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Autoradiographic localization of kainic acid binding sites in the human hippocampus

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Autoradiographic localization of kainic acid binding sites has been determined in postmortem human hippocampi. The results reveal that these binding sites are present in regions vulnerable to epilepsy, in particular the terminal field of the mossy fibers.

Systemic administration of the potent excitatory agent kainic acid (KA)[21] to rats produces a seizure and brain damage syndrome[4,23] which constitutes a particularly suitable experimental model of human temporal lobe epilepsy[1,18]. The lesions caused by the neurotoxin include a sclerosis of the hippocampus[1,4,18,23] which is also the most frequent pathological sequela found in epileptic patients[15]. Several lines of evidence suggest that the neurotoxic action produced by systemic administration of KA is due to a cascade of events which include interaction with specific receptors, epileptogenesis and subsequent damage in brain structures vulnerable to the recurrent paroxysmal activity[1–4,18,25]. Autoradiographic studies in the rat have shown that specific high-affinity KA receptors[13] are particularly enriched in some vulnerable regions, notably in the CA3 mossy fiber zone of the hippocampus[5,8,17,27]. In contrast, the distribution of KA binding sites in the human brain is unknown. Using a newly-available, highly-specific radiolabeled KA and autoradiographic procedure, we now report that in the human brain, the mossy fiber zone of the hippocampus has a particularly high content in specific KA binding sites.

Sections from human and rat hippocampi (30 μm) were cut in a cryostat (−22 °C), thaw-mounted onto gelatin-coated slides and treated concomitantly. The visualization of high-affinity KA receptors was obtained using a previously described technique[5] with minor modifications. In brief, the slices were preincubated in 50 nM Tris-acetate buffer (pH 7.1) first at 4 °C for 1 h and then at 30 °C for 15 min to remove competing endogenous ligands from the tissue. Thereafter, the slices were incubated for 1.5 h in the same buffer (4 °C) containing 20 nM of [Vinylidene-^{3}H]KA (NEN, 60 Ci/mmol), then for 2 min in the buffer containing unlabeled KA (10 μM) to replace the binding sites which have a fast dissociation rate[5,12]. The sections were then rinsed in buffer and then in distilled water. Control for non-specific binding was made by incubating alternate sections with the radioactive material plus an excess of cold KA[5,27]. After drying, the slices were exposed to [^{3}H]sensitive ultrofilm (LKB) for 18 days, simultaneously with internal standards prepared from rat brain homogenates containing known concentrations of the radioligand[25]. Quantification of the density of binding sites was performed using an image analyzer (Imanco Quantimet 720, System 23) and relying on the comparison with the optical density of the internal standards[26]. To examine the distribution of the mossy fiber system, Timm's stain[12] was used. Two

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rats were intracardially perfused with a 0.37% sulphide solution followed by 4% formalin and the procedure described by Sloviter[24] (i.e. Fig. 1E). In addition, a modification of the procedure was adapted for unperfused, unfixed material; in this aim, sections of human and rat hippocampi were postfixed with 70% alcohol for 2 days, then dipped for 1 h in 0.37% sulphide and revealed according to Timm's procedure.

In the rat, in agreement with earlier studies[5,8,17,27], the greatest density of KA binding sites was seen in the terminal field of the mossy fibers of CA3 (Fig. 1). High levels of KA binding sites were also present in the inner part of the molecular layer of the fascia dentata (i.e. immediately above the granular layer) and in the deep layers of the piriform cortex[5,8] (also see Fig. 1). As shown in Table I, in control cases (0 h postmortem delay), the content in KA binding sites in the mossy fiber zone of CA3 (see Fig. 1A) was 4 times higher than those found in CA1 (significantly different for $P < 0.01$).

To determine if differences in the postmortem delay could influence the distribution of KA binding sites, we have compared the hippocampi of rat brains which had been maintained after sacrifice at 4 °C for 0 (control), 24 or 48 h before freezing (Fig. 1B–D, Table I). We found an increase in density of binding sites in particular with 24-h delay in CA3 and the granular layer of the fascia dentata; the values were reduced with 48-h delay, although they remained higher than with 0 h (Table I). The reasons for this in-

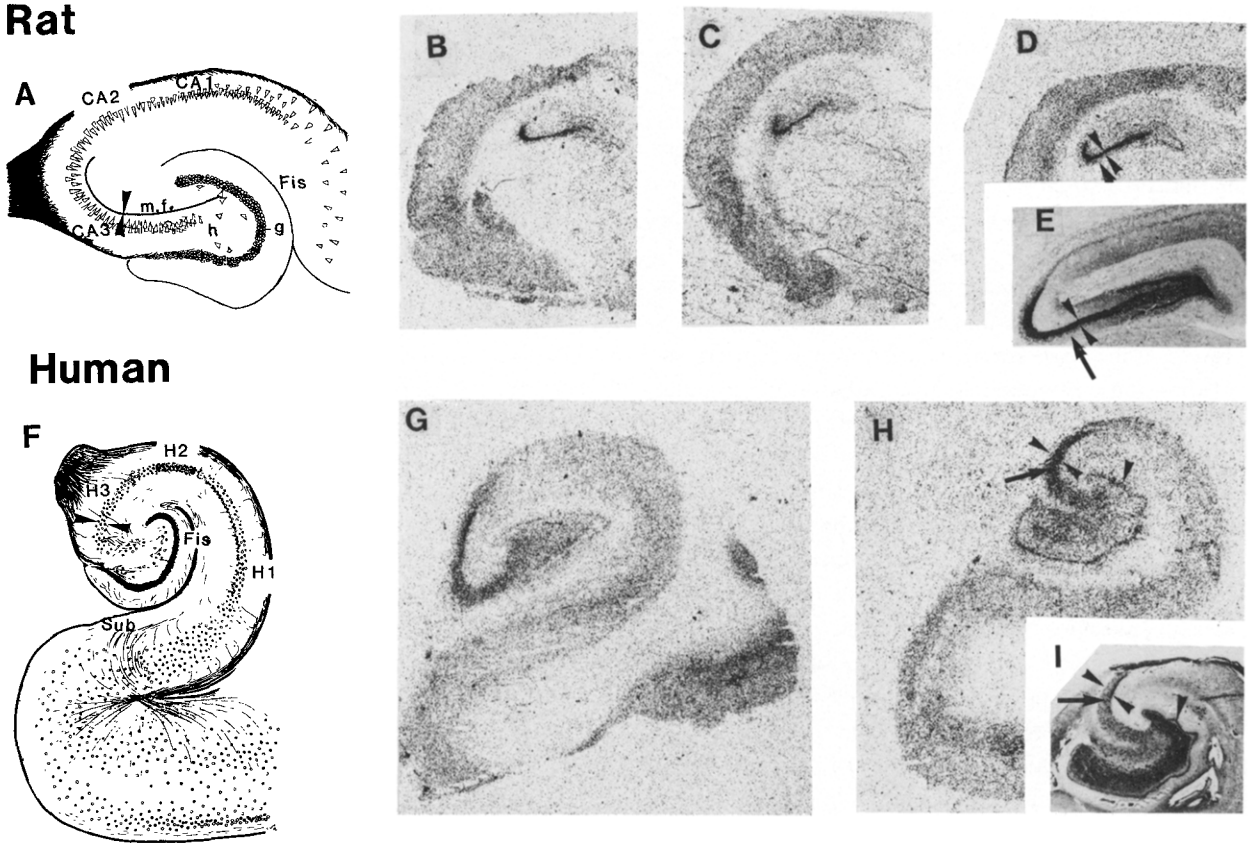

Fig. 1. Autoradiographic localization of [^{3}H]KA receptors on slide-mounted sections from rats (B–D) and human hippocampi (G, H). Schematic representation of the corresponding sections are illustrated in A and F. The postmortem survival times before freezing were 0, 24, 48, 30 and 48 h for B, C, D, G and H, respectively. Note that the greatest labeling is found in the CA3/H3 region in every case. CA1/H1–CA3/H3, fields of the Ammon's horn[14]; Fis, fissura hippocampi; g, granular layer of the fascia dentata; h, hilus, m.f., mossy fibers; Sub, subiculum. E shows the typical dark zone produced with the conventional Timm's stain in the rat[12]. I shows the brown product produced in the zinc-enriched zone with the modified Timm's procedure (used with postfixed material, see Methods). The pyramidal and granular neurons are also stained in a red color. Note that in the rat, the zinc-containing mossy fibers (double arrowheads) are immediately above the pyramidal layer (arrow); in the human, the zinc stain is within the pyramidal layer, but also the endfolium (hilus) and the granular layer (single arrowhead).

TABLE I

Concentration of KA binding sites (in fmol/mg protein) in the rat and human hippocampus

Quantitative determination of KA binding sites in the hippocampus. Each value is the mean ± S.E.M. of the values determined from 9 sections. Note the highest values in CA3/H3 than in CA1/H1 (P at least < 0.05 in each case: Student's t-test).

Cases	*Age (years)*	*Post-mortem delay (h)*	*Cause of death*	*CA3/H3*	*CA1/H1*	*Fascia dentata*
Rats						
1	adult	0	–	327 ± 16	88 ± 9	105 ± 9
2	adult	0	–	324 ± 68	76 ± 28	161 ± 37
3	adult	24	–	685 ± 96	131 ± 31	250 ± 13
4	adult	48	–	536 ± 85	59 ± 8	126 ± 14
Humans						
1	57	5	Cardiac failure	78 ± 15	39 ± 12	44 ± 12
2	58	17	Cardiac failure	90 ± 22	40 ± 13	50 ± 11
3	64	22	Pulmonary embolism	78 ± 16	57 ± 12	55 ± 14
4	27	30	Pulmonary embolism	101 ± 21	60 ± 33	102 ± 22
5	79	48	Gastric hemorrage	159 ± 17	31 ± 13	95 ± 16

crease which is also observed in human material (see below) are not clear.

Five human temporal lobes were obtained from patients who died without known neurological disorders; the postmortem delays varied between 5 and 48 h (Table I). In all cases, it was found that the H3 (CA3) sector of the Ammon's horn[14] (see Fig. 1F) is also the most enriched in KA binding sites (see Fig. 1G, H and Table I). Other hippocampal regions, including the endfolium (hilar zone) and the granular layer of the fascia dentata (single arrowhead in Fig. 1H), as well as the deep layer of the adjacent temporal lobe, were also enriched in KA binding sites (Fig. 1G, H). With the image analyzer (Table I), the following indications were obtained: (1) the KA binding sites were lower than in rats in every hippocampal subregion; interestingly, the values found in this study as well as the differences between human and rat hippocampi are comparable to those found earlier (in striatum) in biochemical studies[6]; (2) as in the rat, there is an increase in density of binding sites with longer postmortem delays, in particular in H3 and the granular layer of the fascia dentata; and (3) the H1 region contains a lower density of binding sites; furthermore, this region is clearly more heterogeneous than in the rat (Fig. 1).

To determine more precisely the localization of KA binding sites with reference to the mossy fibers, we have used the Timm stain. This method[12,24], which reveals transition divalent metals, has been extensively used to study the mossy fibers which have a particularly high content of zinc in the rat and human brains[9,10]. With the conventional procedure (i.e. intracardial perfusion with silver sulphide) there is, in the rat, a dense zone (Fig. 1E, between the arrowheads) which is located immediately dorsal to the pyramidal layer of CA3 (Fig. 1E, arrow); this corresponds to the suprapyramidal bundle of the mossy fibers[12], and it has been shown by electron microscopy that zinc is present in mossy fiber terminals[11], and also that endogenous zinc is released in situ by depolarization of these fibers[7]. This zone terminates abruptly at the CA3/CA2 boundary zone and corresponds precisely to the KA binding enriched zone[5,8].

With postfixed human and rat hippocampi, the pattern of stain was less reproducible, often of poor quality, and included zones of dense material which are not as strongly stained as in perfused material. Nevertheless, in human (Fig. 1I) as in rat (not illustrated), the CA3/H3 zone showed dense precipitates, in keeping with atomic absorption measurements of zinc concentrations in human and rat hippocampi[10]. These observations are, therefore, in agreement with the suggested association between Timm-positive material in the mossy fibers and KA binding sites[1].

In both rats and primates, the H3 (CA3) zone and the hilus are highly vulnerable to the seizure and brain damage syndrome produced by KA[1,4,16,18,20,23]. Extensive investigations made primarily in the rat suggest that the mossy fibers play a particular role in

the sclerosis of the CA3 pyramidal neurons which appears to be causally related to the paroxysmal discharge per se. Thus: (1) a lesion of the mossy fibers[19] or transection of the perforant path[3] prevents the damage in the vulnerable zone after KA injected in the brain; (2) repetitive electrical stimulation of the main input to the granules of the fascia dentata also produces sclerosis[25], (3) during maturation, the damage in the CA3 zone is only produced once the mossy fibers are mature, even if this zone is metabolically activated by the neurotoxin at an earlier stage[2]. These observations, as well as the demonstration that the sclerosis of the CA3 zone produced, in the rat, by parenteral KA is not due to local anoxic conditions[22] raise the possibility that the mossy fibers release, during the seizure, factors, including zinc and possibly an endogenous KA-like agent, which are toxic to postsynaptic neuronal elements[1]. Whatever the exact mechanism, the present study shows that KA binding sites can be readily studied in human brains. Our observations show that KA binding sites are particularly enriched in hippocampal regions which are highly vulnerable to the epilepsies. A better understanding of the relationship between KA-like agents and mossy fibers may provide new insights into the aetiology of temporal lobe epilepsy.

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