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GAMMA-AMINOBUTYRIC ACID METABOLISM AND RADIATION-INDUCED SEIZURES

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FOREWORD
(Nontechnical summary)

The compound gamma-aminobutyric acid (GABA) is known to exist primarily in the central nervous system and to act as an important regulatory agent in controlling brain activity. Alterations in the metabolism of GABA play a role in the onset of convulsions resulting from a variety of stresses. Earlier work at this laboratory studied the biological effects of supralethal doses of ionizing radiation. Such exposures were shown to alter brain GABA metabolism in rats and, therefore, suggest that these alterations may play a role in the neurologic radiation syndrome. Miniature swine exposed to supralethal doses of ionizing radiation exhibited tremors and convulsions shortly after exposure. The purpose of this study was to determine if radiation-induced alterations in brain GABA metabolism occur in miniature swine and to elucidate the role of any observed changes in the convulsions shown by this species after radiation. Such information could lead to possible pharmacological protection against radiation-induced seizures.

Seven brain regions were chosen and the concentration of GABA determined in each. Additionally the activity of the enzyme which synthesizes GABA and provides sensitive control of its supply, L-glutamic acid decarboxylase (GAD), was determined in each brain region. These measurements indicated that the hypothalamus and midbrain had the greatest capacity to synthesize GABA and that, correspondingly, GABA concentrations were highest in these areas. The other areas examined, in decreasing order of GABA concentration, were the caudate nucleus, thalamus, hippocampus, cerebral cortex, and cerebellum. These results agreed with reported distributions in other species.

Radiation doses of 10,000 rads of high-energy electrons were delivered to the brains of 12 miniature swine, resulting in tremors and convulsions in each of the animals within seconds after radiation. These symptoms terminated 2 to 5 minutes later. Animals were euthanatized at various times after irradiation, and regional brain GABA concentrations and GAD activities were determined. The following findings were made: (1) GAD activity in the thalamus was significantly increased by 32 percent at 4 minutes but returned to normal levels at 30 minutes and remained so at 3 hours after irradiation. In no other brain region was GAD activity significantly altered after irradiation. (2) GABA concentrations were not significantly altered at 4 minutes after irradiation in any brain region examined. The largest changes observed were a 9 percent rise and a 13 percent decrease in GABA concentration in the hypothalamus and thalamus, respectively. (3) A small decrease in GABA concentration occurred by 3 hours postirradiation in four brain areas, the caudate nucleus, thalamus, cerebral cortex and cerebellum.

These alterations may have some influence on the neurologic symptoms exhibited by miniature swine after irradiation. However, inhibition of GAD, accompanied by significant reduction in GABA concentrations, is generally associated with convulsive disorders. Therefore the results of this study are inconsistent with the hypothesis that alterations in brain GABA metabolism are involved in the onset or course of radiation-induced seizures. The etiology of these seizures and of the associated behavioral incapacitation observed in other species remains a question requiring extensive investigation.

ABSTRACT

Miniature swine convulsed spontaneously within 30 seconds after a 10,000-rad dose of 30 MeV electrons was delivered to the brain. The seizures ended 2 to 5 minutes later. Since alterations in gamma-aminobutyric acid (GABA) metabolism occur in and may be an underlying cause of a variety of convulsive disorders, regional brain concentrations of GABA and activities of L-glutamic acid decarboxylase (E. C. 4.1.1.15) (GAD) were determined in control and irradiated miniature swine. The hypothalamus and midbrain had the highest GABA concentration and GAD activity, followed, in decreasing order of GABA concentration, by the caudate nucleus, thalamus, hippocampus, cerebral cortex and cerebellum. At 4 minutes after irradiation, GABA concentrations did not change significantly in any brain region examined; the largest apparent change was a decline from $2.31 \pm .13$ to $2.04 \pm .11$ μmoles/g wet weight in the thalamus. Concurrently, GAD activity in the thalamus increased significantly from 238 ± 7 to 315 ± 29 μmoles/h per g protein. Since inhibition of GAD and significant reduction in GABA concentrations are generally associated with convulsive disorders, these data are not consistent with the hypothesis that alterations in brain GABA metabolism play a role in the etiology or course of radiation-induced seizures in miniature swine.

I. INTRODUCTION

Miniature swine exhibit tremors and convulsions after receiving rapidly delivered doses of ionizing radiation. These seizures occur within seconds to minutes after radiation doses in excess of approximately 2000 rads.[2] This response, which has not been reported in rodents, dogs or monkeys, seems to be unique to miniature swine. The rapid onset of these seizures and the fact that irradiation of only the brain produces symptoms identical to those induced by whole-body irradiation,[11] indicate a probable central nervous system involvement.

The inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and the enzyme which synthesizes GABA, L-glutamic acid decarboxylase (E. C. 4. 1. 1. 15) (GAD), play an important role in regulation of neuronal activity in brain.[12] Alterations in brain concentrations of GABA or inhibition of GAD occur in a variety of convulsive disorders.[14,15] In particular, seizure activity is induced by exposure to high-pressure oxygen, a stress known to induce altered brain GABA concentrations[15] and which is thought to affect basic mechanisms similar to those involved in radiation exposure.[7]

Ionizing radiation has previously been shown to induce changes in brain GABA concentrations in rats.[3,4] The reported increase in brain GABA concentrations during the first 20 minutes after 10, 000- and 20,000-rad doses was accompanied by signs of ataxia, lethargy and somnolence, while the subsequent decline at 1 to 3 hours after 20, 000-rad doses occurred during the time in which convulsive activity became apparent at slightly higher doses. These findings suggested that alterations in brain GABA levels may play a role in the seizure patterns seen after irradiation.

Therefore, this study was designed to determine if the unique postirradiation response exhibited by miniature swine is related to postirradiation alterations in brain GABA metabolism different from those observed in rats. In this report, data are presented on the distribution of GABA and GAD in seven miniature swine brain areas and on the regional changes induced by a 10,000-rad dose of ionizing radiation. Particular attention was paid to the time interval immediately following irradiation during which convulsive activity is most apparent. In addition, observations were made and data obtained at 30 minutes and 3 hours postirradiation.

II. METHODS

Animals. The animals were young adult miniature swine weighing 20 to 30 kg. Since no differences in biochemical parameters or postirradiation responses were noted, males, females and barrows of the Pitman-Moore strain (Vitavet, Inc., Marion, Indiana) or of mixed breed (procured locally) were used.

Radiation. The AFRRI electron linear accelerator (LINAC) was used as the radiation source. The experimental animals were restrained in a Plexiglas box and positioned so that a dose of 10,000 ± 1,000 rads of 30 MeV electrons was delivered to the brain. The average dose rate was approximately 10^7 rads/minute, and the total exposure time about 60 msec. Only a minimal dose was delivered to the remainder of the body. The details of the LINAC radiation field, dosimetry techniques and depth-dose distribution patterns within the whole animal have been previously reported.[2]

Brain dissection. After completion of the radiation exposure, the animals were removed from the restraining boxes. They were euthanatized either as soon as possible (4 minutes), 30 minutes or 3 hours postexposure by jugular exsanguination or

electrocution, and then decapitated. The top of the skull was then rapidly removed and the whole brain excised. Seven brain regions (thalamus, hypothalamus, midbrain, hippocampus, caudate nucleus, cerebellum, and cerebral cortex) were dissected out (Figure 1) and immersed in liquid nitrogen. The dissection procedure was completed within 10 to 20 minutes after euthanasia. Frozen brain tissues were stored at -80°C until time of assay

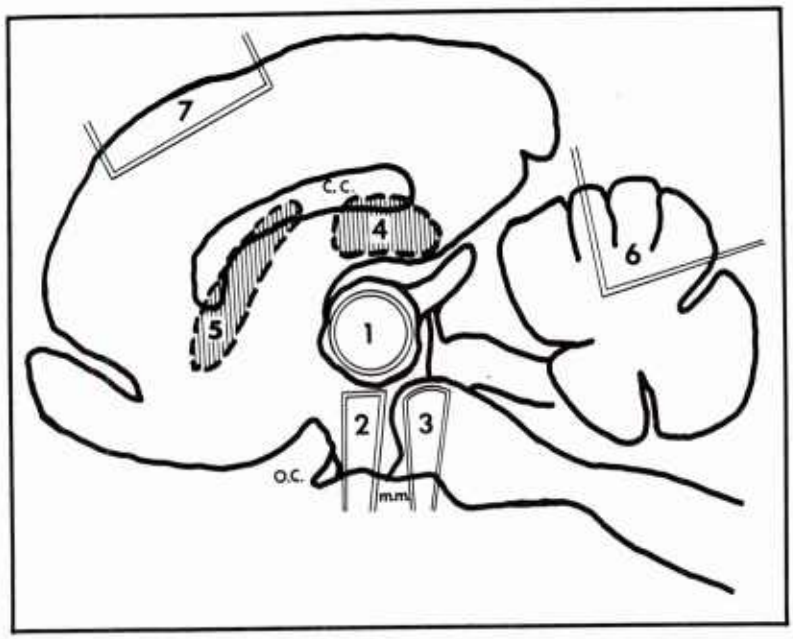

Figure 1. Medial view of the miniature swine brain. Immediately upon removal of the whole brain from the skull, the cerebral hemispheres were separated and the following gross regions excised: (1) thalamus, (2) hypothalamus, (3) midbrain, (4) hippocampus, (5) caudate nucleus, (6) cerebellum, (7) cerebral cortex. Shaded areas represent lateral dissected regions. Except for cerebral cortex, regions of both hemispheres were included in the assays. Approximately 1 g of tissue was obtained from each region. Abbreviations: c.c., corpus callosum; m.m., medial mammillary bodies; o.c., optic chiasm.

GABA and GAD assays. GABA concentrations were determined enzymatically by the method of Scott and Jakoby[10] and GAD activity was determined by an isotopic

assay similar to that employed by Roberts and Simonsen[9] as modified by Wilson et al.[13] The details of the tissue homogenization, extraction and assay procedures have been previously described,[3] except that the glutamic acid concentration in the GAD assay incubation mixture was increased from 5 to 40 mM. Additionally, the GABA homogenate for some samples was prepared by diluting a portion of the ice-cold GAD homogenate in 4 volumes of 95 percent (v/v) ethanol. This technique permitted assay of both GABA concentration and GAD activity in the same tissue sample, and yielded analytical results identical with the previously described method. Measurements of total protein content of the GAD extracts were made using an automated modification (Catravas, G. N., in preparation) of the procedure of Lowry et al.[6]

III. RESULTS

GABA and GAD in control swine brain. The two methods of euthanatizing the animals, jugular exsanguination or electrocution, were chosen because neither required pharmacological involvement and each resulted in rapid demise (15 to 30 seconds). However, each method subjected the animal to different physiological stresses which may have affected GABA concentrations or GAD activity. Postmortem increases in GABA concentrations were minimized by performing the dissections as rapidly and systematically as possible, and comparison of the data (Tables I and II) indicates that animals euthanatized by the two different methods yield fairly reproducible regional GABA concentrations and GAD activity in most brain regions. No significant ($p<0.05$) differences in GAD activity were noted in any regions, and only in the hippocampus was there a significant difference in GABA concentration.

Table I. Regional Brain Gamma-Aminobutyric Acid Concentration in Miniature Swine Euthanatized by Two Methods. Data represent the mean ± S. E. M. for five animals. Method 1 and Method 2 indicate animals euthanatized by jugular exsanguination and electrocution, respectively. Dissection and assay procedures were identical for the two groups (see Methods).

Brain region	GABA concentration (μmole/g wet weight)	
	Method 1	Method 2
Hypothalamus	3.21 ± .21	3.80 ± .20
Midbrain	3.05 ± .25	2.86 ± .22
Caudate Nucleus	2.70 ± .11	2.85 ± .25
Thalamus	2.33 ± .24	2.29 ± .14
Hippocampus*	2.00 ± .11	1.50 ± .19
Cerebral cortex	1.56 ± .10	1.74 ± .07
Cerebellum	1.28 ± .08	1.50 ± .03

* Significant difference ($p < 0.05$)

Table II. Regional Brain L-Glutamic Acid Decarboxylase Activity in Miniature Swine Euthanatized by Two Methods. See Table I for details.

Brain region	GAD activity (μmole/h per g protein)	
	Method 1	Method 2
Hypothalamus	486 ± 30	416 ± 29
Midbrain	434 ± 24	409 ± 31
Caudate nucleus	271 ± 19	304 ± 27
Thalamus	229 ± 8	247 ± 10
Hippocampus	190 ± 7	208 ± 25
Cerebral cortex	231 ± 20	208 ± 7
Cerebellum	237 ± 12	240 ± 22

Postirradiation course. All of the miniature swine receiving brain doses of 10,000 rads developed generalized convulsions within 30 seconds after irradiation.

The seizures abated within 2 to 5 minutes. The animals remained comatose and demonstrated labored irregular breathing for the ensuing 15 to 40 minutes, at which time the animals regained consciousness but were ataxic and disoriented for the rest of the 3-hour observation period. No deaths occurred during this time. Chaput and Berardo[2] reported median survival times of 5 hours to 21 days for similarly irradiated miniature swine.

GABA and GAD after radiation. Eight miniature swine were euthanatized 4 minutes after receiving brain doses of 10,000 rads. Postirradiation seizures occurred in three of these animals until the time of death but had abated in the remaining five. An additional two animals were euthanatized at 30 minutes and 3 hours postirradiation to elucidate the time course of any metabolic alterations observed. (These data are presented collectively in Tables III and IV.) The following changes in GABA concentrations and GAD activity after radiation were found: (1) GAD activity in the thalamus was significantly ($p < 0.05$) increased by 32 percent from 238 ± 7 to 315 ± 29 μmoles/h per g protein at 4 minutes but returned to control levels at 30 minutes and remained so at 3 hours postirradiation. In no other brain region was GAD activity significantly ($p < 0.05$) altered after radiation; (2) GABA concentrations were not significantly ($p < 0.05$) altered at 4 minutes after radiation in any brain region examined. The largest apparent changes in GABA concentrations which occurred were a 9 percent increase from 3.54 ± .17 to 3.85 ± .11 μmoles/g wet weight in the hypothalamus and a 13 percent decrease from 2.31 ± .13 to 2.04 ± .11 μmoles/g wet weight in the thalamus; (3) an apparent decrease in GABA concentration occurred by 3 hours postexposure in the caudate nucleus, thalamus, cerebral cortex and cerebellum. However, only in the

Table III. Regional Gamma-Aminobutyric Acid Concentration in Miniature Swine Brain after a 10,000-Rad Dose of Ionizing Radiation. GABA concentrations are expressed as μmoles of GABA per gram wet tissue weight, and represent the mean value obtained from 10 control animals, 8 animals euthanatized at 4 min postexposure and 2 each at 30 min and 3 h postexposure. The S. E. M. for the control and 4-min groups is shown in parentheses. Animals were euthanatized by either electrocution or jugular exsanguination. Postirradiation changes are essentially the same when animals euthanatized by either method are referred to appropriate controls (Tables I and II).

Brain region	Control	4 minutes	30 minutes	3 hours
Hypothalamus	3.54 (0.17)	3.85 (0.11)	3.26	3.76
Midbrain	2.95 (0.16)	2.83 (0.15)	2.94	2.86
Caudate nucleus	2.77 (0.13)	2.89 (0.15)	2.66	2.28
Thalamus	2.31 (0.13)	2.04 (0.11)	2.28	1.86
Hippocampus	1.75 (0.13)	1.57 (0.15)	1.93	1.91
Cerebral cortex	1.65 (0.07)	1.54 (0.08)	1.70	1.34
Cerebellum	1.39 (0.06)	1.41 (0.06)	1.49	0.97

Table IV. Regional L-Glutamic Acid Decarboxylase Activity in Miniature Swine Brain after a 10,000-Rad Dose of Ionizing Radiation. GAD activity is expressed as μmoles of L-glutamic acid decarboxylated per hour per gram protein. For further details see Table III.

Brain Region	Control	4 minutes	30 minutes	3 hours
Hypothalamus	451 (23)	434 (41)	400	438
Midbrain	421 (19)	375 (22)	368	382
Caudate nucleus	287 (16)	306 (23)	242	230
Thalamus	238 (7)	315 (29)*	239	230
Hippocampus	199 (13)	222 (14)	200	215
Cerebral cortex	219 (11)	254 (11)	264	242
Cerebellum	238 (12)	232 (12)	229	203

* Significant difference ($p < 0.05$)

caudate nucleus and cerebellum did these changes appear to result from impaired GAD activity.

IV. DISCUSSION

Regional GABA concentrations and GAD activities were fairly resistant to high doses of ionizing radiation. The immediate postirradiation changes observed, although statistically significant in one brain region, do not appear to be of physiological significance when considered in light of the alterations induced by other convulsive agents. For example, Wood et al.[15] reported reductions in brain GABA concentrations in the guinea pig, rat, hamster, rabbit and mouse by approximately 6 to 38 percent of control values after exposure to high-pressure oxygen, and showed that the magnitude of the decrease was related to species susceptibility to induced seizures. Inhibition of GAD by approximately 80 percent was also reported[15] in the above species during *in vitro* exposure of brain homogenates to high-pressure oxygen, and in the rat, by 25 percent following *in vivo* exposure to high-pressure oxygen.[16]

Our data therefore indicate that alterations in brain GABA metabolism do not accompany radiation-induced seizures in miniature swine. Two factors, however, must be considered when interpreting *in vivo* relationships from our *in vitro* data. First, during the 10- to 20-minute dissection procedure employed, postmortem hypoxia probably resulted in increased GABA concentrations. The postmortem increase in GABA concentration has been shown to be about 20 percent in excised brain tissue kept at room temperature for 30 minutes.[5] Dissections were conducted systematically in all animals to eliminate differences due to this effect, but other unknown postmortem effects may have masked any radiation-induced alterations. Second, although inhibition of GAD has been demonstrated in rats treated *in vivo* with high-pressure oxygen or other convulsive agents through the use of an *in vitro* assay,[16] the

use of an in vitro assay for GAD activity must be considered. If ionizing radiation were to affect GAD through cofactor depletion or SH-group oxidation, the use of an assay reaction mixture containing added cofactor and an SH-protecting agent could tend to eliminate inhibitory influences. Data indicating the absence of significant inhibition must therefore be interpreted with great care.

It is interesting to speculate that the significant decrease in GABA concentration noted in the hippocampus of control animals euthanatized by electrocution may have been due to depletion of neurotransmitter supply as a direct result of electroplectic convulsions. However, the relative reproducibility of the results obtained by the two methods in all other brain regions indicates that handling stress and mode of death probably did not influence GABA concentrations and GAD activities. Because of the relatively large brain areas encompassed in our procedures, GABA distribution did not show the high regional specificity reported through the use of more precise dissection procedures.[8] However, both GABA and GAD distribution patterns appear to agree reasonably well with those reported in other species.[1,8]

The fact that the two animals euthanatized at 3 hours postirradiation showed decreased regional GABA concentrations in four brain areas parallels our findings in rats, that brain GABA concentrations decrease by 2 hours after 20,000 rads.[4] At this time the rat began to show signs of convulsions at slightly higher doses.[4] However, since seizure activity in miniature swine terminates within a few minutes postirradiation, even at much higher doses (unpublished results), both the mechanism and implication of the late alterations remain unknown.

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13. ABSTRACT

Miniature swine convulsed spontaneously within 30 seconds after a 10,000-rad dose of 30 MeV electrons was delivered to the brain. The seizures ended 2 to 5 minutes later. Since alterations in gamma-aminobutyric acid (GABA) metabolism occur in and may be an underlying cause of a variety of convulsive disorders, regional brain concentrations of GABA and activities of L-glutamic acid decarboxylase (E. C. 4.1.1.15) (GAD) were determined in control and irradiated miniature swine. The hypothalamus and midbrain had the highest GABA concentration and GAD activity, followed, in decreasing order of GABA concentration, by the caudate nucleus, thalamus, hippocampus, cerebral cortex and cerebellum. At 4 minutes after irradiation, GABA concentrations did not change significantly in any brain region examined; the largest apparent change was a decline from $2.31 \pm .13$ to $2.04 \pm .11$ μmoles/g wet weight in the thalamus. Concurrently, GAD activity in the thalamus increased significantly from 238 ± 7 to 315 ± 29 μmoles/h per g protein. Since inhibition of GAD and significant reduction in GABA concentrations are generally associated with convulsive disorders, these data are not consistent with the hypothesis that alterations in brain GABA metabolism play a role in the etiology or course of radiation-induced seizures in miniature swine.

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