Detoxification Enzymes Following Intrastriatal Kainic Acid

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A complete explanation of the neurotoxicity that follows kainic acid (KA) injection into the rat striatum is lacking. An assessment of the chronological course after intrastriatal KA injection of the activities of enzymes preferentially concentrated in glia or involved in the detoxification of oxygen metabolites is accomplished. An enhancement of the specific activities of glutathione peroxidase (GP) and catalase is found without an alteration in the specific activity of superoxide dismutase (SOD). There is no increase in the in vivo striatal levels of malondialdehyde, a putative indicator of lipid peroxidation, the expected result of cell membrane damage from oxygen metabolites. Understanding the mechanism and importance of the preferential induction of the activities of the detoxification enzymes will require further study.

KEY WORDS: Kainic acid; striatum; glutathione peroxidase; catalase.

INTRODUCTION

Common forms of human cognitive and motor deterioration, such as dementia of the Alzheimer type (DAT), Huntington’s chorea and Parkinson’s disease, share with aging a preferential pathogenic effect on neurons as compared to glia (1-5). The injection of kainic acid (KA) into the striatum of the albino rat also produces a lesion with preferential damage to neurons (6). Therefore understanding the biochemical mechanisms of KA-induced damage may aid the understanding of human central nervous system (CNS) degenerative diseases.

The mechanism of KA toxicity for striatal neurons is not well understood and is apparently complex in requiring an intact corticostriatal glutamatergic excitatory input (7). However, the sparing of glia suggests that enzymes relatively concentrated in glia as compared to neurons may be of particular interest for biochemical study. In addition, the role of neuronal excitation would support the hypothesis that an overload on neuronal metabolic requirements and/or a disruption of redox reactions leading to free radical oxygen formation may be involved (8). Thus enzymes involved in the detoxification of oxygen metabolites, at least some of which are relatively concentrated in glia, could have an important role in the pathogenesis of KA-induced striatal damage (9).

In this study, the effects of KA induced striatal damage on the striatal levels of malondialdehyde (MDA, a proposed indicator of lipid peroxidation and an end product of free radical reactions with cellular membranes) (10), and the enzyme activities of superoxide dismutase (SOD), glutathione peroxidase (GP), catalase, and glutamine synthetase (GS) are evaluated.

EXPERIMENTAL PROCEDURE

Surgery. Male Sprague-Dawley rats (170-230 grams) are anesthetized with pentobarbital. Using a stereotaxic, 1 μl of a
fresly prepared saline solution of either 5 or 10 nm/µl KA is injected into the right striatum via a 34 gauge cannula.

Accuracy of cannula placement is substantiated by gross inspection of trypan blue staining and contralateral head turning in the postanesthetic hours. The validation of brain damage is further confirmed by the loss of acetylcholinesterase (AChE) activity in the right striatum compared to the left (see Results) (6). Following surgery, animals are provided heat until their own thermoregulatory responses return; otherwise, no special postoperative care is provided. The mortality of the procedure is less than 10%.

Animals are sacrificed 1, 4–5, 14, and 42 days following surgery by decapitation without anesthesia. The brain is immediately excised and rinsed in cold normal saline. Gross brain dissection is carried out on a glass plate supported by ice. The right and left striatum are excised by the method of Glowinski and Iversen (11), frozen on dry ice and stored at −70°C. At the time of chemical assay, homogenates are made from whole right or left striatum using a Brinkmann polytron.

**Chemical Assays.** AChE (acetylcholine acetylhydrolase EC 3.1.1.7) activity is determined by the colorimetric method of Ellman et al. (12) using a commercially purified bovine erythrocyte AChE (Sigma) standard. For the typical group, undamaged (left) striatal mean ± SEM AChE = 40.2 ± 1.4 nm/mg wet wt.

SOD (superoxide oxidoreductase (EC 1.15.1.1)) activity is determined by the method of Cohen et al. (15). On each assay day, a linear relationship between the log concentration of a standard bovine erythrocyte SOD (Sigma) solution and % inhibition of cytochrome c reduction is established and tissue activities determined from their % inhibition effects using this relationship. For the typical group, undamaged (left) striatal mean ± SEM SOD = 146 ± 10 ng/mg, wet wt.

GP (glutathione peroxidase, GSH:H₂O₂ : oxidoreductase, EC 1.11.1.9) activity is measured by the standard assay system described by Splittgerber and Tappel (14). Tissue activities in units of µm/min are determined from a linear standard using commercially purified bovine erythrocyte GP (Sigma). For the typical group, undamaged striatal mean ± SEM GP specific activity = 31.2 ± 1.4 units/gram protein.

Catalase (H₂O₂ : H₂O oxidoreductase, EC 1.11.1.6) activity is determined by titrating residual hydrogen peroxide with peroxidase as demonstrated by Cohen et al. (15). Because of the low activity present in brain tissue, a blank determination using heat inactivation is required for each tissue assayed. From absorbance above blank, catalase activity is calculated in units of µm/min from a standard curve utilizing commercially prepared bovine liver catalase (Sigma). For the typical group, undamaged striatal mean ± SEM catalase specific activity = 1.1 ± 0.2 units/mg protein.

GS (Glutamine synthetase, l-glutamate: ammonia ligase (ADP), EC 6.3.1.2) activity is evaluated by the spectrophotometric method described by Rowe et al. (16). A standard curve utilizing the product, gamma-glutamyl-hydroxamic acid is established on each assay day to determine tissue GS activity (unit = µm/15 mins). Absorbance from tissue assayed without the addition of hydroxylamine is used as blank. Typical group mean ± SEM GS specific activity = 16.2 ± 0.4 units/gram protein.

MDA is measured by the method of Placer et al. (17). To determine in vivo levels, it is necessary to prevent in vitro peroxidation caused during homogenation whether by polytron or motor driven teflon-glass. 30 mM EDTA is found to maximally inhibit peroxidation without interference with the MDA determination. Homogenates are made in an ice cold solution of 0.2 M tris-maleate buffer and 30 mM EDTA, pH 5.9, using a Brinkmann polytron for 5–7 seconds at speed setting 5. Prior to assay, nuclear and cellular debris are removed by centrifugation at 1000 g × 10 min and 0–4°C. On each assay day, a linear regression equation between standard MDA concentrations and absorbance is established and tissue levels calculated from their absorbance and this equation. For the typical group, undamaged striatal mean ± SEM MDA = 138 ± 19 nm/gram wet wt.

Protein determinations are accomplished by the method of Lowry et al. (18) or from absorbance of the peptide bond at 205 nm (19). For both procedures, a bovine serum albumin standard is utilized.

With the exception of protein determination utilizing UV absorbance, KA at tissue concentrations <10⁻³ M, is found not to interfere with the chemical evaluations.

**Statistical Analysis.** To minimize intra as well as inter assay variability, chemical concentrations of the left and right striatum of an individual animal are determined consecutively and the evaluation for the entire group accomplished within a single assay. Differences between the results of chemical assays on left and right striatal homogenates are attributed to the effect of KA. The following definition of percentage concentration loss (% LOSS) is used: %LOSS = 100 x [(Left striatal concentration–Right striatal concentration)/Left striatal concentration]. Since Left and Right striatal wet weights did not significantly differ, % (striatal concentration) LOSS is also the % LOSS of the striatal content from normal. The negative % LOSS = % GAIN.

The statistical significances of % LOSSES or % GAINS are determined by paired (left and right striatal values of an individual animal) t-tests; p-values are 2-tail. In groups of normal or saline injected animals (N = 5), there is no significant % LOSS or GAIN for any of the chemicals reported in this paper.

The statistical analysis is limited by the finding that experiments conducted at different times, albeit using KA injections of the same dose to animals of the same strain and nearly the same weight, nevertheless produce quantitatively variable chemical effects. Although qualitatively similar, the quantitative variability of the effects precludes the use of techniques such as analysis of variance to evaluate the chronological course of KA effects that are found in animals studied at different times. For example, experiments conducted to evaluate at 14 days after KA, changes in GP, find the group mean percentage induction of GP (% GAIN) to vary from 70 to 140%. In the Results section, the mean values for representative groups (N ≥ 5) are presented. Each chemical is evaluated in at least 2 groups damaged at different times. To assure specificity, alterations found following a 10 nm dose are confirmed using a 5 nm dose.

**RESULTS**

KA injections of 5 or 10 nm induce a considerable loss of striatal AChE activity evident at 4–5 days following injection and still present at 6 weeks. A consistent loss of AChE activity is not demonstrable within the first 24 hours. No increase in striatal MDA level is found. In fact, the MDA level decreases concomitantly with an overall loss of protein concentration. Values for representative animal groups are found in Table I.

In contrast to the effects on AChE activity and