

# Kainic Acid Induced Seizures and the GABA System in the Substantia Nigra<sup>1</sup>

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**ABSTRACT.** Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter implicated in the control of generalized seizures induced by various convulsants. A specific anatomical site of action, the substantia nigra (SN), has been shown to be involved in the GABA mediation of seizures. It was the objective of this study to investigate the GABA system in the SN and its response to seizures induced by the neuroexcitant kainic acid (KA). Since there have been conflicting reports of the effects of convulsants on GABA-related measures in some brain regions, with some reporting increases and others showing decreases, one purpose of this investigation was to examine the directionality of changes in GABA content of the SN, along with the magnitude of the effects of KA and the time course over which these effects were manifested. Rats were injected intraperitoneally with either 12.5 mg/kg KA or saline. GABA and protein contents of the SN were measured at 2, 16, 24 and 48 hrs subsequent to injection. The levels of GABA in the SN were found to increase in KA-injected animals over control levels for all time intervals except the 16 hr condition. These results are generally in conflict with those obtained by many researchers using the activity of the GABA synthetic enzyme glutamic acid decarboxylase (GAD) as a measure of GABA levels. It is suggested that GABA levels may actually increase in response to convulsants and serve to negatively feed back to its synthetic enzyme. Therefore, a decrease in GAD activity would not necessarily reflect a decrease in GABA levels. Future research directions include enzyme studies of both GAD and GABA transaminase and their regulation by products and substrates.

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## INTRODUCTION

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system. It is synthesized by the decarboxylation of glutamic acid via the action of the enzyme glutamic acid decarboxylase (GAD). In the brain, GABA is synthesized by shunting glutamate away from the Krebs' cycle at the energetic expense of the cell, supporting the importance of maintaining appropriate levels of the neurotransmitter. After release from the presynaptic terminal, GABA is either taken up into the mitochondria of neurons and glial cells, or is degraded to succinic semialdehyde by the actions of GABA transaminase (GABA-T). Succinic semialdehyde can be oxidized to succinic acid and can reenter the Krebs' cycle, maintaining a self-regenerating synthesis loop (Stryer 1988).

GABA is found in relatively high concentrations in the pituitary gland and in many brain regions, as well as in hypophyseal portal blood. Mugnaini and Oertel (1985) demonstrated the distribution of GABA cell bodies and presynaptic terminals in the rat brain by immunohistochemical localization of the GABA synthetic enzyme GAD. Using these techniques, GABAergic cell bodies and nerve terminals were found in a wide variety of brain structures and regions, with high levels of GAD immunoreactivity measured in the substantia nigra (SN), among other areas. The high GAD content in this structure synthetically provides the GABA contained in terminals within the striatum. The SN has been implicated as the primary site of GABA-mediated control of induced seizures, and in the pathology of Parkinson's disease and Huntington's chorea (Kandel and Schwartz 1985).

The role of GABA in the control of generalized induced seizures in laboratory animals has been well documented. A number of studies have shown that GABA or its analogues such as muscimol, when administered centrally, have controlled seizures induced by GABA-antagonistic convulsants such as bicuculline (Iadorola and Gale 1982) and kainic acid (KA) (Albala et al. 1984). Kainic acid (a glutamate analogue) has been used by many researchers to induce seizures that are thought to mimic the pathological state of epilepsy and other convulsive disorders (e.g., Armstrong et al. 1986, Lothman and Collins 1981).

In the present study, the focus was directed to determination of the relationship between seizures induced by systemic administration of the glutamate analogue kainic acid and the GABA system in the substantia nigra. The relationship between kainic acid and GABA-related measures has been reviewed by Coyle et al. (1978). It was suggested that KA administration consistently reduced GAD levels, and by implication GABA levels, in the brain regions studied. However, this view of GABA reduction in response to convulsants has recently been challenged. Ruth and Seimen (1988) found that GABA concentration in the hippocampus was 19% higher in rats treated with systemic KA than in control rats.

The objective of the present study was to more directly assess the changes in GABA content of the substantia nigra in response to systemic kainic acid administration and subsequent seizure activity. By making a direct estimate of GABA content of the SN, as opposed to the activity of its synthetic enzyme, the magnitude and directionality of any change in GABA content could be examined. It was also of interest to examine the course of these changes over time.

## MATERIALS AND METHODS

**ANIMALS.** Male albino rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN) at a body weight of 200 g and were housed in the Life Sciences Animal Research Facility at Bowling Green State University, where they were maintained and provided

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with food and water ad libitum and subjected to a 12 hr alternating light/dark schedule until the time of the experiment.

Sixteen experimental (kainic acid injected) and sixteen control (saline injected) rats were divided into groups of four for testing at 2, 16, 24, or 48 hrs after injection. This gave a total of four experimental and four control rats per time point studied. The number of animals at each time point was minimized because of the obvious noxious effects of KA administration.

Rats were injected intraperitoneally with 12.5 mg/kg body weight kainic acid (Sigma Chemical Company, St. Louis, MO) or with an equivalent volume of injection vehicle (0.9% saline) and tested for GABA content of the substantia nigra at the appropriate time after injection. The rats were killed by decapitation, brains were quickly removed from the skull, and a 3.0 mm slice of brain was excised using the posterior hypothalamus as the rostral edge and the anterior pons as the caudal edge of the slice. Substantiae nigrae were visualized in the slice by pigmentation, dissected free, weighed and homogenized for use in protein and GABA assays.

**EXTRACTION.** The substantiae nigrae were homogenized in 150  $\mu$ l of ice-cold 0.5M perchloric acid containing 1 mM EDTA. The homogenate was then neutralized with an equal volume of cold  $\text{KHCO}_3$ . After 50  $\mu$ l of homogenate was removed for determination of protein concentration, the remainder was centrifuged at  $16,400 \times g$  for 30 min. The supernatant was used for GABA determination.

**ASSAYS.** Protein content was determined using the method of Lowry et al. (1951).

GABA content in the tissue extracts was determined using the spectrophotofluorimetric method of Okada et al. (1971). Briefly, 100  $\mu$ l of supernatant from each sample was added to 150  $\mu$ l of the following solution: 0.1M tris-HCl buffer (pH 8.6), 3.2 mM  $\alpha$ -ketoglutarate, 0.5 mM NADP, 8 mM 2-mercaptoethanol. Sixty-nine  $\mu$ l of Gabase (2.55 units/ml) was added to each tube. After incubation for 50 min at 22°C, 800  $\mu$ l of 0.1 M tris-HCl buffer (pH 7.6) was added. GABA, Gabase, NADP and  $\alpha$ -ketoglutarate were obtained from Sigma Chemical Company (St. Louis, MO).

Fluorescence of samples and standards was read in a volume of 1 ml using a SPEX 212 FLOUROLOG spectrofluorimeter with excitation wavelength set at 350 nm and emission wavelength set at 460 nm. Intensity of fluorescence, expressed as counts/sec, was measured at one sec intervals for 60 sec, then averaged. After subtraction of the blank from each sample and standard, a standard curve was generated and GABA content of the sample was derived. The blank consisted of 50  $\mu$ l of distilled water, in place of homogenate, added to the assay reagents. Standards contained from 2-100 nmoles GABA and were run in parallel with samples.

Concentrations were calculated and statistical analyses were performed on the data set expressed as nmoles GABA per substantia nigra, since an overall analysis of variance (ANOVA) showed no significant difference between experimental and control groups with respect to protein content. With respect to GABA content, ANOVA was also performed and showed significant differences between the saline-injected and the KA-injected groups. Thus, paired t-tests were performed to find which groups contributed to these significant differences. Since the GABA content of the experimental and control groups did not follow parallel functions, an analysis of trend (method of orthogonal polynomials) (Keppel 1982) was conducted to determine which component of the experimental function was significant.

## RESULTS

Kainic acid did not significantly modify tissue protein content, which ranged from 390  $\mu$ g to 470  $\mu$ g protein per SN (Table 1). Analysis of variance revealed that the KA-injected rats exhibited significantly higher levels of GABA in the substantia nigra than the control rats ( $p < 0.025$ ), and that there were significant differences in GABA content among rats in the different time conditions ( $p < 0.01$ ) (Fig. 1). When collapsed across treatments, the 2 hr and 16 hr rats had higher GABA values than the 24 hr and 48 hr rats. Analysis of trend revealed that the linear component was the significant trend in the function ( $p < 0.05$ ), indicating that the change in GABA content in the SN in response to KA generally followed a linear decrease over time, while still remaining above control values.

TABLE 1

Mean protein content in  $\mu$ g protein/substantia nigra in kainic acid and saline-injected rats. Each value represents the mean  $\pm$ S. E. for 8 substantiae nigrae.

	Hours after injection			
	2	16	24	48
Saline:	420 $\pm$ 70	410 $\pm$ 40	430 $\pm$ 30	470 $\pm$ 50
Kainic Acid:	440 $\pm$ 50	390 $\pm$ 30	410 $\pm$ 50	410 $\pm$ 30

## DISCUSSION

The average protein content found in the substantiae nigrae in this study was consistent with those in other investigations. For example, Gale and Iadorola (1980) found that a single substantia nigra contained approximately 400  $\mu$ g protein as compared to 390  $\mu$ g to 470  $\mu$ g in the present study. The control levels of GABA in the SN found here are generally lower than those reported in other studies, although some values approached those previously reported. The lower values may be explained by the rapid degradation of GABA immediately after death. At any rate, factors which contributed to the degradation would have been consistent throughout the course of this study.

The significant increase in the GABA content of the SN seen 2 hr following KA injection in the present study is not consistent with the findings of some researchers. This short time interval for the initial manifestation of KA effects has not been previously described. Several studies included measures taken many hours or days after treatment. The direction of the effect is also different from that described in previous studies. Coyle (1983), when using GAD activity as a measure of GABA levels, found decreases in response to injected convulsants. The contradictory results found here may be explained by intracellular mobilization of the inhibitory neurotransmitter in response to the neuroexcitant. There have been documented increases in the metabolic activity of the SN in response to KA (Lothman and Collins 1981), suggesting a compensatory mechanism. That is, it may be that GABA is being synthesized and released in large amounts to compensate for the excitatory effects

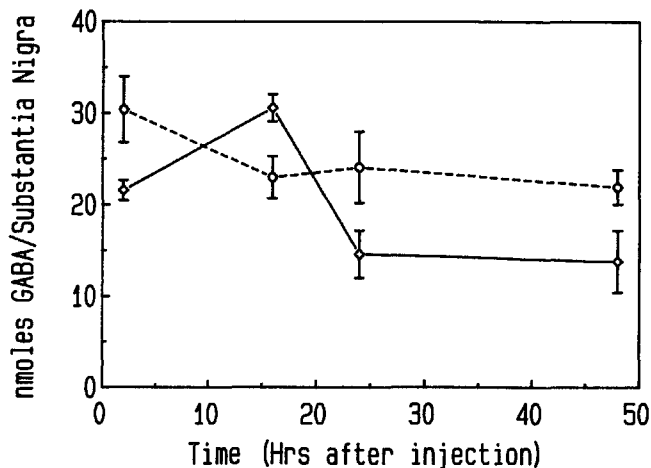


FIGURE 1. Mean GABA content in substantiae nigrae of kainic acid injected (dashed line) and saline injected (solid line) rats.

of KA. Following this line of reasoning, the establishment of a plateau of GABA content in the treated rats at a concentration well above control values may indicate a longer term mechanism by which the system protects itself from subsequent exposures to the excitotoxin.

A previously described consistently irreversible decrease of GABA in response to convulsants (Coyle 1983) is not confirmed by the present study. The decreases in GAD activity found by other researchers may not accurately indicate GABA availability. If oversynthesis of GABA serves to negatively regulate its own synthesis, it is conceivable that an increase in GABA might decrease the activity of its synthetic enzyme.

The results of this study are in agreement with those of Ruth and Seimen (1988), who found GABA concentration in the hippocampi of rats treated with systemic KA to be higher than that in control rats. As in the present study, these effects were observed at relatively short time intervals after injection of KA, indicating that they may be of shorter latency and duration than the previously indicated results. It is clear that this problem deserves closer examination to resolve apparent discrepancies. Histological and localization studies may reveal the source of the neurotransmitter and related substances which mediate the action of KA in the brain, along with an idea of any degeneration which may occur in response to the excitotoxin. Enzyme studies may shed more light on the effects of systemic KA on the GABA system in the substantia nigra. Changes in GAD and GABA-T levels and activity would indicate a mechanism whereby GABA levels may be altered by the treatment. The excitotoxin could act at the level of the synthetic or degradative enzyme, affecting the final measure of GABA content in the substantia nigra.

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