INTRA-AMYGDALOID INJECTIONS OF KAINIC ACID: REGIONAL METABOLIC CHANGES AND THEIR RELATION TO THE PATHOLOGICAL ALTERATIONS

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Abstract—Kainic acid was injected unilaterally in the amygdala of the rat. Following various delays, 2-deoxy-D-[14C]glucose was given intravenously. Autoradiographs of frontal brain sections showed increased glucose uptake in a number of cerebral structures as compared with controls. Most of these structures belong to or are closely related to what is traditionally called the 'limbic system'. The structures that show an increased glucose consumption subsequent to kainic acid injections are, with few exceptions, identical to those that are sensitive to the toxic effect that kainic acid exerts on structures distant to the site of injection. The findings are discussed in relation to the hypothesis that the latter effect is secondary to the epileptogenic properties of kainic acid.

Kainic acid (KA), the potent excitatory analogue of glutamic acid has been extensively used as a tool in neurobiology and particularly as a lesion producing drug. Soon after the introduction of this compound, however, it became evident that its effects were not restricted to the brain structure in which it was injected. The mechanism of the local toxic action of KA and the pathogenesis of its deleterious effects on structures distant of the site of injection have not been elucidated.

In a series of investigations performed in this laboratory, it has been suggested that the damage inflicted upon the brain by intracerebral KA has a twofold aetiology: (1) a direct toxic effect of the toxin at the site of injection and (2) a distant pathological damage which is related to the epileptogenic effect of the drug. It follows that experiments based on local administration of KA may shed light on the mechanisms underlying the pathological changes known to accompany certain types of epilepsy. Using the 2-deoxyglucose autoradiographic method we have studied the metabolic alterations which occur following intracerebral application of KA. The amygdaloid complex was chosen as the target of the injection since it was previously found to be very sensitive to the epileptogenic properties of local KA. The metabolic effects of KA applied systematically have been described in detail elsewhere.

EXPERIMENTAL PROCEDURES

Ten male Wistar rats (250–300 g) were subjected to 2-deoxyglucose autoradiography.

In 5 animals, kainic acid (0.6–1.2 μg dissolved in 0.15–0.3 μl phosphate buffer, pH 7.4) was unilaterally injected into the amygdala in the freely moving animal. The injection was made via a stainless steel delivery cannula inserted through a previously implanted guide cannula (see refs 3, 5 for details). The electrographic activity in cortical and subcortical structures was recorded by means of chronically implanted animals in two cases (Fig. 6 and refs 3, 5). In one additional case, the animal was briefly anaesthetized with equithesin (3 ml/kg, Jensen Salbury) and the injection made via a micropipette (tip 20 μm) stereotaxically inserted into the amygdala. Following a delay of 50 min to 3 days, a pulse of 100 μCi/kg of 2-deoxy- D-[14C]glucose (2DG) (C.E.A. Saclay, specific activity 40–50 μCi/μM) was injected through an indwelling catheter in one jugular vein. Just afterwards, a similar volume of saline was injected via the same catheter. Forty-five min after the 2DG injection, the brain was rapidly removed (following cervical dislocation) and dipped for 1 min in isopentane at 0°C. The brain was then transferred to isopentane maintained at −50°C and kept therein for 2 min. It was stored at −20°C until sectioning. Frontal sections were made of the entire brain using a cryostat kept at −22°C. Three consecutive 16 or 32 μm thick sections cut every 200 μm were picked up on glass slides, dried on a hot plate at 60°C for at least 5 min and thereafter placed in an X-ray cassette. Autoradiographs were obtained using Mammoray T3 films (Agfa Gevaert, 3 weeks exposure). The sections were stained with Kluiwer–Barrera or cresyl violet. Four animals were used as controls. In 3 cases, saline was administered systematically and in one additional case, the vehicle solution was injected into the amygdala in the freely moving animal, as described above.

In a second experimental series, KA was injected into the amygdala as described above, but the animals received no 2DG injection. Instead, they were allowed to survive for 15 min to 2 months. The animals were then deeply anaesthetized and perfused through the heart with saline followed by a 10% formalin solution. The entire brain was sectioned in the frontal plane. Alternate sections were processed according to Fink & Heimer or stained with cresyl violet. In several animals of the latter series, the electrographic activity in cortical and subcortical structures was also recorded (vide supra).
RESULTS

Pattern of 2-deoxy-D-[14C]glucose uptake in control cases

The regional distribution of labeling in control una-
nesthetized rats is in keeping with earlier observations (see ref. 6 and references therein). In brief, in spite of some variability, in both the systemic vehicle cases (3
cases, see Fig. 1, case D 64) and in the rat which received an intra-amygdaloid injection of vehicle (Fig. 1, case D 47) a similar general pattern of distribution was noticeable. Intense labeling was noted in the olfactory system, in particular the anterior olfactory
nucleus (Fig. 1A), the medial and lateral anterior limbic cortices (Fig. 1B); the cortical mantle and particularly the anterior cingulate (Fig. 1C)—was heavily labeled as well as the caudate putamen (compare to the globus pallidus in Fig. 1J), the medial geniculate

* Status epilepticus may be broadly defined as a convul-
sive episode lasting for prolonged periods or which is
repeated at brief intervals so as to create a fixed and dur-
able epileptic condition (i.e. ref. 3).

Fig. 1. Regional glucose utilization in two control cases. Photomicrographs in this and the following
Figures are taken on the X-ray film negative, so that dark areas represent areas of high radioactivity,
signifying high accumulation of the label. Case D 64, received a systemic administration of saline,
whereas case D 47 received an intraamygdaloid injection of the vehicle solution through a chronically
implanted cannula (see arrow). Asterisks point to deformations of the section during the cutting process
(level K). For abbreviations, see list.

List of abbreviations used on Figures

| AAC | anterior limb of the anterior commis-
sure |
| AC | anterior commissure |
| AID | agranular insular cortex, dorsal sub-
division |
| AIP | agranular insular cortex, posterior sub-
division |
| AL | lateral amygdaloid nucleus |
| AON | anterior olfactory nucleus |
| AV | nucleus anterior ventralis thalami |
| BL | basolateral nucleus of the amygdala |
| CA | see AC |
| CEm | central amygdaloid nucleus, lateral part |
| CEm | central amygdaloid nucleus, medial part |
| CH | cortex cerebelli |
| CI | capsula interna |
| CIF | inferior colliculus |
| CL | claustrum |
| CP | caudate-putamen |
| CX | capsula externa |
| ENv | endopiriform nucleus, ventral subdiv-
ision |
| FD | facia dentata |
| FH | fimbria hippocampi |
| FMT | fasciculus mammillothalamicus |
| G | granular layer of the facia dentata |
| GP | globus pallidus |
| GE | nucleus gelatinosus thalami |
| H | hilus of area dentata |
| HL | hypothalamus lateralis |
| IL | infralimbic area |
| L | lateral preoptic area |
| LEA | lateral entorhinal area |
| LDG | lateral geniculate body, dorsal subdiv-
ision |
| LOT | lateral olfactory tract |
| LP | nucleus lateralis posterior thalami |
| LU | stratum lucidum of Ammon’s horn |
| M | stratum moleculare of fascia dentata |
| MD | nucleus medialis dorsalis thalami |
| MEA | medial entorhinal cortex |
| MG | medial geniculate body |
| NHDB | nucleus of the horizontal limb of the |
| NTOL | nucleus of the lateral olfactory tract |
| O | stratum oriens of Ammon’s horn |
| OT | olfactory tubercle |
| P | stratum pyramidale of Ammon’s horn |
| PAC | periamygdaloid cortex |
| PAR | parapallidum |
| PC | piriform cortex |
| PL | periamygdaloid cortex |
| PR | pretectal area |
| PRE | presubiculum |
| RE | nucleus reuniens |
| RH | nucleus rhomboides |
| RI | region inferior of Ammon’s horn |
| RS | region superior |
| S | septal area |
| SG | supraependymal nucleus |
| SM | stria medullaris |
| SN | substantia nigra |
| ST | stria terminalis |
| SU | subiculum |
| TO | tractus opticus |
| TT | taenia tecta |

Regional metabolic changes after intra-amygdaloid injection of kainic acid

The clinical signs induced by intra-amygdaloid injection of KA in naive rats have been described in detail previously and will only be briefly mentioned at this point. After a delay which depends on the dosage injected as well as the experimental par-
parameters, e.g. type of anaesthetics, the toxin induces recurrent limbic motor seizures which may culminate in status epilepticus.* To obtain a representative im-
Fig. 2. **Regional glucose utilization in case D11.** Level A represents the rostral-most, Level G the caudal-most transverse section. On level D, only the hippocampal region with adjacent structures is shown. Arrowhead in right half of C points to dark spot practically coextensive with insula Calleja magna. In E, the white quadrangle represents the center of the KA injection, as judged by the end of the injection cannula track. Asterisk in F indicates the perirhinal area.
Fig. 3. Pattern of regional glucose consumption in the ipsilateral hippocampal region and adjacent structures in D11. (A) Photomicrograph of the autoradiograph at a level corresponding to that represented in Fig. 2(D) showing increased glucose metabolism in the fascia dentata. (B) Same as in (A), except that the autoradiograph has been put on top of the mounted cresyl violet-stained section from which the autoradiograph was originally obtained. Care was taken to ensure a precise alignment between the autoradiograph and the section. The photomicrograph was obtained by focusing on the section. By using this technique it is evident that the strong labeling in fascia dentata mainly involves the outer two thirds of the molecular layer, whereas the granular layer is virtually unlabeled. The same pattern is also found at a slightly more caudal level (C). The latter photomicrograph (obtained as B), shows, in addition, a large increase in glucose uptake in the CA3 field of the hippocampus (involving, in particular, subfields CA3a and b). In contrast, the CA1 is only weakly labeled. The asterisk indicates a high concentration of the label in the stratum lacunosum-moleculare of CA3.
Fig. 4 Regional glucose utilization in case D48 in which the toxin was injected via a micropipette in anaesthetized conditions. The animal recovered from anaesthesia approximately 1 h later and displayed one limbic motor seizure before the 2-DG injection (see Table 1). The regional differences in labeling are similar to other (i.e. anaesthetized) cases although the general level of labeling is lower.
Fig. 5. Regional glucose utilization in D 45. Conventions as in Fig. 1. Asterisks indicate in D, the dorsal subdivision of the lateral geniculate body; in E, the deep layers of the temporo-occipital neocortex, and in F, the perirhinal area.
CASE D12

Fig. 7. Pattern of (14C)2-deoxyglucose accumulation in D 12. (A) The asterisk indicates the dorsal subdivision of the agranular insular area. (B) Photomicrograph showing an increased glucose consumption in the contralateral ventral striatum, at a level just caudal to that represented in A. The white arrow points to one of the cell bridges connecting the olfactory tubercle with the ventral caudate-putamen. (C) Illustrates the labeling in allocortical and thalamic structures in both sides of the section. Note the intense labeling in the side of injection (right part). Note also the symmetrical labeling of the rostral hippocampus of both hemispheres. (D) Shows a very localized accumulation of the tracer in the contralateral nucleus of the lateral olfactory tract (larger magnification). (E) and (F) Photomicrographs, obtained as described in the text to Fig. 2(B), from the contralateral amygdaloid region, and the medial thalamus, respectively. Note that the metabolic alterations follow cytoarchitectonically defined borders. Asterisks in (E) represent artefacts.
Fig. 8. Photomicrographs of the amygdaloid injection site in D 8. The boxed area in (A) is shown in larger magnification in (B). The asterisk denotes the cannula track, whereas the arrowheads point to the border between the area of shrunken, darkly-staining neurons (dorsally) and normal-appearing neuropil (ventrally). Klüver-Barrera strain.

Fig. 9. Photomicrographs of cerebral pathological alterations as revealed by the Fink-Heimer method, after intra-amygdaloid injections of kainic acid. (A) argyrophilic pyramidal neurons in the ipsilateral CA3 3 h after KA administration. Both basal and apical processes are impregnated. Arrowheads point to the sharp border between normal and degenerating cells. (B) same as in A, but at a more caudal level. Note extensive vacuolization of the neuropil in stratum lucidum. (C) degenerating neurons and fibers in the ipsilateral hippocampal region following a survival time of 4 days. The pathological alterations are far more extensive than in the case illustrated in (A) and (B), involving the entire regio inferior as well as regio superior. There is no evidence of neuronal degeneration in the fascia dentata (the boxed area in C is shown in larger magnification in (D). (E) argyrophilic neurons in layer V of the ipsilateral neocortex just dorsal to the rhinal sulcus. Two days survival. (F) degenerating neurons in the contralateral basolateral amygdaloid nucleus. Note that the area of pathological alterations closely follows the contour of the nucleus. Two days survival.
Fig. 9.
Fig. 6. Electrographic records to illustrate the effects of kainic acid application into the right amygdala (rA) in case D 12, before and following the injection of $^{14}$C-2-deoxyglucose (2-G) Derivations of the records are indicated only for the initial tracings; CA3 and CA1 are fields of the Ammon's horn of the right (r) and left (l) dorsal hippocampi: Cx, electroencephalogram. At short delays (upper tracing), the rat manifested 'wet-shakes' (refs 3, 4) followed by stage 3 or 4 amygdaloid seizures ($S_3$ and $S_4$, see refs 3, 4) alternating with immobility (imm.). At longer delay repetitive generalized seizures were displayed; these included circling behavior (cg) and intense agitation (i.e. ref. 3). Note that the paroxysmal activity which first appears in amygdala (not illustrated) rapidly propagates to the cortex as well as bilateral hippocampal fields CA1 and CA3. Note also the typical bursts of spikes recorded synchronously in all the leads during circling behaviour (cg).
age of the metabolic alterations, we have used various dosages of KA as well as time-intervals between the local administration of the toxin and the systemic injection of 2-DG (Table 1). Thus, in case D 45, the 2-DG administration preceded the occurrence of the first limbic seizure; in cases D 48 and D 11, several individual seizures were displayed but these did not culminate in status epilepticus whereas, in cases D 12 and D 8, the 2-DG was injected after the animals had displayed a severe status epilepticus. In one additional case (D 41), the pulse of 2-DG was injected 3 days after KA administration; this rat had displayed a severe status epilepticus for over 6 h before receiving an injection of diazepam (20 mg/kg, i.p.) to prevent the death of the rat due to prolonged status epilepticus (i.e. ref. 5).

The metabolic maps obtained for D 11 will be described with some detail and major differences seen in the other representative cases illustrated thereafter.

The pattern of labeling in D 11 is illustrated in Fig. 2: it is quite different from the control cases. Thus, there is a high rate of glucose utilization in the infra-limbic and prelimbic areas of the ipsilateral frontal cortex (Figs 2A, B), and in the taenia tecta (see ref. 19 for nomenclature). In the prelimbic area, the superficial layers show a more modest labeling than the deeper ones. At the dorsal border of this area, the density drops abruptly so as to render the anterior cingulate area virtually unlabeled at this level (Fig. 2E). At these levels, there is no increase in glucose consumption on the contralateral side, except near the site of injection (quadrangle, Fig. 2F), except near the site of injection (quadrangle, Fig. 2E), where the tissue seems to be indiscriminately engaged in an increase in functional activity.

More caudally, the concentration of label ipsilaterally remains high both dorsal and ventral to the rhinal sulcus, i.e. in the posterior agranular insular area and the prepiriform cortex (Fig. 2C). Medially, there are high densities in the septal area, except in its dorsomedial part, and in the anterior extension of the lateral preoptic area. On the contralateral side, there is an intermediate concentration of label in the claustrum and septum (Fig. 2C). A dark spot basal to the latter (arrowhead, Fig. 2C) appears to be partially co-extensive with the insula Calleja magna.

The remaining levels in Fig. 2 show an increased glucose utilization in various allocortical and thalamic structures. The labeling of allocortex is more pronounced ipsilaterally whereas that of the thalamic nuclei is almost symmetrical with respect to the midline. The lateral posterior nucleus and the medial geniculate body show, however, a predominant ipsilateral increase in metabolism. The pattern of metabolic changes in the medial thalamus is highly reminiscent of that in D 12 (to be described later; see Fig. 7).

Suffice it to mention that the highest densities are found in the mediodorsal nucleus and nucleus reuniens (Fig. 2E), as well as in the paraventricular and parataenial nuclei (not illustrated).

The caudal allocortical areas show a complicated pattern of labeling. A high grain density is found over Ammon's horn, fascia dentata, subiculum, perirhinal cortex, lateral entorhinal cortex, parasubiculum, and area 29b of the retrosplenial cortex. Adjoining areas, such as the presubiculum and medial entorhinal cortex, exhibit low densities (Fig. 2G). If the autoradiographs are superimposed on the stained sections (see Experimental Procedures), the pattern of labeling in the rostral hippocampal formation can be analysed in greater detail (Fig. 3). There is a strong labeling in the outer two-thirds of the molecular layer of the fascia dentata, whereas metabolic activity appears to be decreased in the inner third in comparison to the control cases (Fig. 1). The granular layer is not appreciably darker than background. Also in Ammon's horn, the cell layer (stratum pyramidale) appears lighter than the fiber layers, among which the stratum lacunosum-moleculare of CA3 displays the highest rate of glucose consumption (asterisk in Fig. 3C). A rise in labeling is also conspicuous in the stratum lucidum suggesting an increased metabolism along the mossy fiber system (not shown, also see Discussion). The caudate–putamen is generally weakly labeled (Fig. 2C), except near the site of injection (quadrangle, Fig. 2E), where the tissue seems to be indiscriminately engaged in an increase in functional activity.

In D 11 as well as in the other experimental animals, neocortex as a whole shows a lower rate of metabolism than it does in the controls. An increased labeling is, however, found over some areas, most notably area 41 (the primary auditory cortex), area 18, and the cortical regions immediately adjacent to these. The changes are most pronounced in the deep cortical layers on the ipsilateral side (Fig. 2F).

It should, finally, be noted that the lateral part of the ipsilateral substantia nigra has accumulated small amounts of the label. The cerebellum shows a homogenous low grain density (not illustrated).

Case D 48 (Fig. 4) is of interest since KA was injected via a micropipette (under anaesthesia) and the diffusion of the toxin thus considerably limited. The animal displayed upon recovery from anaesthesia limbic motor seizures (see Table 1 and refs 3, 4). The pattern of labeling in these conditions was quite similar to that of case D 11 (i.e. an increase in the hippocampal formation, septum, and limbic cortex. Fig. 4) although the general level of labeling was lower than in other cases due probably to the prolonged effects of anaesthesia.

Injection of 2-DG a few minutes before the occurrence of the first limbic motor seizure (case D 45) also leads to an accumulation of label at the injection site as well as the immediately adjacent prepiriform cortex and ventral border of the caudate-putamen (Fig. 5C). The pattern of labeling in more distant brain regions
such as the caudal allocortex including the hippocampus, fascia dentata, lateral entorhinal cortex, paraseubiculum and perirhinal cortex, is virtually identical to that seen in case D 11 (Fig. 5D–F). As in case D 11, the neocortex is not labeled except in the temporal and occipital regions. In these areas (Fig. 5D), layer VI is very dark, the superficial layers are light, whereas the intervening ones are immediately dense. Furthermore, the medial geniculate body, substantia nigra (Fig. 5E) and lateral posterior thalamic nucleus (Fig. 5D) have increased their metabolic rate. In the rest of the brain, however, the metabolic changes were either absent or more modest than in case D 11. Thus, no contralateral structure, except in the caudal hippocampus (Fig. 5E) shows a labeling significantly above background. The enhanced rate of glucose consumption in contralateral hippocampus is well correlated with the rapid propagation of paroxysmal activity to this structure following intra-amygdaloid application of KA (see Fig. 6 and ref. 3). Furthermore, there is no accumulation of label in the medial thalamus. Similarly, the prelimbic area and the prepiriform cortex show a lower rate of glucose consumption than in the preceding case, and the insular cortex is insignificantly labeled.

The metabolic maps obtained from D 8 and D 12 were very similar and revealed a striking increase in glucose uptake in both ipsilateral and contralateral structures. Both rats had displayed severe status epilepticus before the injection of the radioactive marker. As shown in Fig. 6, the electrographic records made in case D 12 reveal the presence of severe paroxysmal discharge in the injected amygdala and, bilaterally, in the CA1 and CA3 fields of the Ammon’s horn. Initially, the animal displayed well individualized recurrent limbic seizures (i.e. stage 3 or 4, see refs 3, 6); at a later stage (i.e. Fig. 6, 120 min), the animal displayed severe status epilepticus accompanied by repetitive circling, facial movements, jump, etc. (i.e. ref. 3). The pattern of labeling from D 12 (Fig. 7) reveals that the entire hemisphere is virtually labeled on the side of injection (Fig. 7C). At rostral levels, the metabolism is greatly increased in contralateral structures, viz. in the anterior olfactory nucleus and parts of the medial frontal cortex (Fig. 7A). The pattern of labeling on the ipsilateral side at this level resembles that in D 11 (Fig. 2B). More caudally, there is a clear-cut increase in glucose consumption in the contralateral septal area, claustrum (not illustrated), and nucleus of the lateral olfactory tract (Fig. 7D). The contralateral ventral striatum, which comprises the nucleus accumbens, olfactory tubercle, and ventral parts of the caudate-putamen, as well as the striatal cell bridges between the latter two structures is labelled in its entirety (arrow, Fig. 7B).

The non-injected amygdala shows an increase in functional activity in the lateral and basolateral nuclei, and in the lateral parts of the central nucleus. By superimposing the radiographs on the stained sections, one clearly sees that the accumulation of the label respects the cytoarchitectonically-defined borders of the amygdaloid nuclei (Fig. 7E). This is particularly evident for the basolateral nucleus. The ventral limit of the dark area associated with this nucleus corresponds to its border towards the ventral endopiriform nucleus, and the lateral limit of the area corresponds to the capsula externa.

Using the same technique, it is readily appreciated that the metabolic increase in the thalamus is similarly confined to anatomically-defined structures. The entire mediiodorsal nucleus, except it lateralmost (paralameolar) part, is labeled. More laterally, the anteroventral and anterodorsal nuclei have taken up small amounts of the label only. The autoradiographs also show a sharp border between the mediiodorsal nucleus and the stria medullaris. A similar border is not found at the ventral aspect of the mediiodorsal nucleus, since the subjacent rhomboid and reunions nuclei are also heavily labeled. Other thalamic nuclei with an increased uptake include the nucleus gelatinosus (Fig. 7F) and the paraventricular and parataenial nuclei (not illustrated). In the remaining parts of
D 12 the metabolic changes parallel those in D 11 and D 45, except that they tend to occur bilaterally.

Finally, 3 days following KA application (case D 41), only few structures showed an enhanced increase in glucose uptake. Thus, a faint labeling could be depicted in the amygdala and mediadorsal thalamic nucleus on both sides as well as in the ipsilateral septum; this in keeping with previous observations made following systemic administration of the toxin (ref. 6).

The pattern of regional metabolic changes compared with the distribution of pathological alterations

At the site of the injection. In most cases, the center of the injection was located in the centromedial part of the amygdala. An example of such an injection is shown in Fig. 8 (case F8, 2 days survival). The injection area is characterized by shrunken, triangular darkly-stained neurons, which sharply delimit the injection area from the surrounding normal-appearing neuropil (arrowheads, Fig. 8A). Generally, the pathological alterations involve a smaller area than does the local increase in metabolism at shorter survival times. This can be illustrated by comparing case D 11 (Fig. 2) and D 45 (Fig. 5) with F8 (Fig. 8). D 11 and F8 received equivalent doses of KA.

Hippocampal region. The time-course of the development of KA-induced pathological changes in the hippocampus has been described previously. In most cases, the center of the injection was located in the CA3 a and b fields (Fig. 9A), whereas the entire Ammon's horn is afflicted after longer survivals (Fig. 9C). This corresponds well with the metabolic changes, which are most pronounced in the CA3 field in cases where the hippocampus is moderately involved (Fig. 3). Of particular interest is the heavy labeling of the stratum lacunosum-moleculare (asterisks, Fig. 3C), inasmuch as dendrites in this location are among the first elements to show argyrophilia after intramygdaloid KA application (Fig. 9B; also see refs 3, 4).

The fascia dentata is never pathologically altered after intraamygdaloid KA injections. The significance of its high metabolism is a topic for the discussion.

Other structures. Figure 9E shows argyrophilic cells in the deep layers of the neocortex just dorsal to the rhinal sulcus. The affected part of the cortex corresponds to that showing an increase in metabolism in Fig. 5E (asterisks). Similarly, the basolateral amygdaloid nucleus is susceptible to the pathological (Fig. 9f) as well as the metabolic consequences (Fig. 6E) of KA injections in the contralateral amygdala. This is also in keeping with electrophoretic data which reveals the presence of severe paroxysmal discharge in the contralateral amygdala (not illustrated, i.e. ref. 3).

Other structures that exhibit degenerative changes include several medial and midline thalamic nuclei (the mediadorsal, reuniens, parataenial, and paraventricular nuclei), the lateral posterior thalamic nucleus, the septum, the insular area, the medial frontal cortex, and the claustrum (see refs 3 and 4). As presently shown, all these areas enhance their rate of glucose utilization after intraamygdaloid KA injections.

Three areas show a high metabolic rate, but minimal pathological changes; viz. the substantia nigra, principal medial geniculate nucleus, and the anterior olfactory nucleus. It should be noted that these areas are heavily labeled in the control cases as well.

Discussion

Anatomical substrate of the metabolic maps

The structures that enhance their metabolism in response to KA administration are, with few exceptions, either parts of the allocortex, or strongly related to it. Most of the structures would be considered parts of the 'limbic system', as traditionally defined. As such, they are closely interconnected by axonal projections. Illustrating this point is the fact that a majority of the areas with an increased glucose consumption receive projections from the amygdala. This is true for the prelimbic, infralimbic and perirhinal cortices, the agranular insular and lateral entorhinal areas, the subiculum, parasubiculum, nucleus of the lateral olfactory tract, medio-dorsal nucleus of the thalamus, and ventral striatum. Several of these areas exhibit reciprocal connections with the amygdala. The structures which show an increased functional activity in the absence of any direct projections to or from the amygdaloid complex are, as a rule, connected to other areas with a high concentration of the label. Examples are the retrosplenial cortex, which receives a massive projection from the subiculum and the fascia dentata, which is an important termination area of fibers from the septum and the lateral entorhinal area.

The pattern of labeling in the thalamus closely reflects the distribution of label in the cortex. The high rate of glucose consumption in the dorsal part of the lateral geniculate body and in the principal medial geniculate nucleus (cf. Fig. 5; case D 45) parallels the metabolic changes in the visual and auditory cortices, respectively. The posterolateral part of the neocortex that is labeled in D 45 is also connected to the lateral posterior and suprageniculate nuclei (see e.g. case D 16 in ref 17). The prelimbic and agranular insular areas, which are related to the heavily-labeled mediadorsal nucleus, are characterized by an increased glucose consumption, whereas the anterior cingulate and medial precentral areas, which are related to the unlabeled paralamellar part of the mediadorsal nucleus show a very sparse increase in glucose consumption. The claustrum, which in most cases is conspicuously labeled, receives fibers from the mediadorsal nucleus projection cortex on both sides. It is also bilaterally and reciprocally connected with the visual cortex and the acoustically responsive association cortex. Similarly, the striking increase in glucose consumption seen in 2 cases in the contralateral basolateral and lateral part of the central nucleus of the amygdala is also substantiated by anatomical data.
However, a number of brain structures that have extensive afferent and efferent connections to the amygdaloid complex are not metabolically activated. The lack of labeling of the pontine and tegmental amygdaloid complex are not metabolically activated. The extensive afferent and efferent connections to the kinase and his colleagues are particularly intriguing since these structures are particularly good candidates to mediate several motor signs which are typical of amygdaloid epilepsy.

When injected intraperitoneally, KA induces a pattern of metabolic changes that is very similar to the one presently described. Thus, the metabolic alterations rapidly involve the septal and hippocampal region, and from there spread to medial, frontal, piriform, and insular cortices, amygdala, claustrum, ventral putamen, nucleus accumbens, olfactory tubercle, and several thalamic nuclei including the lateral dorsal, lateral posterior, mediodorsal, reuniens, paraventricular, and parataenial nuclei. The affected structures show a bilateral increase in metabolism. Nothing is known about how the KA enters the brain when systematically applied. It is, however, improbable that it first gets access to the amygdala to exert its effect there, since other structures exhibit metabolic changes well in advance of the amygdala. It follows that the initial site of KA effect is not critical for obtaining the pattern of metabolism presently described. It remains, however, that some structures are clearly less sensitive towards the epileptogenic properties of KA than others.

**Relationship between epilepsy and brain pathology**

The areas that show an increased rate of glucose utilization are to a remarkable extent identical with those that show degeneration after amygdaloid injection of kainate (also see ref. 3). A number of studies have shown a good relationship between local epileptiform discharge and increased glucose utilization. Electrophographic recordings made in this laboratory have revealed the existence of intense epileptiform activity in the hippocampal formation and other limbic structures, in particular the contralateral amygdala (i.e. ref 3 and the present report), after local injection of KA in the amygdala. Furthermore, there is an excellent relationship between the severity of the local paroxysmal discharge and the pathological alterations subsequently induced in the hippocampus. Taken altogether, these observations therefore confirm and extend our previous suggestion that the pathological alterations—at least those seen in distant structures in which a quantitative evaluation of the electrographic record has been made—are causally related to an excessive increase in neuronal activity and metabolism. The other alternative, i.e. a direct action of the toxin, cannot be reconciled with several lines of evidence which have been extensively discussed elsewhere. It is worth noting here that ventricular diffusion of the toxin can be excluded since (a) lateral or oblique injections of the toxin in the amygdala produce a similar hippocampal damage (unpublished observations) whereas injections in the immediate vicinity of the ventricle (in the septum or preoptic area) do not readily produce such damage (viz. ref. 4); (b) similar metabolic (case D 48, Fig. 4) and pathological alterations are produced after injections made via a micropipette, i.e. with minimal diffusion of the toxin; (c) transection of the perforant path reduces the hippocampal damage produced by intra-amygdaloid injections of KA (ref. 4) but not these resulting from intraventricular administration of the toxin (i.e. refs 24, 25). Additional support for our hypothesis has been reported more recently; thus (a) local administration of folate in the amygdala reproduces the KA type limbic seizures as well as the distant pathological alterations but little or no damage at the site of injection, (b) sustained electrical stimulation of the perforant path produces, in anaesthetized animals, seizure activity and pathological alterations with a similar distribution in the hippocampal formation, i.e. a resistant fascia dentata is more susceptible CA3–CA4 fields. Therefore, the typical image of hippocampal damage which is also seen in post mortem studies of chronic epileptics can be induced by excessive activation of the synaptic circuitry without using neurotoxins.

If the spread of the epileptiform activity depends on the rich network of pathways that interconnect the involved areas, an implication of our hypothesis is that lesions of crucial pathways should confer a protection against the deleterious effects of KA on distant brain sites. This has been shown by our previous experiment of unilateral transection of the perforant pathway (see above). More recently, Nadler & Cuthbertson have studied the effects of transection of the mossy fiber just prior to an intraventricular injection of the toxin. This procedure was found to prevent the CA3 neuronal degeneration that was otherwise observed after such an injection (also see ref. 25).

The mossy fibers constitute a link in the major impulse pathway through the hippocampal formation (viz. entorhinal cortex, fascia dentata, mossy fibers, regio inferior, regio superior). In keeping with our earlier observations made following systemic administration of KA a clear-cut rise in labeling was noted along this impulse pathway, in particular in the entorhinal cortex, the lacunosum moleculare of regio inferior and the mossy fiber system. It is, therefore, tempting to propose that this impulse pathway is activated by intra-amygdaloid injections of KA and that the pathological changes that develop result from an excessive enhancement in neuronal activity and metabolism in the component structures. It remains, however, to be explained why one of the links in the impulse pathway, i.e. the fascia dentata, does not show any neuronal degeneration. This could be related to the fact that in control cases the fascia dentata appears to have a high metabolic rate, in particular the molecular layer (i.e. Fig. 1). Alternatively, the fascia dentata may possess a higher resistance.
towards the deleterious effect of metabolic stress than other structures. It should also be kept in mind that an increase in glucose consumption under paroxysmal activity may be due to each of a number of processes, such as transmitter release and uptake, ion pumping, intracellular transport, macromolecular synthesis and others, and that the relative importance of these processes may differ among the structures involved. Provided that the various energy-requiring processes differentially influence the development of pathological alterations, a disconnection between pathology and metabolism in certain structures could be explained.

Conclusions
This study has shown that intra-amygdaloid injections of KA induce an increase in metabolism in numerous cerebral structures that are strongly interconnected by axonal projections. It is proposed that the metabolic changes reflect local epileptiform activity. The close spatial correlation between the pathological and metabolic alterations is taken to support the hypothesis that local epileptiform discharge plays a role in the pathogenesis of the KA-induced distant lesions.

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