminal nerve (NTS). (C) is enlarged from the boxed area in (D) to depict the cortical layers.

Fig. 5. Regional brain glucose consumption in a control case (P24). Note the high labelling in the region of inferior thalamic peduncle (C) and basolateral nucleus of amygdala (D and E) at this age. Double arrow, parietal cortex (B-E); single arrow-head, auditory cortex (F); double arrow-head, visual cortex (F).

Fig. 6. Optical densitographs reflecting the differences in metabolic activity of hippocampal subfields in control (A) and kainate-treated animals (B). (A) The optical density of the radiograms was measured with a Leitz microphotometer device; a rectangular beam of light with a cross-section of 40 \times 400 \mu m was automatically moved along a path which is shown by arrows in the right diagrams of the hippocampal formation. Light transmittance (T) was recorded in 40 \mu m steps and the logarithmic relation to film background (T0) plotted against the distance covered. The particular dots represent every second individual value (i.e. every 80 \mu m of beam pathway). Note that the highest optical density by day 3 is in the granular (g) and pyramidal (p) layers as well as in ventricular region (v) including the plexus choroides and the subependymal germinal tissue. There is a progressive shift from the main cellular layers (i.e. pyramidal and granular) to dendritic subfield during the time of maturation (compare P3, 12 and 24). (B) Effects of KA in a rat at P12; the beam pathways (square and triangle) are shown in the diagram. Note the large increase in the CA3 field; at this septotemporal level, the rise in the more medial parts of CA3 and CA1 (triangle) was smaller.

Fig. 7. Hippocampal glucose utilization after saline (A) or KA (1 mg/kg i.p.; B and C) in two rats (P3). 2-Deoxyglucose was injected 35 min after these injections; at this time, the KA-treated rat presented continuously tonico-clonic seizures which persisted until sacrifice. Note the rise in labelling in the Ammon's horn, particularly in CA3.

Fig. 8. Alterations in regional brain glucose consumption after KA injection (2 mg/kg) at P12, in two rats. (A) and (D) Frontal sections from case 1285 which received 2DG 24 min after KA; (F) and (F) sagittal sections from case 1291 which received 2DG 35 min after KA. Both cases presented continuous clonic seizures before and after 2DG. Note the conspicuous increased glucose consumption in septum (A, B) and hippocampal formation (C-F). In contrast to the effects of KA injection by day 3, the temporal pole of the hippocampal formation has also an increased labelling (D, E).

Fig. 9. Detailed pattern of labelling in various brain structures after KA injection at P12. In this figure, the 2DG autoradiographs were superposed on the Nissel-stained sections from which they had been obtained (see Experimental Procedures). Note the high labelling in the Ammon's horn (C-F), lateral septum (B) and vertical limb of the diagonal band (A). Star in (F) indicates the amygdalohippocampal transitional area located laterally to the rostral pole of the medial entorhinal area.

Fig. 10. Alterations in local glucose consumption following KA injection in a rat at P24 (case 15/72 received 9 mg/kg i.p. KA and 2DG 90 min later, i.e. at a time when stage 4 limbic motor seizures were already elicited at a mean frequency of 1.5-7 min). Note the increased labelling in infra-limbic cortex (B), amygdala (D, E) and entorhinal area (E). The prelimbic (B) and cingulate cortices (E) are the only structures not showing an increased labelling after KA as compared to adult cases. Asterisk (in F) indicates the amygdalohippocampal transitional area. Double arrow: parietal cortex.
Fig. 10.
transitional amygdalo-hippocampal area (Figs 8D–F and 9E, F); the hippocampal rudiment (taenia tecta) was also labelled (Fig. 9A). In contrast, as in younger animals, the entorhinal cortex was not activated. The densitographs obtained in the temporal pole of the hippocampus after KA are illustrated in Fig. 6(B).

In addition to the hippocampal formation, KA now induced a clear-cut metabolic rise only in the lateral septum (Figs 8A, B and 9B). The other labelled regions which are noted in Figs 8 and 9—notably the vertical and horizontal limbs of the diagonal band (Figs 8A and 9A), magnocellular preoptic nucleus (Fig. 8B) and ventrobasal complex, particularly its lateral part (Fig. 8C)—were also labelled in control cases (Figs 3 and 4).

Age 24 days.

A typical 2DG map after KA at P24 is illustrated in Fig. 10. In addition to the hippocampal formation and lateral septum, the following structures developed a high 2DG labelling: (1) amygdaloid nuclei, notably the medial and cortical nuclei, and the areas which are reciprocally interconnected with the amygdala, notably the bed nucleus of the stria terminalis (not shown) and the stria terminalis fibre tract as well (Fig. 10D); (2) piriform cortex and underlying claustrum (ibid.), the deep layers of the cortex in the vicinity of the rhinal fissure, infralimbic cortex and ventral pallidum (Fig. 10C, D); (3) medial and midline thalamic nuclei (Fig. 10D, E), preopticohypothalamic centres and ventral tegmental area (Fig. 10D–F). Interestingly, the development of labelling of some of these latter structures (e.g. thalamic and preopticohypothalamic nuclei) seemed to follow the process of maturation of the hippocampal efferents emerging through the post-commissural part of fornix (collumanae fornix), whereas the presence of high labelling in lateral septal nucleus at P12 could indicate the earlier maturation of the terminals of the precommissural fornix. In contrast to adult patterns, no rise in labelling was observed in the prelimbic and cingulate cortices (Fig. 10B–E).

The maturation of the metabolic changes produced by KA is also illustrated in Fig. 11. In this experimental series, control and kainate-treated rats at different ages were sacrificed 45 min after 2DG and the radioactive material counted in several brain structures (see Experimental Procedures). At P12, the hippocampus was the only structure in which an increase in labelled material was found ($P < 0.01$); there was no significant change at that age in the remaining dissected structures which included the amygdala, thalamus, cingulate cortex (Fig. 11) but also (not shown) the frontoparietal cortex, cerebellum, pons and caudate putamen. At P30, the metabolic rise in the amygdala ($P < 0.01$) and thalamus ($P < 0.05$) was apparent whereas the cingulate cortex was activated only in adult animals ($P < 0.05$).

Fig. 11. Changes in metabolic rise following KA administration at various ages (P12, P30 and adults, $n = 4$ for each group). Animals received saline or KA parenterally followed by 2DG (25 $\mu$Ci/kg). Forty-five minutes after 2DG, the brains were rapidly removed and the following structures rapidly dissected on an ice-cold plate: hippocampus (Hip), caudate putamen, amygdala–piriform cortex region (Amyg.), frontoparietal cortex, cingulate cortex (Cing.), thalamus (Thal.), pons and cerebellum. The mean cpm/mg (wet wt) in each structure was expressed in per cent of the average cpm/mg in the whole brain. The bars represent SEM. At P12, a metabolic rise was apparent only in the hippocampus; at P30 and in adults, there was a rise in labelling in the amygdala and thalamus. Mean values $\pm$ SE for controls, 12 days old, 30 days old and adult, respectively: hippocampus: 95.3 $\pm$ 1.5, 82 $\pm$ 4.8, 88.8 $\pm$ 5.4; amygdala: 91.6 $\pm$ 2.3, 90 $\pm$ 1.5, 86.6 $\pm$ 0.9; thalamus: 102 $\pm$ 2.0, 95.2 $\pm$ 4.7, 116.6 $\pm$ 1.5; cingulate cortex: 102 $\pm$ 1.5, 112.7 $\pm$ 0.5, 110.6 $\pm$ 2.
DISCUSSION

Epidemiological studies indicate that seizure disorders in humans frequently have their onset early in life. However the behavioural manifestations of the seizures are quite different from those observed in adults; thus, seizures are more frequently of the generalized type (primarily or secondarily) in children younger than 15 years, whereas partial epilepsy, for instance that associated with temporal lobe symptoms, is much less frequent in the latter group (21% as compared to 56% in adults) and notoriously non-existent in children younger than 3 years. Since parenteral or intracerebral administration of KA produces experimentally a limbic seizure and brain damage syndrome which reproduces several features of human temporal lobe epilepsy, it was warranted to study the maturation of kainic actions.

Maturation of electrographic and motor signs produced by kainic acid

There is a dramatic change in the motor syndrome produced by KA between P16 and 19, in agreement with a recent study. This change occurs with almost no transitional stage, suggesting a critical period of maturation of the cortex which mediates the limbic motor syndrome produced in adult rats by KA. Until P16, KA produces clonic or tonico-clonic seizures never seen in adults. It also produces automatic movements, notably scratching, which probably have the same functional meaning as the wet-dog shakes (also see Ref. 6) which are only observed after P19 with the same temporal course as the scratching movements (see Fig. 2, this report). As extensively discussed elsewhere, wet-dog shakes in adult rats probably constitute an adaptive mechanism to maintain body temperature; thus, parenteral KA produces a fall in body temperature and wet-dog shakes most often begin concomitantly with the maximum of this fall. Interestingly, this fall in body temperature also occurs in immature animals and is followed, as in adults, by a conspicuous rise in temperature. The anatomical correlate of this action is not known; however, we have suggested elsewhere the involvement of endorphin-containing arcuate neurons since (a) naloxone reduces the KA-induced fall in temperature and the wet-dog shakes (E. Tremblay, G. Charton and Y. Ben-Ari, unpublished observations), also see Ref. 22a) and (b) intracerebroventricular administration of endorphins produces a drop in temperature followed by a motor syndrome which has some similitude to the KA-induced one.

Between P3 and 16, the main changes in the syndrome concerned the latency of the signs and the doses needed to induce them, which were progressively increased (see Fig. 1 and Table 1). This is probably due to a maturation of the blood–brain barrier. In keeping with this possibility, pentetrazole—which probably crosses the blood–brain barrier readily—produces tonico-clonic generalized convulsions with an identical delay in immature and adult animals (i.e. 1–2 min. E. Tremblay, G. Charton and Y Ben-Ari, unpublished observations). However definitive conclusions can only be drawn once the amounts of KA crossing the blood–brain barrier have been determined.

Since animals of less than 1 week of age are not readily amenable to chronic electrographic studies, we have relied on the 2DG observations to evaluate the actions of KA. These, even by P3, show a conspicuous rise in 2DG consumption, restricted to the CA3 region of the Ammon’s horn (Fig. 7). In keeping with this, Cherubini and coworkers observed paroxysmal discharges in the hippocampus of curarized animals when rather large doses of KA are used. During the second week of age, both electrographic (this report and Cherubini et al.) and 2DG studies (this report) reveal the presence of seizure-like activity in the hippocampus and cortical electroencephalogram. The hippocampal spikes and polyspikes were rather isolated and showed little correlation with either the cortical paroxysmal discharges or with the motor signs (also see Cherubini et al.). There was also no simple relation between the former and the latter. However the electrographic records were made in animals which had just recovered from anaesthesia and thus delayed effects on the pattern of activity should be kept in mind.

These observations raise the following problems.

(1) Since in immature animals, KA often produces primarily cortical epileptic activity which secondarily spreads to the hippocampus, Cherubini and coworkers have suggested that the hippocampal seizure discharge may be due to the damage produced by the sustained cortical activity and not to a direct action of KA on this structure. This is unlikely since (a) high affinity KA receptors are present in the CA3 region of the hippocampus early in life (at any rate at P10) and (b) there is no cortical damage before P18, even with subsequently lethal doses of KA.

(2) Another major issue concerns the anatomical correlates of the tonic-clonic syndrome produced by KA before P19. Our study does not provide any indication in this respect, since the enhanced 2DG labelling of the hippocampus is unlikely to bear any relation to the syndrome produced by the toxin at that age. Bearing in mind the limitations of the presently used 2DG method (see below), it is possible that another brain structure mediates this action but also other explanations are possible. The possibility of a peripheral site of action of KA has been suggested as well as an interaction at the level of the spinal cord, however, in our experiments, limited observations of sections of the anterior pole of the cord failed to reveal any particular rise in labelling. Clearly, this anomaly of behaviour vs 2DG uptake in young animals is not yet understood.
2-Deoxyglucose observations

The 2DG pictures also suggest a major change in the brain's reactivity to KA and helps in better defining the anatomical correlates of this change. The following points related to the 2DG methodology deserve however emphasis prior to discussing our main observations.

The method developed by Sokoloff and coworkers\(^b\) applies to adult animals to which the radioactive material is given i.v., arterial samples removed during the assay period and the rate of phosphorylation of 2DG calculated by means of an equation which is valid under certain circumstances. To the best of our knowledge, consequently to several difficulties, this entire methodology has never been applied to immature rats. In the present as in earlier studies\(^{31,41}\) in which metabolic alterations were estimated in immature rats, 2DG was administered i.p. (also see Refs 40 and 51) and the distribution of labelled examined autoradiographically and quantified with a photodensitometer device. In spite of the possible complications and errors of this approach, the following observations suggest that this use can give valid results.

(a) In adult animals, i.p. and i.v. administration of 2DG yield essentially identical 2DG maps following parenteral KA (E. Tremblay and Y. Ben-Ari, unpublished results); this is in keeping with other observations.\(^{40,51}\) Discrepancies between i.v. and modified techniques have been however reported.\(^{36}\)

(b) In control conditions, the regional distribution of labelled material shows an excellent correlation with earlier observations obtained with histochemical mapping of oxidative enzymes during early postnatal life.\(^{23,44,54}\) Thus, oxygen consumption and oxidative enzyme activity in newborns are highest in the choroid plexus and ependyma, and high levels have been described in the locus coeruleus and in other brain regions (ibid.). There is also—a again in parallel to the 2DG pictures—a somatodendritic shift of metabolic levels in the fascia dentata, which presumably corresponds to the maturation of dendritic arborization and ingrowth of afferent inputs and synaptogenesis\(^{24,57,44,67}\) (and ibid.).

(c) Before the third week of age, KA produces a rise in labelling in the hippocampus, this correlates with the paroxysmal discharges recorded electrophysiologically in this structure\(^{31}\) (also see Fig. 2). After the third week of age, the rise in labelling in the amygdala and other limbic structures is identical to that seen in adult animals and is likely to be associated with the propagation of paroxysmal discharges along axonal connections which have been extensively characterized.\(^{5,36}\) Identical observations have been made both using the qualitative autoradiographic 2DG methodology\(^a\) and the measure of glucose consumption\(^b\) with the method of Sokoloff and coworkers.\(^a\) Similar observations have been made with other experimental models of epilepsy (in which the qualitative autoradiographic and the method of Sokoloff have been used), such as parenteral bicuculline which does not produce a rise in metabolism in unanaesthetized animals in several brain regions including the hippocampus.\(^{6,40}\)

(d) In both adult\(^a\) and immature animals (E. Tremblay, M. Berger and Y. Ben-Ari, unpublished observations), administration of an anti-convulsant (diazepam) prevenus KA-induced seizures and reduces the rise of labelled material in the hippocampus, again in keeping with the notion that this rise reflects an increased glucose consumption associated with the seizure discharge.

A second problem concerns the observations that in immature animals, substrates other than glucose can provide alternative sources for brain metabolism.\(^{17,18,31,50}\) In the rat, it has been estimated that up to 30% of the metabolic substrates can be provided by ketone bodies.\(^{11}\) If there are major regional differences between utilization of glucose and other substrates, the metabolic changes will not be estimated reliably with the 2DG method. However, such regional differences have not been found with ketone bodies (ibid.), and whenever such differences have been described with other metabolic sources, they appear to implicate primarily regions which are not protected by the blood-brain barrier.\(^{23}\) This, as well as the excellent correlations between our 2DG maps and the developmental changes in the distribution of oxidative enzymes (see Results section), speak against a major distortion of our main observations by this factor.

The first message which stems from our 2DG experiments is that the Ammon's horn is activated by KA by 3 days after birth. Within the Ammon's horn, the rise of labelling preferentially involves the septal CA3 region and the hilus with lateromedial and septotemporal gradients; this is somewhat reminiscent of the developmental gradients of hippocampal neurons\(^b\) and their connections, notably acetylcholinesterase-containing elements.\(^{39,42}\) Other components of the hippocampal formation, notably the entorhinal cortex—which plays a crucial role in the onset and propagation of the paroxysmal discharge induced by parenteral KA in adult animals—are not labelled up to at least P12. This observation is particularly important since the entorhinal cortex, by means of the perforant path–mossy fibre system, provides a major input to the CA3 region. Furthermore, the granular layer of the fascia dentata through which this input is conveyed has a delayed maturation, and the mossy fibres are not operational at this early stage, both in anatomical and electrophysiological terms (see Discussion in the following paper). Thus, the simplest explanation of the aforementioned observations is that a sufficient amount of KA may reach hippocampal KA receptors which are present in the CA3 region at an early age.\(^5\) However, at this stage of maturation, the enhancement of neuronal discharges (as revealed by the 2DG pictures) remains restricted to the Ammon's horn (and lateral septum), and does not appear to involve the other components of the limbic circuitry, to which the hippocampus projects, and which will be labelled at a later age, once the toxin produces limbic motor seizures.

The labelling of the amygdala in more mature animals deserves particular emphasis since there is now good evidence that this structure plays a crucial role in the symptomatology of experimentally induced limbic motor seizures, after both repetitive electrical stimulation of limbic structures (i.e. kindling)\(^{3',5'}\), or intracerebral or parenteral\(^a\) administration of KA.

Also data from human epilepsy suggest that the amygdala occupies a central position in the symptomatology of partial complex or temporal lobe epilepsy.\(^24,46\) Thus, some signs of this syndrome are always found associated with paroxysmal discharges in the amygdala (for instance oro-alimentary manifestations) or they can be produced by electrical stimulation of this structure, but not by hippocampal stimulation.\(^24,46\) Buser and coworkers\(^a\) have furthermore shown that in patients suffering from temporal lobe epilepsy, stimulation of the hippocampal for-
motion produces evoked potentials in the amygdala which are not observed in patients suffering from other types of epilepsy; this illustrates the importance of the hippocampo-amygdaloid axis in the aetiology of temporal lobe epilepsy. With regard to the motor signs, the amygdala is in a suitable position to play a predominant role. Thus, in rats, cats and monkeys, the amygdala (but not the hippocampus) directly and massively project to pontine and tegmental structures involved in facial and masticatory movements. Therefore, even though one cannot completely exclude the possibility that the lack of activation by KA of the amygdala (and other structures) up to at least P12 was due to methodological complications discussed above, these various observations strongly suggest that the late occurrence of the limbic activations discussed above, these various observations strongly suggest that the late occurrence of the limbic cognitive processing and learning in temporal lobe epilepsy. With regard to the motor signs, the amygdala is in a suitable position to play a predominant role. Thus, in rats, cats and monkeys, the amygdala (but not the hippocampus) directly and massively project to pontine and tegmental structures involved in facial and masticatory movements. Therefore, even though one cannot completely exclude the possibility that the lack of activation by KA of the amygdala (and other structures) up to at least P12 was due to methodological complications discussed above, these various observations strongly suggest that the late occurrence of the limbic motor syndrome is due to a late maturation of the limbic circuitry, in particular in the amygdala. This conclusion is reinforced by the observation made in one companion paper that high affinity KA binding sites are present in the amygdala starting from P18–19, whereas these sites are present much earlier in the hippocampus.

**Conclusion**

We suggest that the change in the type of seizures produced by kainate (around P19) is associated with a concomitant development of some features of amygdaloid organization and connectivity. The anatomical correlates of this development remain to be investigated. In the following paper, we show that in spite of the early induction of seizures in the hippocampus, there will be no brain damage in this as well as other brain structures until KA induces limbic seizures, i.e. after P19.

**Acknowledgements**—We are greatly indebted to Drs. C. Battini, J. M. Besson for permission to use the quantitative microphotometer. We thank G. Ghilini, G. Charton and J. P. Bouillet for technical assistance. Supported by the “Fondation pour la Recherche Médicale”, Ministère de la Recherche et de l’Industrie, INSERM (C.R.E. No. 1093), and by the Austrian Science Research Foundation (for M. B., project E0002).

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