EFFECTS OF MIXED GAMMA-NEUTRON RADIATION ON THE ACTIVITY OF BRAIN ENZYMES INVOLVED IN NEUROTRANSMITTER METABOLISM

G. N. Catravas
C. G. McHale

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

Approved for public release; distribution unlimited
Research was conducted according to the principles enunciated in the "Guide for Laboratory Animal Facilities and Care," prepared by the National Academy of Sciences - National Research Council.
EFFECTS OF MIXED GAMMA-NEUTRON RADIATION ON THE ACTIVITY OF
BRAIN ENZYMES INVOLVED IN NEUROTRANSMITTER METABOLISM

G. N. CATRAVAS
C. G. McHALE

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

Approved for public release; distribution unlimited
ACKNOWLEDGMENT

The authors wish to express their appreciation to R. L. Long and J. K. Widmayer of mechanical services for the design and fabrication of the guillotine; to the members of the reactor, radiological physics and radiological safety groups for their assistance during exposures and to O. Z. Williams for his capable technical assistance.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreword (Nontechnical summary)</td>
<td>iii</td>
</tr>
<tr>
<td>Abstract</td>
<td>v</td>
</tr>
<tr>
<td>I. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>II. Materials and Methods</td>
<td>1</td>
</tr>
<tr>
<td>Materials</td>
<td>1</td>
</tr>
<tr>
<td>Animals</td>
<td>1</td>
</tr>
<tr>
<td>Radiation source</td>
<td>2</td>
</tr>
<tr>
<td>Guillotine description</td>
<td>2</td>
</tr>
<tr>
<td>Procedures</td>
<td>4</td>
</tr>
<tr>
<td>III. Results</td>
<td>6</td>
</tr>
<tr>
<td>IV. Discussion</td>
<td>7</td>
</tr>
<tr>
<td>References</td>
<td>9</td>
</tr>
</tbody>
</table>

# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>Front view of guillotine device</td>
<td>3</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Rear view of guillotine device</td>
<td>3</td>
</tr>
<tr>
<td>Figure 3</td>
<td>Side view of guillotine device</td>
<td>3</td>
</tr>
<tr>
<td>Figure 4</td>
<td>Radiation-induced changes in activities of brain enzymes involved in neurotransmitter metabolism</td>
<td>7</td>
</tr>
</tbody>
</table>
Animals such as monkeys, miniature pigs and rats almost immediately after exposure to supralethal doses of pulsed ionizing radiation undergo a period of transient incapacitation which is manifested by impairment in performance, incoordination and other neurological symptoms and is often accompanied by convulsions. After a temporary recovery which follows this early transient incapacitation, the animals become irreversibly incapacitated shortly before death. It is known that the levels of acetylcholine and other neurotransmitters are affected by the functional state of the animal and during drug-induced convulsions. It was, therefore, of interest to investigate if any changes occur in the activity of brain enzymes which are involved in neurotransmitter metabolism during and after the early transient incapacitation. We selected three brain areas, i.e., cerebellum (synergic action of voluntary movement), cerebrum (voluntary motor activity) and hippocampus (learning and recent memory) and the following enzymes: choline acetyl transferase (acetylcholine synthesis), acetylcholinesterase (acetylcholine hydrolysis), monoamine oxidase (inactivation of biogenic amines) and RNA polymerase (RNA synthesis). It was found that as early as 4 minutes after a pulsed dose of 20,000 rads of mixed gamma-neutron radiation the activities of the enzymes investigated, except acetylcholinesterase, are markedly decreased. Acetylcholinesterase did not seem to be appreciably changed under our experimental conditions.
ABSTRACT

Sprague-Dawley adult male rats were exposed to incapacitating doses (20,000 rads) of mixed gamma-neutron radiation in a single pulse from the AFRRI-TRIGA reactor. The activities of the enzymes choline acetyl transferase, acetylcholinesterase, monoamine oxidase and RNA polymerase were determined in the cerebral cortex, cerebellum and hippocampus. It was found that as early as 4 minutes after irradiation of the animal the activity of monoamine oxidase was markedly decreased in all three brain areas investigated. Choline acetyl transferase and RNA polymerase activities were also decreased but to a lesser extent. No appreciable changes in the activity of acetylcholinesterase were observed.
I. INTRODUCTION

Although the central nervous system has been generally regarded to be fairly radiosensitive, physiologic and biochemical changes have been reported in brain tissue even after relatively low doses of ionizing radiation.\(^1,6,7,10,12\) Investigations concerning the ability of animals such as monkeys and miniature pigs to perform learned tasks have revealed that these animals, shortly after exposure to supralethal doses of pulsed ionizing radiation, experience a period of transient performance decrement or early transient incapacitation (ETI) which is often accompanied by convulsions. This is followed by relative improvement in performance and then by a rapid irreversible deterioration and eventual death.\(^2,4\) Reports in the literature\(^3,8\) indicate that the RNA content in nerve cells increases considerably during sensory stimulation and decreases during functional inactivity and drug-induced convulsions. It has also been found that the acetylcholine level and the activities of the enzymes associated with its metabolism change depending on the functional state of the brain.\(^9\)

The purpose of the present investigation was to determine the radiation-induced changes in the activities of enzymes associated with the metabolism of neurotransmitters during and following early transient incapacitation.

II. MATERIALS AND METHODS

Materials. Chemicals utilized in this study were purchased from the Sigma Chemical Company, St. Louis, Missouri. Radioactive compounds were obtained from New England Nuclear Corporation, Boston, Massachusetts.

Animals. Sixty male Sprague-Dawley rats, 8 to 10 weeks old, and weighing 230 to 260 grams were used in five experiments. The animals were kept in a
temperature-controlled room at 22°C and were individually housed in cages. They had free access to food (laboratory pellets) and water. They were divided into two equal groups. The first group was irradiated, whereas the second group was sham irradiated and was used as controls. During irradiation the animals were individually housed in Lucite boxes arranged so that each rat received an equal unilateral exposure. All experimental animals received a single whole-body exposure of 20,000 rads of mixed gamma-neutron radiation in a single pulse.

Radiation source. The AFRRI-TRIGA reactor was used. Its physical characteristics were as follows: the ratio of gamma to neutron kerma, free-in-air, was approximately 1.5. This was measured using a paired chamber technique, i.e., a 50 cm³ tissue-equivalent chamber filled with tissue-equivalent gas (3.1 percent N₂; 32.5 percent CO₂; 64.4 percent CH₄) and a 50 cm³ graphite chamber filled with CO₂. On all Lucite boxes sulfur tablets were used for neutron monitoring. Experimental animals were sacrificed by decapitation at either 4, 40 or 180 minutes postirradiation. Controls were also sacrificed by decapitation. Since early transient incapacitation begins almost immediately after exposure and access to the irradiated animals in the reactor chamber is impossible at this time, a special remote controlled guillotine was developed which decapitated the animals in the exposure room. Rats were irradiated in pairs while restrained in the guillotine device and were guillotined simultaneously.

Guillotine description. Figures 1 and 2 show a front and rear view of the device respectively. Figure 3 represents a side view. The guillotine's steel blade is firmly attached to a rotating aluminum drum by means of metal screws. The blade raises to full cock when pointed straight up and latches into position by means of a small lever.
Figure 1. Front view of guillotine device

Figure 2. Rear view of guillotine device

Figure 3. Side view of guillotine device
which drops into a notch on the aluminum drum. The rats are restrained in specially machined cylindrical acrylic holders (tubes) which can be attached to the guillotine plate and locked into position by means of special adapters. Inside these holders special movable restraining plastic cylinders slide and can be locked in place by means of thumb screws thus immobilizing the rats. The heads of the rats are kept in position by means of specially machined yokes which open and close and can be locked at any position by means of a special thumb screw. When the guillotine blade is released by means of an electronic solenoid which is activated by remote control, it swings down through an arc of approximately $120^\circ$ and decapitates the animals in this travel. The driving force of the blade is supplied by a powerful steel spring which, when the blade is in fully cocked position, is under tension. A special acrylic shield, placed in front of the heads of the rats and attached to a Dewar flask, has a very sensitive trap door that is automatically removed as the blade circumscribes its arc and the heads fall into liquid nitrogen. When in position the door prevents evaporation of the liquid nitrogen and also exposure of the rats to its vapors.

**Procedures.** Instant freezing of the rat heads in liquid nitrogen usually resulted in spontaneous splitting of the skull and brain longitudinally into two almost symmetrical half portions, thus facilitating later dissection. The heads of the rats were removed from the liquid nitrogen within 30 minutes and stored at $-85^\circ$C in a freezer until assay. Assays were done within 24 hours after irradiation in the following manner. The heads were partially thawed in the cold room, and the dorsal hippocampus, cerebral cortex and cerebellum were dissected out and homogenized in the appropriate media described below, using homogenizers of the Potter-Elvehjem type with Teflon
pestle. All operations were carried out at 0 to 4°C unless otherwise stated. To obtain enough material to carry out the activity determinations of all enzymes under investigation, corresponding brain areas from pairs of rats were pooled. Activity determinations of the following enzymes were performed in these experiments: choline acetyl transferase, acetylcholinesterase, monoamine oxidase and RNA polymerase.

Choline acetyl transferase activity was determined according to a modification of the method of McCaman and Hunt. The assay mixture contained 3.5 μmoles phosphate buffer pH 7.4; 0.25 μmole choline hydrochloride; 0.01 μmole eserine; 1.0 μmole MgSO₄; 2.5 μg BSA; 0.5 μmole 1-¹⁴C acetyl coenzyme A (approximately 200,000 counts/min); 0.1 mmole NaCl and 0.2 ml enzyme preparation to a total volume of 0.6 ml. The mixture was incubated for 30 minutes at 37°C and treated as previously described.

Acetylcholinesterase activity was measured colorimetrically according to the method of Ellman et al. as modified by Maletta et al.

Monoamine oxidase was assayed by a modification of the method of Weissbach et al. The assay mixture contained 75 μmoles Tris HCl buffer pH 7.4; 0.45 μmole kynuramine-di-HBr and 0.3 ml enzyme preparation to a total volume of 1.9 ml. Following incubation for 90 minutes at 37°C, the mixture was made up to 3 ml with water. After the addition of 0.2 ml 0.5 N NaOH and 0.4 ml 10 percent ZnSO₄ it was shaken, placed in a boiling water bath for 5 minutes, cooled and centrifuged at approximately 10,000 x g. The concentration of the reaction product 4-hydroxyquinoline was determined in the supernatant spectrophotometrically by measuring the absorbance at 330 nm. A blank cuvette was prepared by replacing kynuramine with water.
RNA polymerase activity was measured according to the method of Weiss with some modifications. The assay mixture contained 5 μmoles Tris HCl buffer pH 7.8; 4 μmoles MnCl₂; 0.5 μmole each ATP, GTP, CTP; 2 μCi ³H-UTP and enzyme preparation to a total volume of 0.55 ml. The mixture was incubated with shaking for 15 minutes at 37°C; 0.15 ml of a 1 percent aqueous casein solution was then added, mixed and followed by 1 ml of 20 percent TCA. The mixture was shaken and allowed to set for 30 minutes in an ice bath. It was then centrifuged at 1500 x g and the precipitate washed three times with cold 5 percent TCA and finally dissolved in 0.7 ml Hyamine. Radioactivity was measured in a liquid scintillation counter (Nuclear-Chicago), using POP and POPOP in toluene. Enzymic activities were expressed per milligram of protein. Protein determinations were performed according to the method of Lowry et al.

III. RESULTS

The radiation-induced changes in the activities of the four enzymes under investigation are shown in Figure 4. It can be seen that monoamine oxidase is the most radiosensitive enzyme and that as early as 4 minutes after irradiation of the animal a pronounced decrease, up to 60 percent, in its activity occurred in all three brain areas examined (p > 0.001 to p < 0.01). In rats sacrificed at 40 or 180 minutes after exposure, the activity of this enzyme, although higher than in animals sacrificed at 4 minutes postirradiation, still remained well below control values (p > 0.005 to p < 0.025). Choline acetyl transferase activity was also found to decrease in the irradiated animals (p > 0.01 to p < 0.05) but to a lesser degree than monoamine oxidase. RNA polymerase activity appeared also to be inhibited by irradiation of the animals especially
in the hippocampus and cerebellum (p > 0.01 to p < 0.05) indicating a decrease in RNA and therefore protein synthesis in these brain areas. No appreciable changes were observed in the activity of acetylcholinesterase in any of the brain areas investigated, although some were perhaps of significant value (p > 0.01 to p < 0.2).

**Figure 4.** Radiation-induced changes in activities of brain enzymes involved in neurotransmitter metabolism. Bars represent standard error.

**IV. DISCUSSION**

Experiments in this laboratory, as well as in others, have shown that in order to produce a visibly distinct and consistent early transient incapacitation in the rat, the radiation dose should be of the order of at least 18,000 to 20,000 rads. Early transient incapacitation, which begins almost immediately after the pulse and lasts up to approximately 20 minutes depending on the radiation dose, is manifested in the rat first by a lack of coordination often accompanied by convulsions or muscle spasms, and later by collapse. The data presented in these experiments indicate that, under
our experimental conditions, monoamine oxidase is the most radiosensitive of the brain enzymes studied. As early as 4 minutes after pulse irradiation of the animal, a pronounced decrease in the activity of this enzyme occurred, especially in the cerebellum, a brain area which is known to be involved in movement coordination, and also, although to a lesser extent, in the hippocampus and cerebral cortex. The fact that choline acetyl transferase activity was found to decrease, especially in the cerebellum of the irradiated rats, whereas no appreciable changes were observed in acetylcholinesterase activity, suggests a decrease in the level of acetylcholine in these animals.

RNA polymerase activity was also found to decrease especially in the hippocampus and cerebellum of the irradiated animals, which indicates decreased synthesis of RNA and therefore of protein. It is of interest to note that, although our experimental and irradiation conditions were different from those of Ordy et al., our results with RNA polymerase are in agreement with their findings that there is a dose-dependent decrease in brain protein, DNA and RNA content of mice whose heads had been submitted to deuteron irradiation.

Radiation-induced changes in the activity of tyrosine hydroxylase, which is the rate-limiting enzyme in catecholamine synthesis, were not determined in this study. Experiments are in progress to determine if and to what extent the activity of this enzyme is altered by irradiation of the animal. Research will also be conducted to determine the radiation-induced changes in neurotransmitter levels.
REFERENCES


Sprague-Dawley adult male rats were exposed to incapacitating doses (20,000 rads) of mixed gamma-neutron radiation in a single pulse from the AFRRI-TRIGA reactor. The activities of the enzymes choline acetyl transferase, acetylcholinesterase, monoamine oxidase and RNA polymerase were determined in the cerebral cortex, cerebellum and hippocampus. It was found that as early as 4 minutes after irradiation of the animal the activity of monoamine oxidase was markedly decreased in all three brain areas investigated. Choline acetyl transferase and RNA polymerase activities were also decreased but to a lesser extent. No appreciable changes in the activity of acetylcholinesterase were observed.