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REGIONAL BRAIN ATROPHY AND REDUCTIONS IN GLUTAMATE RELEASE AND UPTAKE AFTER INTRASTRIATAL KAINIC ACID

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1 Neurochemical changes and tissue weights were measured following intrastriatal injection of 2.5 μg of kainic acid in 2 μl of 0.9% w/v NaCl solution (saline) in the rat.

2 After kainic acid the striatum and neocortex on the injected side showed a progressive reduction in weight, the neocortex showing the greatest absolute weight loss and the striatum the greatest percentage change.

3 Large (80–90%) reductions in choline acetyltransferase (CAT) and L-glutamate decarboxylase (GAD) activities in the striatum occurred within 2–4 days of the injection and persisted at least 10 weeks. At 10 weeks CAT and GAD activities were unaltered in the neocortex.

4 The absolute content of dopamine in the striatum was not different from control 5 days after the injection of kainic acid but was reduced at 2 and 10 weeks. At 2 weeks the concentration (μg/g wet weight) of dopamine also was reduced but at 10 weeks it was near normal due to atrophy of the striatum.

5 The high affinity glutamate uptake into a crude synaptosomal preparation of the striatum was reduced by 64% 5 days after kainic acid and was still reduced by 67% at 10 weeks.

6 The efflux of glutamate from slices of the striatum in the presence of 52 mM K^+ was reduced by approximately 75% 5 days and 15 weeks after kainic acid.

7 *In vitro* kainic acid (10^{-4} M) neither altered the high affinity uptake of radiolabelled glutamate into a homogenate of the striatum, nor released endogenous glutamate from slices of striatum.

Introduction

Kainic acid, an analogue of glutamate and a potent neuroexcitant (Shinozaki & Konishi, 1970; Johnston, Curtis, Davies & McCullough, 1974) has been reported to destroy cholinergic and γ-aminobutyric acid (GABA)-ergic neurones when injected into the striatum without destroying dopaminergic terminals (Coyle & Schwarcz, 1976; McGeer & McGeer, 1976a). Since similar neurochemical changes are seen in the brains of patients dying with Huntington's chorea (Perry, Hansen & Kloster, 1973; Bird & Iversen, 1974; McGeer & McGeer, 1976b) it has been suggested that kainic acid-induced degenerative changes can serve as a model of the disease. Here we investigate whether kainic acid produces brain atrophy like that in Huntington's disease, whether there are changes in the uptake and release of glutamate as markers of glutamatergic neurones, and whether there is degeneration of dopaminergic nerve endings.

Methods

Animals and surgery

Kainic acid (Sigma Ltd.) (2.5 μg in 2 μl of 0.9% w/v NaCl solution-saline) was injected stereotaxically at a rate of 1 μl/min into the right caudate nucleus of adult, male Sprague-Dawley rats. Injection coordinates were A2.0, L3.0, V5.0 according to the atlas of Pellegrino & Cushman (1967).

Dissections

Rats were stunned and killed by decapitation, their brains removed, chilled on an ice-cold glass plate, and dissected into regions as previously described (Glowinski & Iversen, 1966). The substantia nigra was dis-

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sected from a coronal slice between the rostral end of the pons and the caudal end of the mammillary bodies. Knife cuts were made lateral to the interpeduncular nucleus to the base of the brain and along the medial lemniscus to the lateral edge of the brain stem. The tissue bounded by these cuts and the base of the brain was taken as substantia nigra. Slices of the striatum for measuring the K^+-induced efflux of endogenous glutamate were dissected from 1 mm thick frontal sections at the level of the optic chiasm.

Assays

Dopamine. Tissues were homogenized in distilled water and aliquots immediately acidified with an equal volume of 0.4 N perchloric acid containing 0.1 mg/ml disodium edetate (EDTA), centrifuged for 1 min at 10,000 *g* and dopamine in the perchloric acid supernatant was measured by a radioenzymatic method (Moore & Phillipson, 1975) modified from that of Cuello, Hiley & Iversen (1973).

Choline acetyltransferase (CAT). Tissues were homogenized in distilled water and diluted 10-fold with 10 mM EDTA containing 0.5% (w/v) Triton X-100 and assayed for CAT activity by a method similar to that described by Fonnum (1975). To 5 µl of tissue homogenate in 15 ml centrifuge tubes on ice was added 10 µl of incubation mixture containing sodium chloride (450 mM), choline iodide (12 mM), EDTA (30 mM), physostigmine (0.15 mM) and acetyl-[1-^{14}C]-coenzyme A (0.3 mM, 3.33 µCi/µmol) dissolved in 75 mM sodium phosphate buffer, pH 7.4. After incubation at 37°C for 15 min the contents of the centrifuge tubes were rinsed into scintillation vials with two separate 2 ml portions of ice-cold 10 mM sodium phosphate buffer, pH 7.4. Two ml of 0.5% sodium tetraphenylboron in acetonitrile and 10 ml of 0.05% PPO in toluene were added to each vial. After gentle shaking and standing for 30 min, radioactivity was determined by liquid scintillation counting. Radioactivity increased linearly with amount of tissue and incubation time.

L-Glutamate decarboxylase (GAD). GAD was measured by the evolution of $^{14}CO_2$ from L-[1-^{14}C]-glutamic acid. The method was similar to that described by Kanazawa, Iversen & Kelly (1976) modified from that of Albers & Brady (1959). Five µl of tissue homogenate was added to 5 µl of assay mixture in a 0.6 × 5.0 cm glass tube. The reaction mixture contained the following (final concentration): potassium phosphate, pH 6.5 (0.1 M); dithiothreitol (0.5 mM); pyridoxal phosphate (0.5 mM), sodium L-glutamate (25 mM); L-[1-^{14}C]-glutamic acid (10 µCi/ml) (specific radioactivity 55 mCi/mmol, Amersham/Searle). The reaction tube was connected by Tygon tubing to a similar tube containing 150 µl of NCS (Amersham/Searle, tissue solubilizer) to absorb $^{14}CO_2$ evolved. The reaction was stopped after incubation at 37°C for 15 min by injection of 100 µl of 6 N H_2SO_4 into the reaction tube and incubation continued for a further 30 min to complete $^{14}CO_2$ absorption. The tube containing NCS was placed inverted into a scintillation vial and 10 ml of 0.05% PPO in toluene added. Radioactivity was determined by liquid scintillation counting. Under the conditions used, $^{14}CO_2$ evolution was linearly related to incubation time and weight of tissue.

Glutamate. An enzymatic-fluorometric method similar to a modification (Balcom, Lenox & Meyerhoff, 1976) of that described by Graham and Aprison (1966) was used. Tissues were homogenized in 10 volumes of ice-cold 0.05 M sodium acetate, and after centrifugation of 1 min at 10,000 *g* the supernatant was diluted 10 fold with distilled water; 20 µl of the diluted supernatant were added to tubes containing 1 ml of 0.1 M sodium pyrophosphate buffer pH 8.4 and 20 µl of 10 mM NAD. Glutamate dehydrogenase 10 µl (ammonia free, Calbiochem) was added to each tube. The tubes were mixed and left to incubate for 45 min at room temperature. The fluorescence was read in an Aminco Bowman spectrophotofluorometer at 460 nm with an excitation wavelength of 340 nm. A blank containing no tissue gave a slightly higher reading than the samples before addition of enzyme and was therefore used. Fluorescence was linearly related to glutamate standards in the range of 1–5 nmol.

Uptake experiments

The uptake of radioactive glutamate into a sucrose homogenate of the striatum was measured by a modification of the method described by Storm-Mathison (1977). Tissue was homogenized in 50 volumes of 0.32 M sucrose in a glass-Teflon homogenizer; 5 µl of tissue homogenate was added to 485 µl of Krebs phosphate solution (NaCl 140 mM, KCl 5 mM, $CaCl_2$ 1.2 mM, $MgCl_2$ 1.2 mM and glucose 5 mM in 15 mM sodium phosphate buffer pH 7.4). After preincubation for 5 min, 0.5 nmol of [^{14}C-U]-glutamic acid (270 mCi/mmol, Amersham/Searle) was added to a volume of 10 µl bringing the final concentration of [^{14}C-U]-glutamate to 1 µM. After incubation for 3 min the uptake was stopped by addition of 4 ml of ice-cold saline and the tubes placed in ice. The contents were filtered through saline-moistened nitrocellulose membrane filters (0.45 µm pore size, Schleicher and Schuell Inc.,) which were then washed with two separate 4 ml volumes of ice-cold saline. The filters were placed in scintillation vials and dissolved in 1 ml of ethylene glycol monomethyl ether (Piersolve TM, Pierce Ltd., Ill.). Ten ml of 0.5% PPO in toluene/ethanol (70:30 v/v) was added and radioactivity counted in a liquid scintillation spectrometer. To correct for nonspecific binding 0.1 M glutamate was included in some tubes

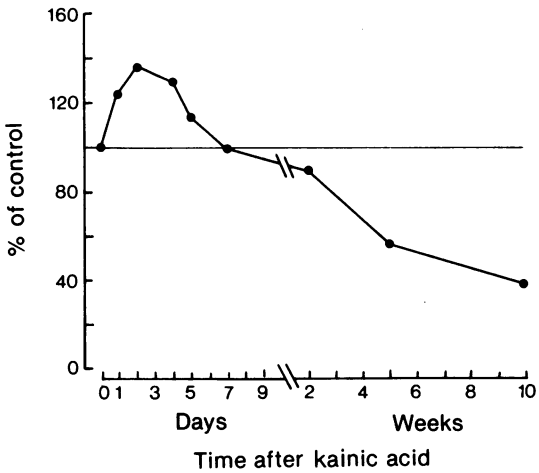

Figure 1 Weight of the right striatum as a percentage of the weight of the left at various times following injection of kainic acid into the right striatum. Each circle represents the mean value obtained from 6–14 rats; the s.e. mean is less than the radius of the circle. The weight of the control side (all time points) was 47.4 ± 0.9 mg (n = 60).

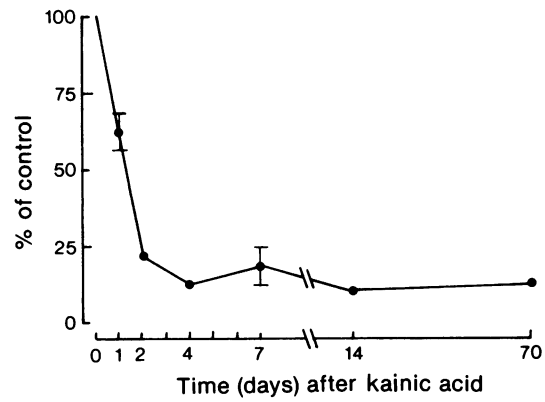

Figure 2 Choline acetyltransferase (CAT) activity in the striatum at various times following the injection of kainic acid into the right striatum. Values on the right side are expressed as a percentage of those on the left. Each circle represents the mean value obtained from 6–9 rats; vertical lines represent ± s.e. mean. Values (100%) for CAT in the striatum on the control side were 21.8 ± 0.6 μmol g^{-1} h^{-1} (n = 49); these control values do not differ significantly from values in non-injected animals (Kelly *et al., 1977)*.

during the incubation and preincubation and these were used as blanks. Uptake was linear for at least 6 min and was proportional to the amount of tissue. Preliminary experiments showed the uptake was sodium-dependent and severely reduced in the presence of 0.1% Triton X-100.

Release experiments

The K^+-induced release of glutamate was measured from striatal slices incubated in Krebs solution. Slices of striatum were removed from 1 mm thick frontal sections at the level of the optic chiasm, and usually weighed 10–15 milligrams. Because of the reduced size of the kainic acid treated striatum 15 weeks after injection, two slices were used to obtain this amount of tissue. In all other cases the release from one slice was measured. Every 2 min the slice was transferred from a gently agitated beaker containing 1 ml of Krebs phosphate solution at 37°C to a similar beaker. In some beakers the K^+ concentration was increased to 52 mM by the addition of 50 μl of 1 M potassium phosphate buffer pH 7.4. The glutamate released into the bathing fluid was determined by assay of 200 μl aliquots by the method described above.

Statistics

The significance of statistical differences was determined by Student's t test.

Results

Tissue weight

Figure 1 shows striatal weight at various times following kainic acid injection into the striatum. Between five days and two weeks after kainic acid injection the wet weight of the striatum was not significantly different from the control side. However, in the first few days following the injection, the striatum showed a transient increase in weight, and between 2 and 10 weeks post-lesion the striatum atrophied to 39% of the weight of the control side. Control injections of saline did not cause atrophy. Table 1 shows the atrophy was not restricted to the striatum but also involved the neocortex and hippocampus. While the striatum showed the largest percentage weight change the neocortex showed a greater absolute weight loss. By gross observation and examination of histological sections the cortical regions showing the largest changes were the frontal cortex and the cortical region below the rhinal sulcus.

Choline acetyltransferase

Striatal CAT activity decreased to 10–20% of control levels within 4 days and remained at this level for 10 weeks (Figure 2). At 10 weeks the cortical CAT activity of six animals was 4.58 ± 0.21 μmol g^{-1} h^{-1} on the right side and 5.22 ± 0.31 μmol g^{-1} h^{-1} on

Table 1 Weights of brain regions at various times after a kainic acid injection into the right striatum

	1 week			2 weeks			10 weeks		
Brain region	*Left (control)*	*Right (kainic)*	*% of control*	*Left (control)*	*Right (kainic)*	*% of control*	*Left (control)*	*Right (kainic)*	*% of control*
Striatum	53.8 ± 1.6	54.0 ± 1.5	100 ± 3	49.9 ± 1.2	44.6 ± 1.3	89 ± 3	60.2 ± 3.6	23.2 ± 1.6*	39 ± 3
Neocortex	389 ± 6	377 ± 7	97 ± 2	403 ± 5	332 ± 4*	82 ± 1	471 ± 12	334 ± 11*	71 ± 2
Hippocampus	56.6 ± 1.1	58.2 ± 1.0	103 ± 2	59.5 ± 2.3	58.1 ± 2.2	98 ± 4	76.7 ± 2.7	60.8 ± 3.1*	79 ± 4
Substantia nigra	8.5 ± 0.5	10.0 ± 0.6	117 ± 7	7.5 ± 0.4	8.8 ± 0.5	111 ± 7	9.2 ± 0.5	8.0 ± 0.4	87 ± 5
Cerebellum	136 ± 4	132 ± 3	97 ± 2	140 ± 3	143 ± 4	102 ± 3	157 ± 4	155 ± 5	99 ± 3
Rest of brain	220 ± 5	227 ± 7	103 ± 3	232 ± 7	218 ± 4	94 ± 2	263 ± 9	255 ± 5	97 ± 2

Values (mean ± s.e. mean) represent the wet weights (mg) determined from 5–8 hemibrains. 'Rest of brain' consists of pons-medulla and midbrain-diencephalon minus substantia nigra. * Brain weights on the kainic-injected (right) side which are significantly different ($P < 0.01$) from the corresponding control (left) side.

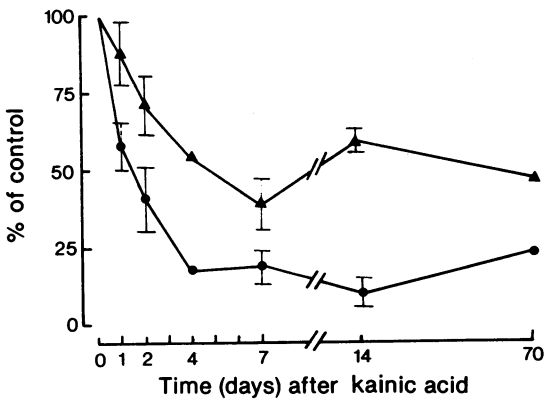

Figure 3 L-Glutamate decarboxylase (GAD) activity in the striatum (●) and substantia nigra (▲) at various times following the injection of kainic acid into the right striatum. Values on the right side are expressed as a percentage of those on the left. Each symbol represents the mean value obtained from 6–9 rats; vertical lines represent s.e. mean. If not shown, the s.e. mean is less than the radius of the symbol. Values (100%) for GAD on the control side were 21.6 ± 0.9 μmol g^{-1} h^{-1} (n = 40) for the striatum and 56.6 ± 1.9 μmol g^{-1} h^{-1} for substantia nigra (n = 29); these control values do not differ significantly from values in non-injected animals (Kelly *et al.*, 1977).

the left. These values were not significantly different ($P > 0.05$).

L-Glutamate decarboxylase

The effects of intrastriatal kainic acid injections on the activity of GAD are depicted in Figure 3. Striatal GAD decreased with a time course similar to that for CAT showing a similar 80–90% decline. Nigral GAD, however, showed a smaller decrease of around 50%. At 10 weeks the GAD activity of the neocortex of six animals was 25.7 ± 1.6 μmol g^{-1} h^{-1} on the right and 27.9 ± 2.4 μmol g^{-1} h^{-1} on the left. These values were not significantly different ($P > 0.05$).

Dopamine

The total amount and the concentration (μg/g) of dopamine in the striatum were unaltered 5 days after the injection of kainic acid but at 2 weeks both values were significantly less than the control (Table 2). At 10 weeks the total amount of dopamine in the striatum was still reduced but, because of the atrophy of the striatum, the dopamine concentration had returned to a level not significantly different from control.

Glutamate

Striatal glutamate concentration was reduced at 5 days and at 10 weeks (Table 3). At 15 weeks striatal glutamate in the kainic acid-treated striatum was 81 ± 5% (n = 3) of the uninjected control ($P < 0.05$).

The reduction of K^+-induced release of glutamate from a striatal slice after kainic acid was larger than the reduction of total glutamate concentrations. Figure 4 shows the K^+-stimulated efflux of glutamate from slices of striatum removed from animals that had received a unilateral intrastriatal injection of kainic acid 15 weeks earlier. The stimulation above baseline in the presence of 52 mM K^+ was reduced by approximately 75% on the kainic acid-injected side. Similar reductions of glutamate release were also observed in striata removed from animals 2 days (58% reduction) and 5 days (75% reduction) after kainic acid injections.

As a measure of the high affinity uptake of glutamate we measured the uptake of [^{14}C]-glutamate into a crude synaptosomal preparation of the striatum suspended in Krebs phosphate buffer containing 1 μM [^{14}C]-glutamate. Table 4 shows the uptake rates for the control and kainic acid-injected striata removed from animals injected with kainic acid 10 weeks before they were killed. The uptake by the kainic

Table 2 Dopamine in striata at various times after an injection of kainic acid into the right striatum

Time after injection	*n*	*Dopamine (ng/striatum)*		*Dopamine (μg/g)*	
		Left	*Right*	*Left*	*Right*
5 days	4	444 ± 29	514 ± 45	10.1 ± 0.8	10.2 ± 0.8
2 weeks	12	444 ± 26	243 ± 21*	9.0 ± 0.5	5.6 ± 0.5*
10 weeks	6	518 ± 44	170 ± 15*	8.7 ± 0.7	7.4 ± 0.5

Values represent mean ± s.e. mean as determined from the number of brains indicated under *n*. * Significantly different ($P < 0.01$) from corresponding control (left) striata which, in turn, were not different from the dopamine concentration in non-injected controls (Kelly *et al.*, 1977).

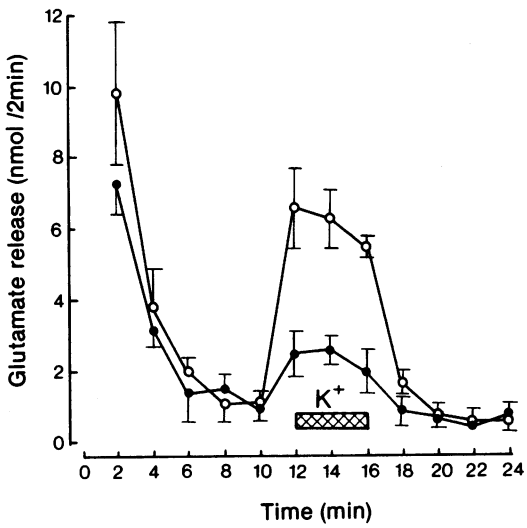

Figure 4 The potassium-induced release of endogenous glutamate from slices of striatum 15 weeks after kainic acid was injected into one striatum. Cross-hatched bar indicates application of potassium. Release was measured from slices of control (○) and kainic acid-treated (●) striata. Each symbol represents the mean amount of glutamate released every 2 min from slices taken from control and lesioned striata ($n = 3$); vertical lines represent s.e. mean. Slice weights were: control 11.1 ± 1.1 mg, injected 11.3 ± 1.1 mg).

acid-treated striatum was decreased by approximately 65%; a similar decrease was observed in animals killed 5 days after a kainic acid injection.

In vitro *effects of kainic acid*

Kainic acid at a concentration of 10^{-4} M did not alter the uptake of glutamate into a crude synaptosomal preparation of the striatum. In absence of kainic acid three samples of striatal homogenate gave an uptake rate of 938 ± 96 nmol g^{-1} h^{-1}, and in the presence of kainic acid (10^{-4} M) the rate for three samples of the same homogenate was 838 ± 23 nmol g^{-1} h^{-1} (89 ± 2%, $P > 0.05$).

Kainic acid at 10^{-4} M did not increase the release of glutamate from a slice of the striatum. For three slices (13.6 ± 0.5 mg) the baseline efflux was 1.24 ± 0.47 nmol per slice/2 min, and in the presence of 10^{-4} M kainic acid was 1.15 ± 0.38 nmol per slice/2 min (not significantly different, $P > 0.05$).

Discussion

The regional brain atrophy and neurochemical changes following injections of kainic acid into the rat striatum resemble, at least in part, those in the

Table 4 Glutamate uptake into sucrose homogenates of sham and kainic acid-injected striata

Solution injected into right striatum	*Left (non-injected)*	*Right (injected)*	*% of non-injected*
Saline	1928 ± 532	1599 ± 301	83 ± 16
Kainic acid	1847 ± 489	639 ± 155*	33 ± 8

Kainic acid or saline (sham) was injected into the right striatum of rats 10 weeks before they were killed. Values (mean ± s.e. mean) represent the striatal uptake of glutamate (nmol g^{-1} h^{-1}) determined from 5 sham and 5 kainic acid-injected animals. * Glutamate uptake in homogenates of kainic-injected striata is significantly different from uptake in sham-injected striata ($P < 0.05$).

Table 3 Glutamate concentrations of brain regions 5 days and 10 weeks after an injection of kainic acid into the right striatum

	5 days			*10 weeks*		
Brain region	*Left (control)*	*Right (kainic)*	*% of control*	*Left (control)*	*Right (kainic)*	*% of control*
Striatum	10.8 ± 0.4	7.6 ± 0.3*	70 ± 3	8.3 ± 0.4	4.9 ± 0.3*	59 ± 4
Neocortex	13.8 ± 0.8	11.2 ± 0.4	81 ± 3	9.1 ± 0.3	8.4 ± 0.3	93 ± 3
Hippocampus	12.4 ± 0.5	11.3 ± 0.8	91 ± 7	9.1 ± 0.1	8.3 ± 0.3	91 ± 3
Pons-medulla	8.4 ± 0.9	8.8 ± 0.6	105 ± 7	6.1 ± 0.2	6.1 ± 0.2	100 ± 3
Midbrain-diencephalon	10.4 ± 1.1	10.0 ± 0.7	96 ± 7	6.8 ± 0.1	6.7 ± 0.2	99 ± 3
Cerebellum	12.1 ± 1.2	12.5 ± 1.0	103 ± 8	8.8 ± 0.1	8.4 ± 0.3	95 ± 3

Values (mean ± s.e. mean) represent the glutamate concentrations (μmol/g wet weight) determined from 5–6 hemibrains. * Glutamate concentrations in the kainic-injected (right) side which are significantly different ($P < 0.01$) from the corresponding control (left) side.

brains of persons with Huntington's chorea. The reduction in weight of the striatum and neocortex of the rat observed in the present experiments is similar to that in the brains of patients with Huntington's disease (Bruyn, 1968).

We have confirmed the previously described decreases in striatal CAT and GAD after kainic acid (Coyle & Schwarcz, 1976; McGeer & McGeer, 1976a) without marked alteration of the dopamine concentration. The values for CAT, GAD and dopamine in the contralateral striatum are similar to those in control rats injected with no neurotoxin (Kelly, Joyce, Minneman & Phillipson, 1977). These neurochemical changes after kainic acid are also seen in the striata of patients who die with Huntington's disease (Bird & Iversen, 1974; McGeer & McGeer, 1976b). Contrasting with the changes occurring in the striatum, cortical CAT and GAD were unchanged 10 weeks after intrastriatal kainic acid. Similarly no significant changes in the CAT and GAD activity in cortical regions have been observed in Huntington's chorea (Bird & Iversen, 1974; McGeer & McGeer, 1976b).

Other results described here suggest the destruction of glutamate neurones by kainic acid. The high affinity glutamate uptake mechanism of the striatum, and the glutamate released from striatal slices by 52 mM K^+ both showed large decrements after kainic acid. The high affinity uptake of glutamate (Logan & Snyder, 1972; Balcar & Johnston, 1972) is believed to be localized in nerve terminals, and in the hippocampus this has been shown by autoradiography (Iversen & Storm-Mathison, 1976). The decrease in the amount of glutamate that could be released by potassium is consistent with degeneration of nerve terminals which use glutamate as a transmitter. In the striatum a corticostriatal pathway appears to use glutamate as a transmitter (Spencer, 1976; Divac, Fonnum & Storm-Mathison, 1977). The loss of striatal markers of glutamate nerve endings and the neocortical atrophy observed in the present experiments suggests degeneration of corticostriatal neurones. Moreover the loss of markers of glutamate nerve endings occurred with a few days and may therefore result from a direct action of kainic acid on the neurones. In contrast, the concentration and content of dopamine in the striatum was normal at five days and reduced at two weeks, which is suggestive of a secondary degeneration of dopaminergic nerve terminals resulting from the loss of striatal interneurones. The total amount of dopamine in the striatum continued to decline between 2 and 10 weeks although at 10 weeks the concentration of dopamine in the severely atrophied striatum was almost normal. It is not possible from our data to know if the reduced dopamine concentration results from a decreased concentration of dopamine in an unaltered number of nerve terminals or whether it represents degeneration of nerve terminals. However, since the number of interneurones available for synaptic contact with dopaminergic terminals is reduced the latter possibility appears more likely.

The mechanism by which kainic acid destroys glutamate nerve terminals is unknown. If glutamate neurones, like other neurones in the CNS, possess autoreceptors it is possible that kainic acid, because of its structural similarity to glutamate, would bind to these and this action could be involved in its neurotoxicity. Whatever the mechanism, it appears not to require use of the high affinity glutamate uptake system as kainic acid did not compete with the high affinity uptake of glutamate, neither did kainic acid (10^{-4} M) cause any release of glutamate from a striatal slice. Probably, therefore, it does not initially depolarize glutamate nerve endings. We have also found that more prolonged exposure (2 h) with higher concentrations (10^{-3} M) produces no release.

There have been few studies of changes in glutamate mechanisms in Huntington's chorea, making comparison with the present results difficult. However, a pronounced reduction of glutamate in the cerebrospinal fluid (CSF) of choreics has been noted (Bruck, Gerstenbrand, Gnad, Gundig and Prosenz, 1967). If CSF glutamate originates predominantly from a transmitter pool rather than from metabolic sources then it is possible that glutamate neurones also degenerate in Huntington's chorea.

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