ALTERED GAMMA-AMINOBUTYRIC ACID METABOLISM EARLY IN THE POSTIRRADIATION RESPONSE OF THE RAT

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The metabolism of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) was studied in rats subjected to 10,000 or 20,000 rads of pulsed mixed gamma-neutron radiation. Whole brain GABA levels were found to rise above control levels by 20 minutes following 10,000 rads and as early as 1 minute following 20,000 rads. L-glutamic acid decarboxylase activity was also observed to rise as early as 1 minute following either dose of radiation. These results are consistent with the hypothesis that GABA is involved in the etiology of the transient central nervous system disorders (apathy, disorientation, ataxia) observed in rats shortly after high doses of ionizing radiation.

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FOREWORD
(Nontechnical summary)

A variety of animals exposed to ionizing radiation doses in excess of approximately 2000 rads exhibit neurologic symptoms including apathy, disorientation, ataxia, hyperexcitability, convulsions, coma and early death. Since the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) has been implicated in several neurologic disorders, its metabolism in rats during the first 20 minutes after exposure to 10,000 or 20,000 rads pulsed mixed gamma-neutron radiation has been investigated. Within minutes after such doses the rat becomes temporarily disoriented and ataxic. Whole brain GABA levels rise significantly by 20 minutes after 10,000 rads. The rise is even more pronounced and begins as early as 1 minute after 20,000 rads. L-glutamic acid decarboxylase, the enzyme which synthesizes GABA, increases in activity as early as 1 minute after either dose of radiation.

Since rats become disoriented and much less active for several minutes after these doses of radiation and since an increased GABA level is known to decrease activity, GABA may be involved in the postirradiation neurologic symptoms of rats. However, the data presented in this report are in contrast with the general decrease in GABA observed in some convulsive disorders. Since convulsions are observed in rats within hours after higher doses of radiation, the metabolism of GABA during radiation-induced seizures should be investigated further.

ABSTRACT

The metabolism of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) was studied in rats subjected to 10,000 or 20,000 rads of pulsed mixed gamma-neutron radiation. Whole brain GABA levels were found to rise above control levels by 20 minutes following 10,000 rads and as early as 1 minute following 20,000 rads. L-glutamic acid decarboxylase activity was also observed to rise as early as 1 minute following either dose of radiation. These results are consistent with the hypothesis that GABA is involved in the etiology of the transient central nervous system disorders (apathy, disorientation, ataxia) observed in rats shortly after high doses of ionizing radiation.

I. INTRODUCTION

Doses of ionizing radiation in excess of approximately 2000 rads produce a variety of clinical and behavioral symptoms cumulatively classified as the central nervous system radiation syndrome. These responses include, over a period of time and in various animal species, apathy, disorientation, ataxia, hyperexcitability, convulsions, incapacitation and early death.[1,3,11-13] The rapid, transient, and species specific nature of many aspects of the CNS syndrome suggests a possible neurochemical involvement. The inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and L-glutamic decarboxylase (GAD), the enzyme which synthesizes GABA, have been implicated in the neurologic dysfunctions associated with epilepsy[14] and exposure to high pressure oxygen or hydrazides.[16,17] However, no studies have been conducted to investigate the effects on GABA metabolism of doses of ionizing radiation sufficiently high to induce neurologic changes. The few studies of radiation effects on GABA metabolism utilized the dose range of 400 to 1000 rads of low dose rate ^{60}Co gamma rays or x rays.[5,6,8]

The purpose of this study was to investigate the possible involvement of an altered GABA metabolism in the neurologic radiation syndrome. In this initial report we present data on the altered GABA metabolism in the rat during the first 20 minutes following exposure to 10,000- or 20,000-rad doses of pulsed mixed gamma-neutron radiation.

II. MATERIALS AND METHODS

Irradiation. Male Sprague-Dawley rats weighing 225-275 grams were exposed in pairs to either 10,000 or 20,000 rads pulsed mixed gamma-neutron radiation from the AFRRI-TRIGA reactor. The rats, which were irradiated while constrained in an

automatic guillotine device, were decapitated at either 1, 5, or 20 minutes postirradiation. The heads were allowed to fall directly into liquid nitrogen for rapid tissue freezing. A detailed description of the radiation source and the automatic guillotine device has been reported.[2] Rat heads were stored frozen (-80°C) until time of assay. Control rats were treated in the same manner as above and were decapitated approximately 10 minutes after a sham pulse of radia ..

GABA assay. Rapid freezing of the rat heads in liquid nitrogen usually resulted in bilateral splitting of the skull and brain facilitating removal of symmetrical half-brain portions (600-700 mg) of frozen tissue. One of these portions was removed and GABA extracted as follows. A 10 percent (w/v) homogenate in 80 percent ethanol was centrifuged 20 minutes at 5000 x g, with the residue being washed twice with 80 percent ethanol. The combined supernates were evaporated under reduced pressure at 60°C overnight and the residue was resuspended in H_2O. This aqueous extract was then clarified by washing with $CHCl_3$. GABA concentrations were then determined enzymatically by the method of Scott and Jakoby.[10] Pseudomonas fluorescens A.T.C.C. 13430 was obtained from International Mining and Chemical Corporation, Skokie, Illinois.

GAD assay. A 12.5 percent homogenate of each remaining half-brain was formed in a solution containing 50 mM potassium phosphate pH 6.8 and 1 mM EDTA potassium salt. After centrifugation for 20 minutes at 35,000 x g, GAD activity in the supernate was determined by an isotopic assay similar to that employed by Roberts and Simonsen[9] as modified by Wilson et al.[15] In an incubation volume of 0.05 ml, final concentrations were 50 mM potassium phosphate pH 6.8, 1 mM EDTA potassium salt, 0.5 mM

pyridoxal phosphate, 0.5 percent Triton X-100, 1 mM β-mercaptoethanol, 1 mM GABA, and 5 mM L-glutamate (1-^{14}C) specific activity 1.25 mCi/mmole (obtained from CalAtomic, Inc., Los Angeles, California). The $^{14}CO_2$ produced was collected and counted with 90 percent efficiency using a Nuclear-Chicago Mark II liquid scintillation spectrometer. Protein content of the extracts was determined by the method of Lowry et al.[7]

Statistics. Data are presented as the average ± standard error, with the number of samples in parentheses. Significance testing was performed using the two-tailed Student's 't' distribution. Significantly different indicates $p < 0.05$.

III. RESULTS

Clinical symptoms. Immediately following exposure to 20,000 rads the rats showed signs of apathy and extreme disorientation, some experiencing obvious loss of the righting reflex. These severe symptoms abated within the first few minutes but the rats remained ataxic and lethargic throughout the first 20 minutes postexposure. After 10,000 rads similar but somewhat less severe symptoms were observed.

GABA levels. Following 10,000 rads, GABA levels were found to rise during the first 20 minutes postirradiation (Figure 1). There was a trend towards an increased GABA level as early as 5 minutes, and at 20 minutes GABA levels had risen to 2.41 ± .05(8) μmoles/g. This was a significant increase over the value obtained in the sham irradiated group of 2.24 ± .05(15) μmoles/g. Following 20,000 rads, the increase in GABA was more pronounced, reaching 2.44 ± .08(6) μmoles/g by 1 minute postirradiation, and 2.51 ± .14(6) μmoles/g by 20 minutes.

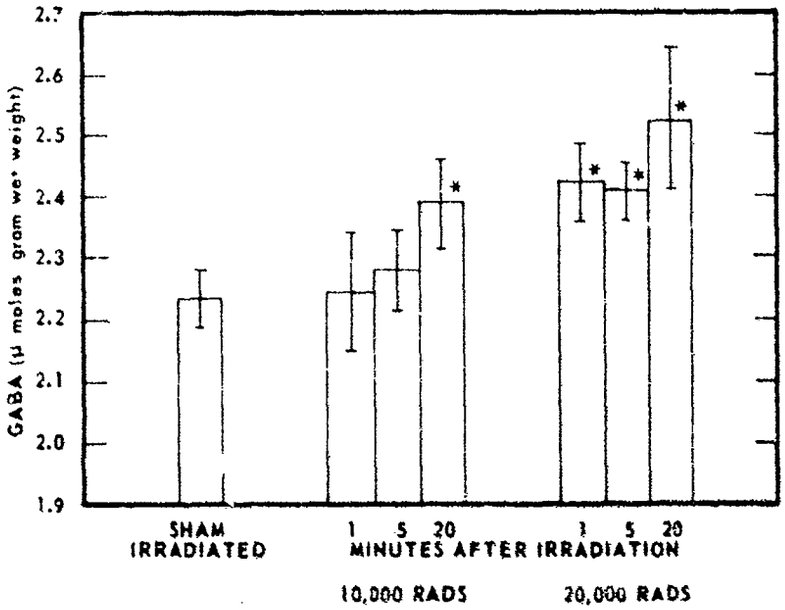

Figure 1. GABA levels in rat brain 1, 5 and 20 minutes after 10,000 and 20,000 rads pulsed mixed gamma-neutron radiation. The sham level is the average for 15 rats; other levels are the average for six to eight rats. Bars indicate standard error ranges (* indicates significantly different from control).

GAD activity. GAD activity also increased, though not significantly, after irradiation (Figure 2). After both 10,000 and 20,000 rads GAD activity was elevated as early as 1 minute postirradiation. At 20 minutes this increase had persisted in the rats receiving 10,000 rads, but returned to control levels in those receiving 20,000 rads.

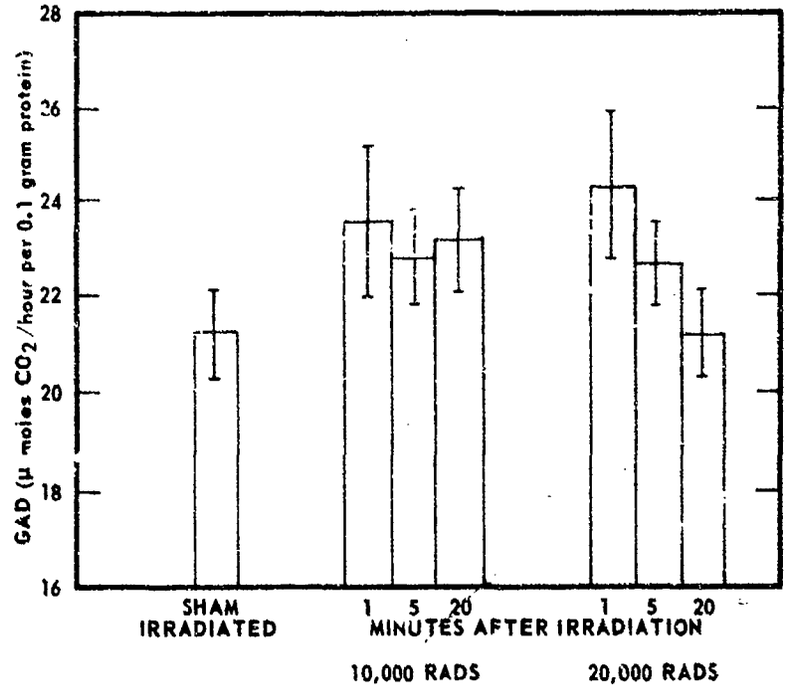

Figure 2. GAD activity in rat brain 1, 5 and 20 minutes after 10,000 and 20,000 rads pulsed mixed gamma-neutron radiation. The sham level is the average for 15 rats; other levels are the average for five to eight rats. Bars indicate standard error ranges.

IV. DISCUSSION

During the first 20 minutes following doses of 10,000 or 20,000 rads, the rats showed apathy, disorientation and ataxia. The data presented in this report indicate that a significant increase of whole brain GABA levels by approximately 10 percent, accompanied by an increase in GAD activity, occurs in conjunction with this state observed early in the postirradiation response of the rat. This trend toward an increase in both GABA levels and GAD activity parallels that reported by others[5,6,8] in mice, rabbits and rats from hours to days after exposure to 400 to 1000 rads.

Increases in brain GABA levels of approximately the same magnitude as were found in this study have also been reported in rats breathing hypoxic gas mixtures

under conditions which result in impaired oxidative metabolism.[18] These findings are particularly interesting when considered in parallel to those of Cohan et. al.[4] who investigated the effect of doses of high energy electrons upon respiration of cerebral mitochondria in rats. They found that at 5 minutes following 20,000 rads respiratory control had decreased below control values, remaining low at 1 hour, and finally returning to control levels by 4 hours postexposure. However, they observed no effect on respiratory control at 5 minutes after 10,000 rads. Therefore the pattern of changes in GABA levels and in oxidative metabolism following these high doses of ionizing radiation is parallel to the changes induced by breathing hypoxic gas mixtures. This suggests that the changes observed early in the postirradiation response of the rat may be due to a radiation-induced state of hypoxia.

Rats begin to show signs of severe tremors and convulsions within 1 to 4 hours after radiation doses above approximately 20,000 rads. Since the impaired respiratory control observed by Cohan et. al.[4] was transient, returning to control levels by 1 to 4 hours postexposure, it is possible that GABA levels may respond similarly. In fact impaired GABA metabolism at this time may be involved in the onset of radiation-induced seizures. Further investigation of this possibility is necessary.

REFERENCES

1. Bond, V. P., Fliedner, T. M. and Archambeau, J. O. Mammalian Radiation Lethality, p. 276. New York and London, Academic Press, 1965.

2. Catravas, G. N. and McHale, C. G. Effects of mixed gamma-neutron radiation on the activity of brain enzymes involved in neurotransmitter metabolism. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR73-17, 1973.

3. Chaput, R. L. and Berardo, P. A. Increased brain radioresistance after supralethal irradiation. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR73-7, 1973.

4. Cohan, S. L., Abbott, J. R. and Catravas, G. N. The effect of ionizing radiation upon mitochondria of the central nervous system. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR72-17, 1972.

5. Jovanovic, M. and Svecenski, N. Effect of high dosage of ^{60}Co gamma rays on gamma aminobutyric and glutamic acid content in brain tissue in mice. Strahlentherapie 125:588-590, 1964.

6. La Grutta, G., Abbadessa, S., Avellone, S., Natale, E., Di Gregorio, I., Montalbano, M. E., Ortolani, E. A. and Zagami, M. T. Azione delle radiazioni ionizzanti su alcune attivita biochimiche e bioelettriche del sistema nervoso centrale. Arch. Fisiol. 65:271-312, 1967.

7. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275, 1951.

8. Nishi, H., Sudow, I. and Ohtsu, T. The free amino acids in rat brain affected by X-irradiation. Yokohama Med. Bul. 10:120-121, 1959.

9. Roberts, E. and Simonsen, D. G. Some properties of L-glutamic decarboxylase in mouse brain. Biochem. Pharmacol. 12:113-134, 1963.

10. Scott, E. M. and Jakoby, W. B. Soluble γ-aminobutyric-glutamic transaminase from Pseudomonas fluorescens. J. Biol. Chem. 234:932-940, 1959.

11. Thorp, J. W. Head shielding protection for beagles exposed to supralethal doses of pulsed mixed gamma-neutron radiations. Aerospace Medicine 40:759-761, 1969.

12. Thorp, J. W., Chaput, R. L. and Kovacic, R. T. Performance of miniature pigs after partial body irradiation. Aerospace Medicine 41:379-382, 1970.

13. Thorp, J. W. and Young, R. W. Monkey performance after partial body irradiation. Aerospace Medicine 42:503-507, 1971.

14. Tower, D. B. Neurochemical mechanisms. In: Basic Mechanisms of the Epilepsies, Jasper, H. H., Ward, A. A., Jr. and Pope, A., editors, pp. 611-646. Boston, Massachusetts, Little, Brown and Company, 1969.

15. Wilson, S. H., Schrier, B. K., Farber, J. L., Thompson, E. J., Rosenberg, R. N., Blume, A. J. and Nirenberg, M. W. Markers for gene expression in cultured cells from the nervous system. J. Biol. Chem. 247:3159-3169, 1972.

16. Wood, J. D. and Peesker, S. J. The effect on GABA metabolism in brain of isonicotinic acid hydrazide and pyridoxine as a function of time after administration. J. Neurochem. 19:1527-1537, 1972.

17. Wood, J. D., Watson, W. J. and Ducker, A. J. Oxygen poisoning in various mammalian species and the possible role of gamma-aminobutyric acid metabolism. J. Neurochem. 14:1067-1074, 1967.

18. Wood, J. D., Watson, W. J. and Ducker, A. J. The effect of hypoxia on brain γ-aminobutyric acid levels. J. Neurochem. 15:603-608, 1968.