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Kainic Acid Alters the Metabolism of Met^5-Enkephalin and the Level of Dynorphin A in the Rat Hippocampus

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Male Fischer-344 rats were given a single intrastriatal injection of kainic acid (KA; 1 μg/rat), which caused recurrent motor seizures lasting 3-6 hr. During the convulsive period, native Met^5-enkephalin-like (ME-LI) and dynorphin A (1-8)-like (DYN-LI) immunoreactivities in hippocampus decreased by 31 and 63%, respectively. By 24 hr after dosing, the hippocampal opioid peptides had returned to control levels, and by 48 hr ME-LI had increased 270% and DYN-LI 150%. Immunocytochemical analysis revealed that ME-LI and Leu^5-enkephalin-like (LE-LI) immunostaining in the mossy fibers of dentate granule cells and the perforant-temporoammonic pathway had decreased visibly by 6 hr and had increased markedly by 48 hr following KA. A visible decrease in DYN-LI in mossy fiber axons within 6 hr was followed by a substantial increase by 48 hr.

To determine whether the increases in hippocampal ME-LI reflected changes in ME biosynthesis, levels of mRNA coding for preproenkephalin (mRNA^{enk}) and cryptic ME-LI cleaved by enzyme digestion from preproenkephalin were measured. Following the convulsive period (6 hr), mRNA^{enk} was 400% of control, and by 24 hr, cryptic ME-LI was 300% of control. Increases in native and cryptic ME-LI and in mRNA^{enk} were also noted in entorhinal cortex, but not in hypothalamus or uninjected striatum. Our data suggest that KA-induced seizures cause an increase in ME release, followed by a compensatory increase in ME biosynthesis in the hippocampus and entorhinal cortex.

Our laboratories have been studying seizure-induced changes in brain levels of opioid peptides. We have been particularly interested in studying the effects of kainic acid (KA)-induced seizures because they are considered to be a useful model of temporal lobe epilepsy (Ben-Ari, 1985). The severity of seizures both in temporal lobe epilepsy and after KA administration is correlated with the extent of cell loss in the hippocampal formation and other limbic areas (Dam, 1980; Lothman and Collins, 1981; Margerison and Corsellis, 1966; Sperk et al., 1985). Opioid peptides are thought to potentiate KA-induced seizures because naloxone pretreatment attenuates and morphine potentiates convulsions and cell loss (Fuller and Olney, 1979).

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We have previously reported that intrahippocampal injections of KA cause a decrease in hippocampal Met^5-enkephalin-like immunoreactivity (ME-LI) (Hong et al., 1980) during the recurrent convulsive period and that intracerebroventricular (icv), intraseptal, intrahippocampal, or intrastriatal injections of KA cause an increase in ME-LI in the rat hippocampus after 2–3 d that lasts for approximately 2 weeks (Hong et al., 1980). We have also observed an increase in Leu^5-enkephalin-like (LE-LI) immunostaining of the mossy fibers and perforant/temporoammonic pathway of the hippocampus 3 d after KA administration (McGinty et al., 1983). Administration of the protein synthesis blocker cycloheximide 6 hr after intrahippocampal administration of KA attenuates the hippocampal ME-LI increase seen 48 hr after dosing (Hong et al., 1980), suggesting that the initial KA-induced depletion of ME-LI is followed by a compensatory increase in ME biosynthesis. Because cycloheximide blocks protein synthesis nonspecifically, one purpose of this study was to provide more conclusive evidence for KA-induced changes in hippocampal ME biosynthesis by measuring changes in preproenkephalin messenger RNA (mRNA^{enk}) and cryptic ME cleaved from preproenkephalin after enzyme digestion. [Four copies of ME, 2 copies of C-terminal-extended ME, and 1 copy of LE are stored in proenkephalin (Udenfriend and Kilpatrick, 1983).]

KA-induced seizures alter hippocampal levels of dynorphin, as well as ME-LI, immunoreactivity. Intra-amygdalar injections of KA cause a decrease in hippocampal dynorphin A 6 hr after dosing, followed by an increase above control levels at 24 hr (Lason et al., 1983). Our immunocytochemical studies indicate that dynorphin A (1–17) returns to control levels within 3 d after the administration of KA (McGinty et al., 1983). A second purpose of this study was to compare the time course of KA-induced changes in dynorphin A (1–8)-like (DYN-LI) and ME-LI immunoreactivities in the hippocampus and other brain regions.

Materials and Methods

Animals

Male Fisher-344 rats (Charles River, Wilmington, MA) between 10 and 12 weeks of age were used. They were housed 4 to a cage in a colony room maintained on a 12/12 hr light/dark cycle at 21 ± 2°C and 50 ± 10% humidity.

Treatment of animals

Rats were randomly assigned to 1 of 3 groups: (1) intact controls, (2) sham-operated saline controls, and (3) KA-injected animals killed 3, 6, 12, 24, 48, or 72 hr postinjection. Different routes of intracerebral injection of KA cause comparable increases in the hippocampal level of ME-LI. Nevertheless, we have found that intrastriatal injection of

KA produces lower mortality (less than 10% at a dose of 1 μg/rat) than that produced by other routes of administration. Therefore, instrastriatal injection was used in this study. The stereotaxic coordinates for intrastriatal cannula implants, according to the atlas of König and Klippel (1963), were as follows: A, 8.2 mm; L, −3.0 mm; V, 0.0 mm. Animals were anesthetized with 2 ml/kg of ketamine plus xylazine (86.2 mg/ml ketamine HCl; Parke-Davis, Morris Plains, NJ and 2.76 mg/ml xylazine; Miles Labs, Shawnee, KA), injected i.p. They were then placed in a stereotaxic holder and given intrastriatal injections of either KA or saline through a 30-gauge cannula. Experimental animals were injected at a constant rate (1 μl/min) for 1 min (Injector Model 355; Sage Instruments, Cambridge, MA) with a single dose of KA (1 μg/μl in 0.9% NaCl) in the right striatum. At the time points specified above, groups of rats were decapitated for radioimmunoassay (RIA) or blot hybridization. Twenty-six rats were perfused with a 4% paraformaldehyde solution for immunocytochemistry (ICC) (see below). For RIA and mRNA extraction, brain regions were dissected according to the method of Glowinski and Iversen (1966). Entorhinal cortex was dissected by cutting a triangular wedge bordered on the dorsal side by the rhinal sulcus, on the anterior side by the amygdala, and on the posterior-ventral side by the curvature of the cortex as it changes from its lateral to its medial extent. Tissue was frozen at −70°C until assays were performed.

Radioimmunoassay

Native and cryptic ME-LI

The tissue levels of native ME-LI were determined by RIA as described previously (Hong et al., 1978). Briefly, tissue was homogenized in 2 M acetic acid and immersed in boiling water for 5 min. After centrifuging at 25,000 *g* for 20 min, the supernatant fluid was lyophilized. The residue was reconstituted in RIA buffer solution and aliquots were used for RIA. The specificity of the antiserum against ME has been described in a previous report (Hong et al., 1978).

The methods of Liston et al. (1984) and Cupo et al. (1984) were followed in the preparation of tissue for RIA or cryptic ME-LI. Brain tissue (30–50 mg) was homogenized in 0.1 *N* HCl (1 mg/10 μl). An aliquot equal to 16–20 mg, wet weight, of tissue was mixed with an equal volume of 10% trichloroacetic acid (TCA), incubated on ice for 15 min, and centrifuged at 10,000 *g* for 2 min. The pellet was washed by sonication in 5% TCA and centrifuged at 10,000 *g* for 2 min. The pellet was rinsed by sonication in diethyl ether (2 ml) and centrifuged at 10,000 *g* for 2 min. The washing and rinsing procedure was repeated twice, and the pellet was dried at room temperature, then reconstituted in twice the original volume of buffer (50 mM Tris-HCl, 5 mM $CaCl_2$, pH 8.4). The solution was incubated with trypsin (final concentration, 10 μg/ml) for 4 hr at 37°C, boiled for 15 min, and cooled on ice. This solution was incubated with carboxypeptidase B (final concentration, 0.1 μg/ml in a mixture 2.5 times the original volume of the aliquot) for 2 hr at 37°C, boiled for 15 min, and cooled on ice. It was then centrifuged at 25,000 *g* for 30 min. The supernatant was used for RIA with Met^5-enkephalin assay kits (Immuno Nuclear Corporation).

Dynorphin

The detailed procedure for the RIA of dynorphin A (1–8) was identical to that of β-endorphin, as described in a previous report (Hong et al., 1984). Briefly, lyophilized tissue extracts were reconstituted in RIA buffer solution (0.02 M phosphate buffer containing 0.15 *N* NaCl, 0.01% (wt/vol) BSA, 0.1% (wt/vol) gelatin, and 0.1% Triton X-100). ^{125}I-Dynorphin A (1–8) was used as a tracer. An antiserum against dynorphin A (1–8) was used that does not cross-react with ME or LE but does cross-react to a small degree with dynorphin A (1–13) (0.02%) and dynorphin A (1–17) (0.01%). Free and bound ^{125}I-dynorphin A (1–8) were separated by charcoal suspension. The sensitivity of this RIA is approximately 5 fmol.

Measurement of $mRNA^{enk}$

Preparation of RNA

Total RNA was extracted from hippocampus and other brain regions as described (Chirgwin et al., 1979), with slight modifications (Sabol et al., 1983). Briefly, the frozen tissue was homogenized in 4 M guanidinium thiocyanate and centrifuged at 13,000 *g* for 20 min. The supernatant was treated with acetic acid and ethanol and kept overnight at −20°C. The precipitate was recovered by centrifugation and treated with guanidine hydrochloride followed by ethanol precipitation. The guanidine hydrochloride extraction step was repeated once. The final pellets were water-extracted and the water extract containing RNA was ethanol-precipitated, recovered by centrifugation, dried *in vacuo* and finally dissolved in water. Absorbance measurements were obtained at 260 and 280 nm using a spectrophotometer. The 260/280 ratio was between 1.7 and 2.0. The RNA samples were stored at −70°C.

Preparation of cDNA probe for hybridization

The plasmids containing the rat brain preproenkephalin cDNA (pRPE2) were isolated from *E. coli* lysates by CsCl-ethidiumbromide equilibrium density-gradient centrifugation. A 941-base-pair fragment was isolated from pRPE2 by endonuclease digestion followed by agarose-gel electrophoresis. The fragments were nick-translated with α-^{32}P-dCTP to specific radioactivities (1–3 × 10^8 cpm/μg DNA). The detailed procedures involved in the above steps have been described by Yoshikawa et al. (1984). A nick-translation kit from New England Nuclear was used for ^{32}P labeling.

Northern blot preparation

Total RNA (40 μg) for a given brain region was denatured in 50% formamide and 20% formaldehyde at 55°C for 15 min and electrophoresed in a 1.0% agarose gel containing 6.6% formaldehyde, 20 mM sodium phosphate buffer, pH 7.0 (Lehrach et al., 1977). After electrophoresis, the gel was soaked in 10 mM sodium phosphate buffer, pH 7.0, and the RNA transferred to a GeneScreen membrane (New England Nuclear) by the capillary blot procedure and left overnight. The membrane was washed twice with 10× SSC (1 × SSC = 0.15 M NaCl, 0.015 M Na citrate, pH 7) for 15 min, air-dried at room temperature, and baked at 90°C for 2 hr.

Dot-blot preparation

Total RNA (hippocampus, 40 μg; striatum, 5 μg; entorhinal cortex, 20–40 μg; hypothalamus, 10 μg) was denatured in 20% formaldehyde, 6× SSC for 15 min at 58°C, and serially diluted with 7.4% formaldehyde, 6× SCC solution. Denatured RNA was filtered under slight vacuum through a GeneScreen membrane held in a Hybri-Dot manifold (Bethesda Research Laboratories). The membrane was washed twice with 10× SSC for 15 min, air-dried at room temperature, and baked at 90°C for 2 hr.

Blot hybridization

Each membrane was incubated for at least 2 hr at 42°C with prehybridizing solution (10 ml 50% formamide, 0.2% polyvinyl pyrrolidone, 0.2% BSA, 0.2% Ficoll, 0.05 M Tris-HCl buffer, pH 7.5, 1.0 M NaCl, 0.1% sodium pyrophosphate, 1% SDS, 10% dextran sulfate, and 100 μg/ml denatured herring sperm DNA). A 941-base-pair rat preproenkephalin cDNA fragment was obtained by digestion of the pRPE2 plasmid with the restriction endonuclease Bsp-1286, and was nick-translated with α-^{32}P-dCTP (1–3 × 10^8 cpm/μg) and added to the prehybridization solution (10^5–10^6 cpm/ml). The solution was incubated for 18–24 hr at 42°C. The membrane was washed twice with 100 ml of 2× SSC, 0.1% SDS for 15–20 min at 42°C, and then washed once with 100 ml of 0.1× SSC, 0.1% SDS for 20 min at 52°C. The GeneScreen membranes were exposed to Kodak XAR-5 film at −70°C in the presence of a Dupont Lightning-Plus intensifying screen. The density of each dot was measured by scanning densitometry (Gilford multimedia densitometer with a Hewlett-Packard 3390 A integrator).

Immunocytochemical method

Rats were perfused with a 4% paraformaldehyde solution, and brains were prepared for immunocytochemistry as described (McGinty et al., 1983). Three serial 50 μm frozen sections were cut at 400 μm intervals from the frontal pole through hippocampus. Two of 3 adjacent sections in a series were incubated with 2% Triton X-100 in PBS, then rinsed for 30 min in PBS, incubated with an antiserum to dynorphin A (1–17) (provided by L. Terenius, Uppsala, Sweden) or to LE (provided by R. J. Miller, University of Chicago), both at a 1:1000 dilution in PBS with 1 mg/ml BSA. Alternatively, tissue sections were incubated with antisera raised against dynorphin A (1–8) or dynorphin B (provided by E. Weber, University of Oregon) and ME (ME-13, raised by J.S.H.). The cross-reactivities of ME-13 antiserum with the following peptides were: LE, 0.47%; dynorphin A (1–8), <0.06%; β-endorphin, <0.2%. The cross-reactivities of the other antisera have been described previ-

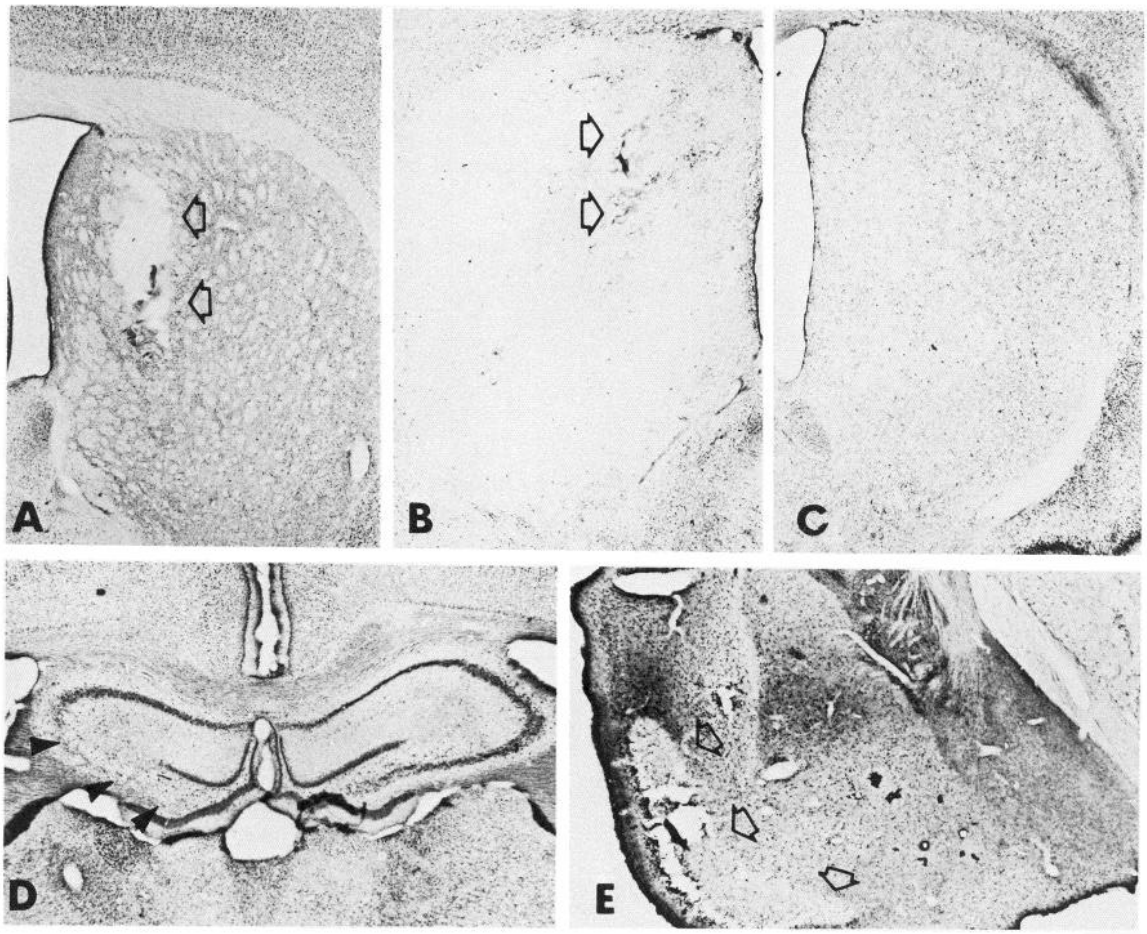

Figure 1. Coronal sections of rat brain injected intrastriatally with kainic acid (KA). Richardson's Nissl stain. *A,* Injection site (*arrows*) in caudate nucleus 6 hr after KA administration. Damage is visible around the injection site only. ×14. *B,* Injection sites (*arrows*) in the caudate nucleus 48 hr after KA administration. Damage is widespread in the ipsilateral caudate, resulting in the lack of Nissl staining. ×9. *C,* Damage is minimal in the caudate nucleus contralateral to the injection site in *B.* ×10. *D,* Forty-eight hours after KA administration, hippocampal degenerative damage is limited to the CA3–4 pyramidical cells (*arrowheads*) ipsilateral to the injection. ×10. *E,* Forty-eight hours after KA administration, some brains exhibited damage (*arrows*) in the ipsilateral pyriform cortex. ×18.

ously (McGinty et al., 1983; Weber and Barchas, 1983; Weber et al., 1982). The primary incubation at 4°C was followed by avidin–biotin–peroxidase immunoreagents as described (McGinty et al., 1983). The third section in a series was stained with Richardson's methylene blue-azure II Nissl stain (Richardson et al., 1960).

Statistics

Analysis of variance (Winer, 1962) was used to test for overall statistical significance. If a significant overall effect of treatment was observed after analysis of variance, post hoc comparisons between group means were made using Fisher's Least Significant Difference test (Miller, 1966). A significance of $p < 0.05$ was required for rejection of the null hypothesis.

Results

Seizures

A single striatal injection of KA caused recurrent seizure activity lasting 3–6 hr. Seizures were characterized by multiple body rolls toward the side of the injection (barrel-rolling), turning in the direction opposite to the injection, clonus of the contralateral forelimb, and side-to-side rhythmic movements of the head. Between bouts of barrel-rolling, animals exhibited body-shaking ("wet-dog shakes"). Severe seizures, characterized by rearing, falling, hypersalivation, or tonic–clonic convulsions, were not observed.

Histology

Histological analysis confirmed that the KA injection sites were located in the dorsomedial portion of the rostral caudate nucleus. Six hours after KA injection, cell damage was confined to the area immediately around the needle track (Fig. 1*A*). However, 48 hr after KA injection, the ipsilateral caudate nucleus demonstrated marked reduction of Nissl staining (Fig. 1*B*), indicative of widespread cellular damage, whereas the contralateral caudate appeared to be intact (Fig. 1*C*). After 48 hr, most of the brains demonstrated CA3–4 pyramidal cell loss, confined to the ipsilateral dorsal hippocampus (Fig. 1*D*), and half of the brains had some cell loss in the ipsilateral pyriform and entorhinal cortical areas (Fig. 1*E*).

Effects of KA on hippocampal enkephalin immunoreactivity

Radioimmunoassay

During the seizure period, there was a 30–40% reduction in native hippocampal ME-LI. This reduction was followed by a return to control levels 24 hr postinjection and a marked rebound (300% of control) by 72 hr (Fig. 2). Cryptic ME-LI increased rapidly beginning 3 hr after KA injection, reached a maximum (300% of control) 24 hr after dosing, and then gradually declined (Fig. 2).

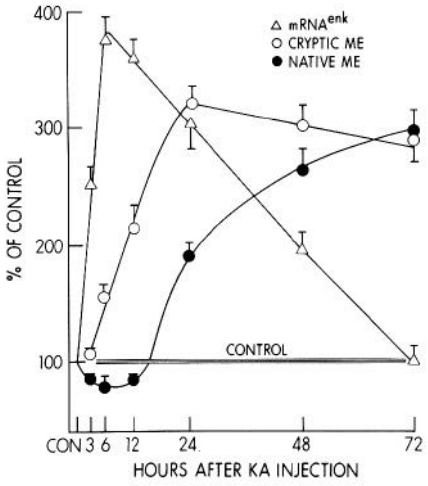

Figure 2. Time course of changes in hippocampal native ME-LI, cryptic ME-LI, and mRNA^{enk} after intrastriatal injection of kainic acid (1 μg/rat). Values are expressed as percentage of sham-operated controls. Native ME-LI was decreased during the convulsive period and increased 48 and 72 hr after KA injection ($F(6,31) = 49.80$, $p < 0.01$). Cryptic ME-LI was increased 24 hr after KA injection and declined gradually thereafter ($F(7,39) = 22.85$, $p < 0.01$). For post hoc tests, * denotes $p < 0.05$ and **denotes $p < 0.001$. Data for mRNA were not subject to statistical analysis. Each value represents the mRNA^{enk} from a pool of 3 animals. The experiment was replicated once.

Because histology revealed cell loss in the dorsal and not the ventral hippocampus ipsilateral to the injected striatum, a follow-up experiment to determine the relative changes in enkephalin immunoreactivity between dorsal and ventral and left and right hippocampi was undertaken. Animals were injected with saline or KA (1 μg/rat) in the right striatum and sacrificed at 6 or 48 hr after injection. As there were no differences between values for animals injected with saline at the 6 and 48 hr time points, the data from these animals were pooled. In both saline- and KA-treated animals, no differences in ME-LI were found between left and right hippocampi ($F(1,13) = 0.19$, $p = 0.6727$), but there was more ME-LI in the ventral than in the dorsal hippocampus ($F(1,13) = 82.03$, $p < 0.0001$). The changes in ME-LI as a result of KA injection were far more striking in ventral than in dorsal hippocampus ($F(2,13) = 24.78$, $p < 0.0001$) (Fig. 3).

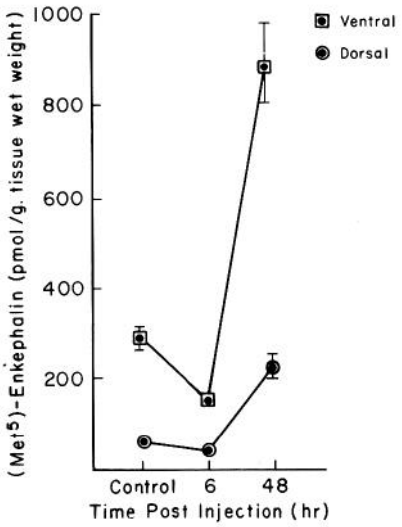

Figure 3. Time course of changes in hippocampal Met^5-enkephalin in dorsal and ventral hippocampus after intrastriatal injection of kainic acid (1 μg/rat). The changes in enkephalin immunoreactivity were greater in ventral than in dorsal hippocampus ($F(2,13) = 24.78$, $p < 0.0001$).

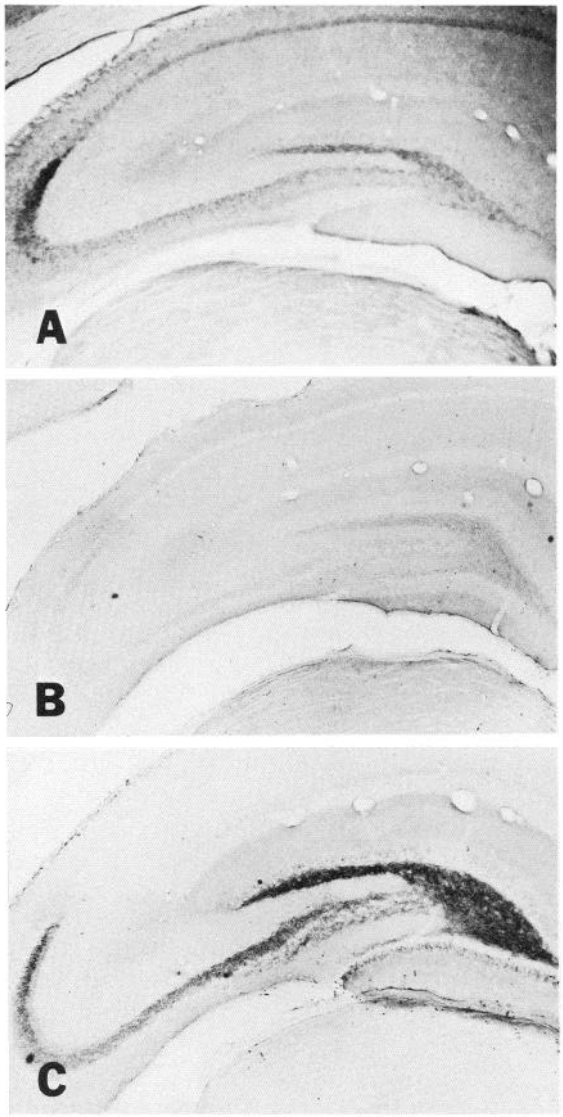

Figure 4. Met^5-enkephalin-like (ME-LI) immunoreactivity in the mossy fibers of the dorsal hippocampus in saline control and KA-treated rats. *A*, Normal intensity of ME-LI in saline control rats. *B*, Diminished ME-LI intensity in a rat 6 hr after KA. *C*, Enhanced ME-LI intensity in a rat 48 hr after KA. *A–C*, × 20.

Immunocytochemistry

The intensities of ME-LI and LE-LI in the dorsal and ventral hippocampus of normal rats were similar (Fig. 4*A* illustrates ME-LI; Fig. 5 illustrates LE-LI). Six hours after KA administration, LE-LI and ME-LI had decreased to a similar extent in the mossy fibers of the dorsal hippocampus (4*B*). In the ventral hippocampus (Fig. 5*B*), however, neither ME-LI nor LE-LI was visibly affected 6 hr after KA. Enkephalin immunostaining (ME-LI and LE-LI) was decreased markedly in the temporoammonic pathway (Fig. 5*B*), which arises in the entorhinal cortex and innervates the pyramidal cells of the hippocampus (Steward and Scoville, 1976). Enkephalin immunostaining (ME-LI and LE-LI) in the perforant pathway innervating the granule cells of the ventral dentate gyrus was decreased slightly after 6 hr (Fig. 5*B*). By contrast, after 48 hr, enkephalin immunostaining (ME-LI

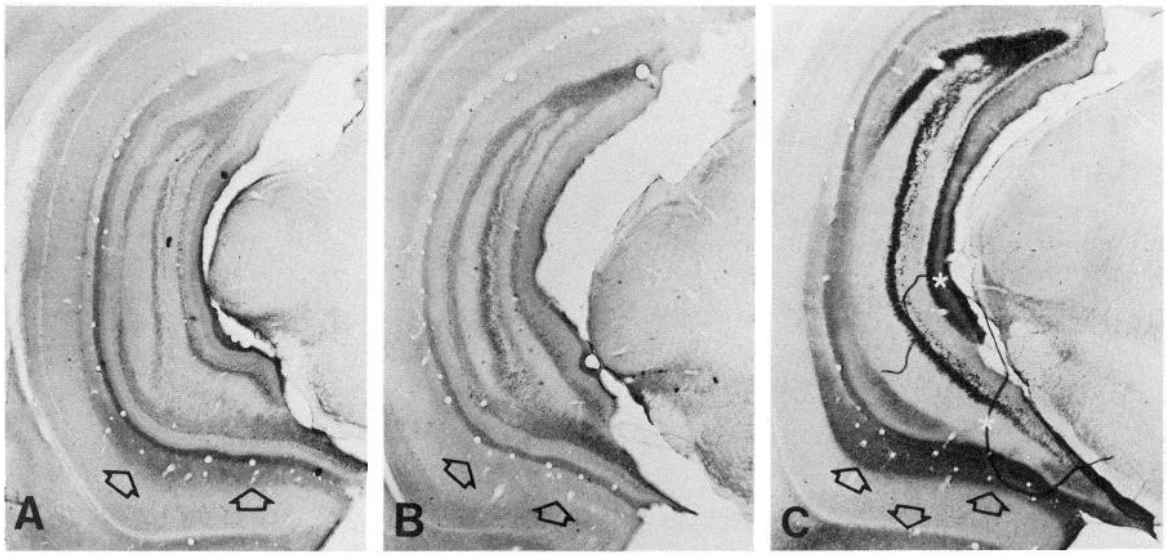

Figure 5. Leu⁵-enkephalin-like (LE-LI) immunostaining in the ventral hippocampus of saline control and KA-treated rats. *A,* Normal intensity of LE-LI in the mossy fibers and perforant-temporoammonic (*arrows*) pathways. ×14. *B,* Diminished intensity of LE-LI in the temporoammonic pathway but not in the perforant pathway or mossy fibers of a rat 6 hr after KA. ×12.5. *C,* Enhanced LE-LI in the mossy fibers and in the temporoammonic pathways of a rat 48 hr after KA. ×14. *Debris on section.

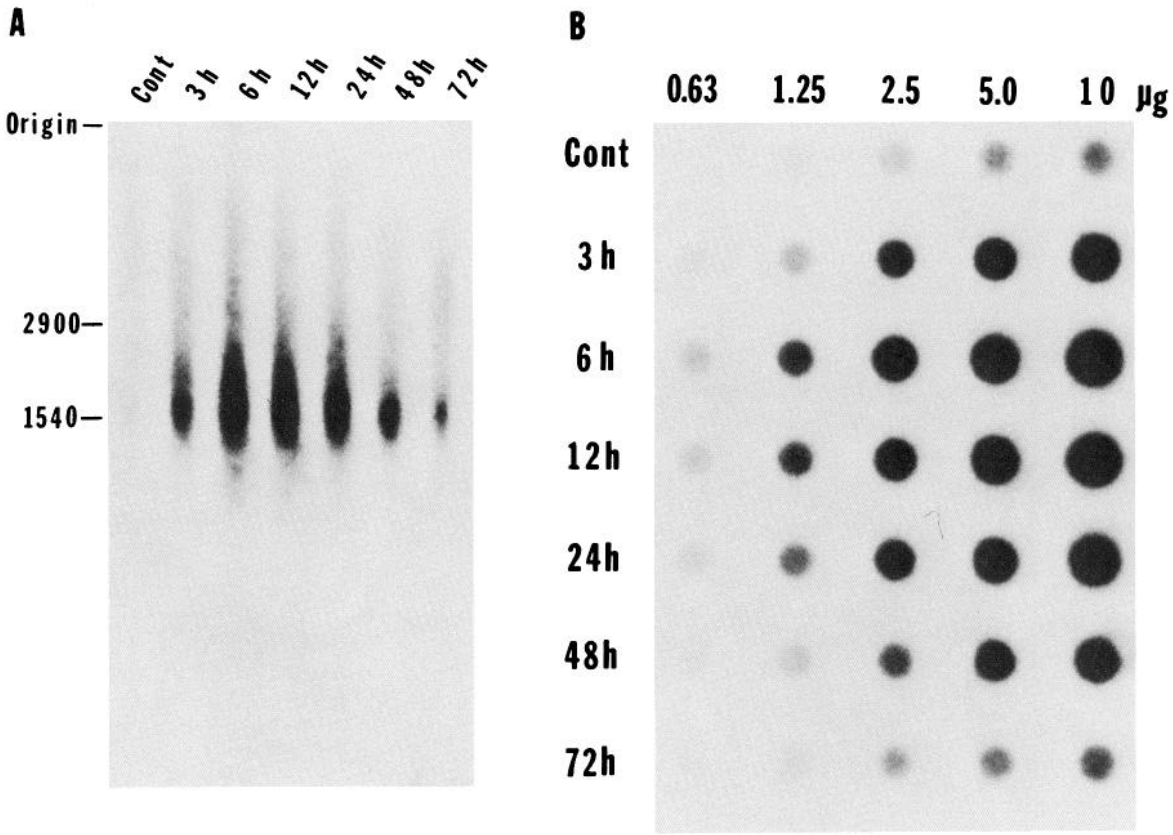

Figure 6. Time course of changes in hippocampal mRNA^{enk} after intrastriatal injection of kainic acid (1 μg/rat). *A,* Northern blot analysis. The ^{32}P-labeled cDNA probe for rat mRNA^{enk} hybridized with a mRNA of approximately 1540 bases. The mRNA^{enk} band increased in intensity during the period of convulsions and decreased thereafter. *B,* Dot-blot analysis. The radiolabeled cDNA probe hybridized with various concentrations of the extracted RNA. Dot intensity increased during the period of convulsions (3–6 hr). A decrease in dot intensity could be observed by 24 hr and a return to control levels by 72 hr.

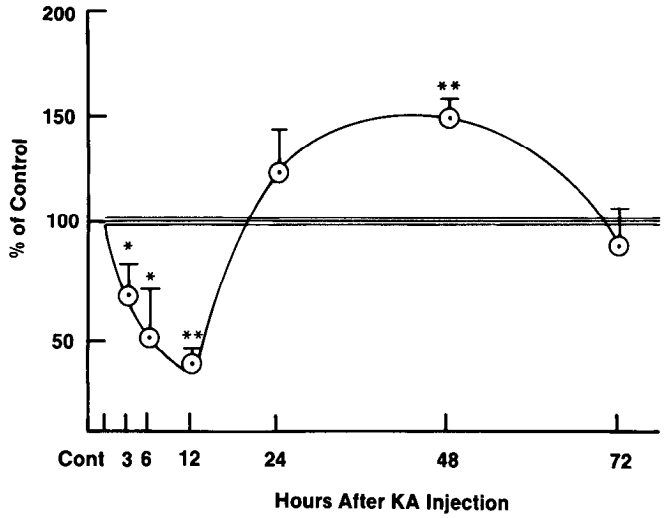

Figure 7. Time course of changes in hippocampal dynorphin A (1–8) after instrastriatal injection of kainic acid (1 μg/rat). Values are expressed as percentage of sham-operated control. DYN-LI was decreased 6 hr after KA and increased 48 hr after KA ($F(7,32) = 11.10$, $p < 0.01$). For post hoc tests, * denotes $p < 0.05$ and ** denotes $p < 0.001$.

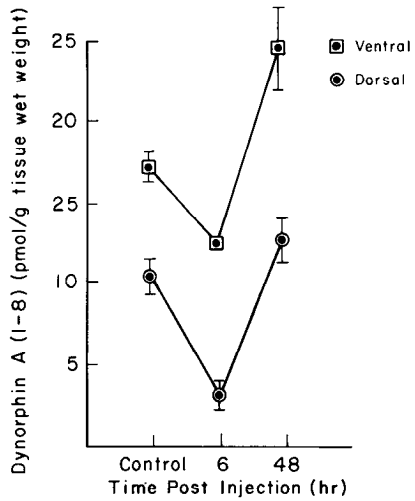

Figure 8. Time course of changes in DYN-LI in dorsal and ventral hippocampus after intrastriatal injection of kainic acid (1 μg/rat). The decrease in DYN-LI in dorsal hippocampus was greater than in ventral hippocampus, but this difference did not reach statistical significance, and the increases in DYN-LI in dorsal versus ventral hippocampus were of approximately the same magnitude ($F(2,13) = 1.06$, $p = 0.3751$).

Table 1. Levels of native and cryptic Met⁵-enkephalin and mRNA coding for preproenkephalin in rat striatum, hypothalamus, and entorhinal cortex after a single injection of kainic acid (1 μg)

		Brain region		
	Time (hr)	Striatum	Hypothalamus	Entorhinal cortex
Native ME-LI	Control	2003 ± 191	1141 ± 82	298 ± 38
(pmol/gm wet wt)	3	1899 ± 296	1207 ± 70	383 ± 42
	6	1986 ± 314	1145 ± 129	371 ± 73
	12	1847 ± 314	920 ± 92	274 ± 24
	24	1951 ± 105	1115 ± 150	397 ± 37
	48	2770 ± 209	1096 ± 139	476 ± 49*
	72	2735 ± 436	1160 ± 150	587 ± 68**
Cryptic ME-LI	Control	655 ± 50	1056 ± 57	329 ± 61
(pmol/gm wet wt)	3	ND	ND	547 ± 85
	6	657 ± 52	1073 ± 162	490 ± 77
	12	834 ± 85	934 ± 73	418 ± 28
	24	885 ± 77	1131 ± 87	561 ± 106*
	48	ND	ND	505 ± 75
	72	855 ± 94	1333 ± 101	831 ± 103**
Preproenkephalin mRNA	Control	100	100	100
(% of control)	3	95.1	115.0	136.0
	6	90.9	140.0	253.4
	12	127.8	150.2	288.2
	24	105.6	124.5	256.4
	48	96.2	109.0	159.4
	72	ND	ND	ND

Three tissue samples were pooled for the measurement of $mRNA^{enk}$. The experiment was replicated once. ND, Not determined. Analysis of variance was not significant for native ME from striatum ($F(7,40) = 1.79$, $p > 0.25$) or hypothalamus ($F(7,40) = 0.57$, $p > 0.25$), but was significnt for entorhinal cortex ($F(7,48) = 4.88$, $p < 0.05$). Analysis of variance was not significant for cryptic ME from striatum ($F(4,24) = 2.00$, $p > 0.25$) or hypothalamus ($F(4,25) = 2.01$, $p > 0.025$), but was significant for entorhinal cortex ($F(6,42) = 3.88$, $p < 0.05$).

* $p < 0.05$, post hoc tests.

** $p < 0.001$, post hoc tests.

Table 2. Levels of dynorphin A (1-8) in rat striatum, hypothalamus, and entorhinal cortex after a single injection of kainic acid (1 μg) (pmol/gm wet weight ± SEM)

Time (hr)	Brain region		
	Striatum	Hypothalamus	Entorhinal cortex
Intact control	35.0 ± 1.7	31.1 ± 0.9	4.5 ± 0.8
Sham control	33.0 ± 2.9	30.8 ± 3.1	3.6 ± 0.6
3	35.0 ± 2.9	29.5 ± 1.6	4.9 ± 0.8
6	29.5 ± 2.3	26.7 ± 1.7	4.8 ± 1.4
12	29.9 ± 3.8	27.6 ± 2.0	2.4 ± 0.3
24	40.9 ± 6.1	31.4 ± 2.2	1.7 ± 0.4
48	39.2 ± 5.6	23.8 ± 2.3	3.1 ± 0.4
72	40.2 ± 2.6	31.3 ± 3.7	3.9 ± 0.7

Analysis of variance was not significant for any of the brain regions: striatum ($F(7,40) = 1.39$, $p > 0.25$), hypothalamus ($F(7,40) = 1.34$, $p > 0.25$), entorhinal cortex ($F(7,45) = 2.52$, $p > 0.10$).

and LE-LI) in the mossy fibers of the dorsal and ventral hippocampus (Figs. 4*C* and 5*C*), as well as in the perforant–temporoammonic pathways (Fig. 5*C*) was markedly increased.

mRNA^{enk}

The abundance of mRNA^{enk} in the hippocampus of control and KA-treated rats was measured by both Northern blot and dot-blot methods. Figure 6*A* shows that, using the Northern blot analysis, ^{32}P-labeled cDNA coding for preproenkephalin A from rat brain hybridized with an apparently single mRNA species of approximately 1450 bases, in accord with previous measurements of brain mRNA^{enk} (Yoshikawa et al., 1984). This same figure shows a time-related increase in the intensity of the band in KA-treated rats. Detailed quantitation of the KA-induced change in mRNA^{enk} was performed by dot blot. Figure 6*B* shows that the dot intensity of the KA-treated rats had time-related alterations. The level of mRNA^{enk} reached a peak (400% of control) 6 hr after KA injection, then underwent a linear return to control values by 72 hr postinjection, with an approximate half-life of 33 hr (Fig. 2).

Effects of KA injection on the levels of native and cryptic ME-LI and mRNA^{enk} in other brain regions

Kainic acid-induced alterations in the levels of native and cryptic ME-LI and of mRNA^{enk} in the entorhinal cortex were smaller than those induced in the hippocampus. Levels of native and cryptic ME-LI increased 100–150% 48–72 hr after KA injection, and these alterations were preceded by an increase in mRNA^{enk} between 6 and 24 hr postinjection (Table 1). At no time point was a depletion of either native or cryptic ME-LI in the entorhinal cortex observed. No KA-induced alterations were observed in native ME-LI, cryptic ME-LI, or mRNA^{enk} in hypothalamus or the uninjected striatum (Table 1).

Effects of KA injection on levels of hippocampal dynorphin immunoreactivity

Radioimmunoassay

KA-induced alterations in hippocampal DYN-LI were similar to those observed for ME-LI. DYN-LI depletion was observed at 3, 6, and 12 hr after KA injection, followed by a rebound between 24 and 48 hr, and a return to control levels at 72 hr (Fig. 7). The depletion of DYN-LI (63% decrease at 12 hr) was more severe than that of ME (31% decrease at 6 hr), but the rebound (151 vs 273%) was not as dramatic. No significant alterations in DYN-LI as a result of KA injection were observed in hypothalamus, uninjected striatum, or entorhinal cortex (Table 2).

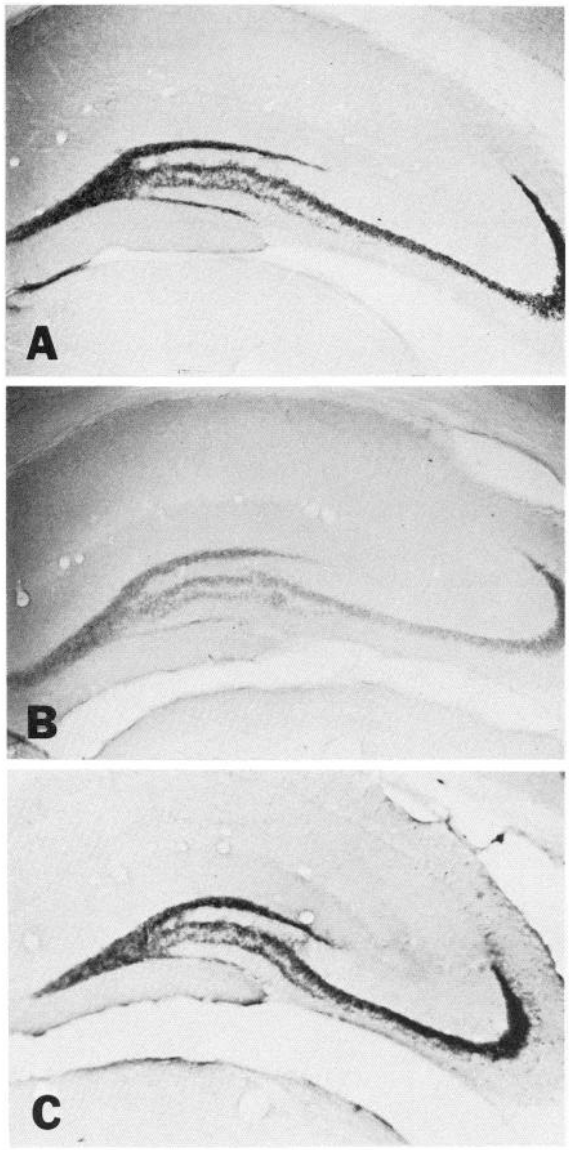

Figure 9. Dynorphin A (1–17)-like immunoreactivity in the mossy fibers of the dorsal hippocampus in saline control and KA-treated rats. *A*, Normal intensity of this DYN-LI in a saline control rat. ×20. *B*, Diminished DYN-LI in a rat 6 hr after KA administration. ×20. *C*, Normal intensity of this DYN-LI in a rat 48 hr after KA. ×18.

As with enkephalin, DYN-LI was measured in left versus right and dorsal versus ventral hippocampi after right striatal injection of KA or saline. In both treated and control animals, no differences in DYN-LI between left and right hippocampi were observed ($F(1,13) = 0.01$, $p = 0.9365$), but there was more DYN-LI in the ventral than in the dorsal hippocampus ($F(1,13) = 50.80$, $p < 0.001$). In contrast to ME-LI, the KA-induced increase in DYN-LI was not significantly greater in ventral than in dorsal hippocampus ($F(2,13) = 1.06$, $p = 0.3751$) (Fig. 8).

Immunocytochemistry

Both dynorphin A (1–17) and dynorphin B antisera were used for immunocytochemical studies. Similar staining patterns were obtained; only the results from dynorphin A (1–17) were presented. Six hours after KA administration, dynorphin immunostaining was depleted in dorsal and ventral hippocampal mos-

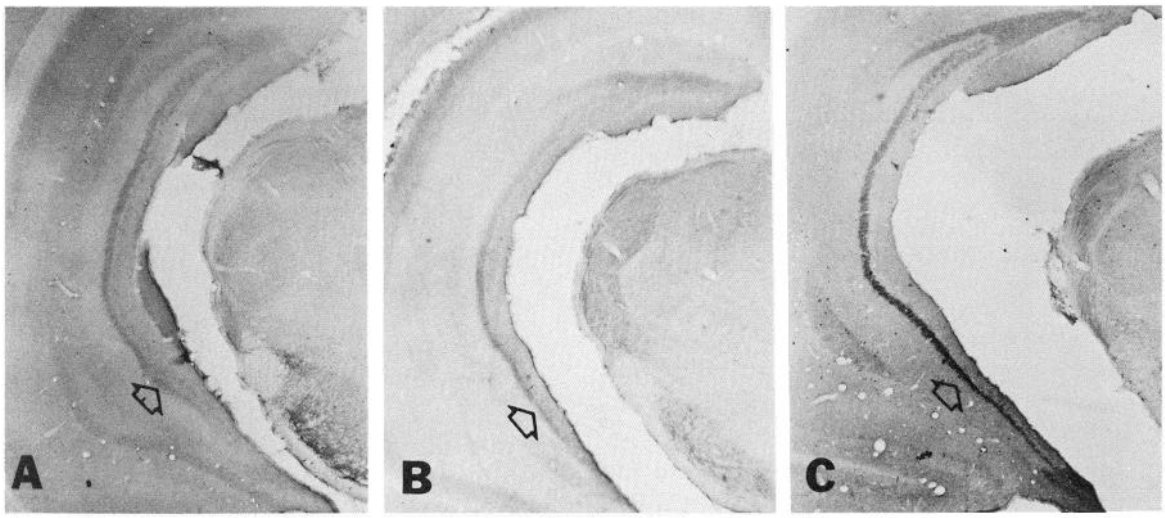

Figure 10. DYN-LI in the ventral hippocampus of saline control and KA-treated rats. *A,* Normal intensity of DYN-LI in a saline control rat. ×12.5. *B,* Slightly diminished intensity of DYN-LI in a rat 6 hr after KA. ×11.5. *C,* Enhanced intensity of DYN-LI, especially in the ventral hippocampus of a rat, 48 hr after KA. ×12.5.

sy fibers. (Compare Figs. 9, *A* with *B,* and 10, *A* with *B.*) After 48 hr, dynorphin immunostaining in the mossy fibers was enhanced, compared to controls (compare Figs. 9, *A* with *C,* and 10, *A* with *C*). The increase in dynorphin immunostaining was not as dramatic as that in enkephalin at 48 hr.

Discussion

We have previously administered KA to different brain regions (striatum, septum, hippocampus, and lateral ventricle) in order to perturb the metabolism of the enkephalin system in the hippocampus (Hong et al., 1980). Nevertheless, we have had only indirect evidence that the KA-induced changes in hippocampal enkephalin indicated an increase in its utilization. Therefore we were interested to determine the effects of KA on enkephalin's biosynthetic pathways. Although we did not originally plan to attempt to clarify the functional relationship between the change in opioid peptides and hippocampal cell loss, the method of unilateral injection into the striatum caused ipsilateral, but not contralateral, limbic cell loss (Wuerthele et al., 1978) and thereby provided us with a means of studying the relationship between these 2 phenomena.

A single intrastriatal injection of KA (1 μg/rat) caused an initial reduction (3–12 hr) in the hippocampal content of ME-LI, followed by a large rebound at 24–72 hr. The initial reduction in ME-LI during the convulsive period was accompanied by an increase in $mRNA^{enk}$ (peak at 6 hr), followed by an increase in cryptic ME-LI cleaved from preproenkephalin (peak at 24 hr). The data suggest that KA induced an increase in the use of the hippocampal opioid peptides and a compensatory increase in production such that, at later time points, opioid peptide levels exceeded control values. The $mRNA^{enk}$ increase may have been the result of seizure-induced enhancement of cellular metabolism or it may have been the result of a feedback mechanism specifically sensitive to ME depletion. Although we speculate that the increase in $mRNA^{enk}$ was due to an increase in transcription, our data do not exclude the possibility of a decreased rate of $mRNA^{enk}$ degradation.

The finding that hippocampal $mRNA^{enk}$ was increased following KA administration is consistent with our previous finding that protein synthesis inhibition attenuated the late increase in hippocampal ME levels of KA-treated animals without affecting ME levels of controls (Hong et al., 1980). The initial seizure-associated DYN-LI decrease and the subsequent rebound at 48 hr resembled the changes in ME-LI. However the DYN-LI rebound was not as dramatic as that of ME-LI, and DYN-LI returned to control levels after 72 hr, as previously reported (McGinty et al., 1983). Work is under way in our laboratory to determine the effects of KA on preprodynorphin mRNA biosynthesis. It is likely, however, that our mRNA studies will indicate an increase in KA-induced dynorphin biosynthesis because cycloheximide, coadministered with KA, has been shown to block the late increase in dynorphin immunoreactivity (Lason et al., 1983).

Although naloxone pretreatment attenuates the convulsions and cell loss induced by high (12 mg/kg i.p.) doses of KA, and morphine potentiates seizures and cell loss after intermediate (7 mg/kg s.c.) doses of KA (Fuller and Olney, 1979), the results of this study suggest that the changes in hippocampal opioid peptides do not play a primary role in KA-induced hippocampal cell death. We found that unilateral intrastriatal injection of KA produced cell death in the ipsilateral dorsal hippocampus, whereas the subsequent changes in opioid peptides were bilateral, and more prominant in the ventral than dorsal hippocampus. It is still possible that KA-induced changes in opioid peptides in brain regions not included in our study could enhance neuronal excitability in the limbic circuit and contribute to cell loss in limbic areas.

Although opioid peptides do not appear to play a primary role in KA-induced neurotoxicity, they may still play an important role in modulating neuronal excitability in the hippocampus. Enkephalins have been shown to cause a naloxone-reversible increase in the population spike response of hippocampal pyramidal cells to orthodromic stimulation in the *in vitro* preparation (Lynch et al., 1981; Valentino and Dingledine, 1982; Vidal et al., 1984). It has been proposed that delta opioid receptors play a key role in enkephalin-induced hippocampal excitability (Valentino and Dingledine, 1982). In contrast to the ME-induced increase in neuronal excitability, dynorphin A (1–17) has been found to have a non-naloxone-reversible inhibitory effect on CA1 population spike responses (Vidal et al., 1984) and on the excitability of CA3 and CA1 pyramidal neurons (Henriksen et al., 1982; Walker et al., 1982). Thus, it is possible that KA induces a release of enkephalin from the projections of the entorhinal cortex and the dentate granule cells that enhances KA-induced neuronal excitability but also induces re-

lease of dynorphin, which may act in a compensatory manner to reduce hippocampal excitability.

Thus, results of this study show that a single intrastriatal injection of Kainic acid (1 μg/rat) caused profound alterations in hippocampal levels of Met⁵-enkephalin, its precursor preproenkephalin, and $mRNA^{enk}$. These alterations support the interpretation that convulsant doses of KA cause an increase in hippocampal utilization of ME, followed by an overproduction of this peptide. Similar alterations in the levels of hippocampal dynorphin A (1–8) were also observed. The KA-induced changes in the levels of hippocampal and entorhinal opioid peptides may affect neuronal excitability of the hippocampus during seizure activity, but do not appear to play a primary role in KA-induced neurotoxicity.

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