ACTIVITY CHANGES OF BRAIN ENZYMES IN RATS EXPOSED TO DIFFERENT QUALITIES OF IONIZING RADIATION

G. N. Catravas
C. G. McHale
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.
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G. N. CATRAVAS
C. G. McHALE

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

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In a previous study we investigated the effect of incapacitating doses of mixed gamma-neutron radiation given in a very short pulse on the activities of brain enzymes responsible for the synthesis and degradation of neurotransmitters. We demonstrated that monoamine oxidase, which is responsible for the degradation of catecholamines and indoleamines, was the most radiosensitive of the enzymes studied and that a marked decrease in its activity occurred during early transient incapacitation. In the course of preliminary experiments we observed that the pattern of incapacitation during the first few minutes after the pulse differed depending on whether the radiation was primarily neutron or gamma. When the rats were exposed to radiation rich in neutrons, the incapacitation was manifested as loss of coordination, drowsiness, and often total loss of consciousness accompanied by convulsions or muscle spasms. In contrast, when the rats were exposed to the same dose of radiation rich in gamma rays, they became hyperactive and showed a high degree of discomfort. These differences in behavior prompted us to investigate possible differences in enzyme activities in the two groups.

It was found that, when the rats received radiation rich in neutrons, a pronounced decrease in the activity of monoamine oxidase occurred; whereas, when the animals were exposed to the same dose of radiation rich in gamma rays, the activity of this enzyme was markedly increased. Minor changes were observed in the activities of the other brain enzymes studied in this series of experiments.
ABSTRACT

The effect of different qualities of ionizing radiation on the activity of brain enzymes involved in the metabolism of neurotransmitters in specific regions of the brain of rats was investigated. Groups of Sprague-Dawley adult male rats were exposed to approximately 20,000 rads of radiation either rich in neutrons or rich in gamma rays from the AFRRI-TRIGA reactor, in the form of a square wave approximately 90 seconds long. It was found that, when the animals were exposed to radiation rich in neutrons, monoamine oxidase activity was pronouncedly decreased in all brain areas studied. In contrast, a very marked increase in the activity of this enzyme was observed when the animals received the same dose of radiation rich in gamma rays. Minor changes were observed in the activities of choline acetyl transferase and RNA polymerase. Acetylcholinesterase activity did not change appreciably.
I. INTRODUCTION

Previous experiments in our laboratory have shown that, when rats were exposed to incapacitating doses of mixed gamma-neutron radiation in the form of a pulse, considerable changes occurred in the activities of brain enzymes responsible for the synthesis and degradation of neurotransmitters. As early as 4 minutes after whole-body irradiation of the animal a marked decrease in the activity of monoamine oxidase, the enzyme responsible for the degradation of biogenic amines, occurred in all brain areas investigated. Less pronounced decreases in the activities of choline acetyltransferase and RNA polymerase were also observed, whereas acetylcholinesterase activity did not appear to be appreciably affected.

Results of previous experiments concerned with the in vivo effects of ionizing radiation on liver enzymes indicated that, when rats were exposed to lethal doses of electromagnetic radiation, the activities of these enzymes were greatly enhanced. In contrast, preliminary experiments had shown that when the animals were exposed to the same doses of 14 MeV neutron radiation, the activity of the same enzyme system was markedly decreased.

The objective of this study was to determine whether the pattern of radiation-induced activity changes of brain enzymes responsible for neurotransmitter metabolism is similar to that observed previously for the liver enzyme system.

II. MATERIALS AND METHODS

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

* Unpublished data
Animals. One hundred and eight male Sprague-Dawley adult rats weighing between 240 and 260 grams were used in six experiments. They were kept in a temperature-controlled room at 22°C and were individually housed in cages. They were given food ad libitum and had free access to water. The animals were divided into three groups of 36 animals each. The first group was exposed to radiation rich in neutrons, the second group received radiation rich in gamma rays, whereas the third group was sham irradiated and was used as controls. During irradiation the rats were individually housed in Lucite boxes which were so arranged that each animal received an equal unilateral exposure. All experimental animals received a single whole-body exposure of approximately 20,000 rads.

Radiation source. The AFRRI-TRIGA reactor was used. To increase the neutron to gamma ray ratio, the core of the reactor was shielded with a 4-inch lead brick wall. A ratio of neutrons to gamma rays of approximately 7.5 to 1 was thus obtained. In the experiments in which the rats were exposed to radiation rich in gamma rays, a space of water approximately 5 inches wide separated the core of the reactor from the half circular aluminum wall of the water tank which projected into the exposure room. A gamma ray to neutron ratio of approximately 7 to 1 was achieved. The ratios of neutrons to gamma rays were measured using a paired chamber technique, i.e., a 50 cm$^3$ tissue-equivalent chamber filled with tissue-equivalent gas (3.1 percent N$_2$; 32.5 percent CO$_2$; 64.4 percent CH$_4$) and a 50 cm$^3$ graphite chamber filled with CO$_2$. Sulfur tablets on all Lucite boxes were used for neutron monitoring. Since in the experiments in which the core of the reactor was shielded with lead bricks it was not possible to
deliver the 20,000 rads in the form of a pulse, the radiation was given in all experiments in the form of a square wave of approximately 90 seconds duration.

**Procedures.** Experimental animals were sacrificed by decapitation at either 4, 40 or 180 minutes after exposure. Control rats were also decapitated. The rats sacrificed 4 minutes after exposure were irradiated in pairs while restrained in a remote controlled guillotine device. The guillotine device decapitated two animals simultaneously with one downward swing of the blade in the exposure room. The heads of all decapitated rats were instantly frozen in liquid nitrogen. They were later removed from the liquid nitrogen and stored at -90°C until assay time. They were analyzed within 24 hours after irradiation.

Instant freezing of the rat heads in liquid nitrogen usually resulted in splitting of the skull and brain longitudinally into two almost symmetrical half portions, thus facilitating the removal of the brain areas under investigation. To remove these areas, the heads were partially thawed in the cold room. The hippocampus, cerebral cortex, cerebellum, and anterior and posterior hypothalami were dissected out and homogenized in the appropriate media using homogenizers of the Potter-Elvehjem type with Teflon pestle. All subsequent operations were carried out at 0°C to 4°C unless otherwise stated. To obtain enough tissue to carry out the activity determinations of all enzymes under study, corresponding brain areas from every two rats were pooled. Activity determinations were performed on the following enzymes: choline acetyl transferase, acetylcholinesterase, monoamine oxidase and RNA polymerase.

Choline acetyl transferase activity was determined according to the method of McCaman and Hunt slightly modified. 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 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 described by McCaman and Hunt.⁷

Acetylcholinesterase was assayed colorimetrically according to the method of Ellman et al.⁴ as modified by Maletta et al.⁶

Monoamine oxidase activity was measured by a modification of the method of Weissbach et al.¹⁰ 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. Then 0.2 ml of 0.5 N NaOH and 0.4 ml of 10 percent ZnSO₄ were added and the mixture 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.

RNA polymerase was measured essentially according to the method of Weiss.⁹ 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 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 for 15 minutes 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. Statistical determinations were performed using Student's two-tail test. Bars in the figures represent standard errors.

III. RESULTS

The radiation-induced changes in the activities of the four enzymes under study in the hippocampus, cerebrum and cerebellum of rats exposed to radiation rich in neutrons are shown in Figure 1. Figure 2 shows the changes in activity observed in the anterior and posterior hypothalami of the same animals. It can be seen in both figures that monoamine oxidase is greatly affected by exposure of the rats to this quality of radiation and that as early as 4 minutes after irradiation rich in neutrons a very

Figure 1. Brain enzyme activity changes in rats exposed to radiation rich in neutrons
pronounced decrease (up to 70 percent) in the activity of this enzyme occurred in all brain areas investigated ($p > 0.001$ to $p < 0.005$). In rats sacrificed at 40 or 180 minutes after irradiation the activity of monoamine oxidase, although higher than in animals sacrificed at 4 minutes, still remained well below control values ($p > 0.005$ to $p < 0.025$). Substantial decreases were also observed in the activities of choline acetyl transferase and RNA polymerase ($p > 0.005$ to $p < 0.05$). No appreciable changes over control values appeared to occur in acetylcholinesterase activity.

The results obtained when rats were exposed to radiation rich in gamma rays are shown in Figures 3 and 4. It can be seen that again monoamine oxidase is the enzyme most affected by irradiation of the animals and that, in contrast to the effects shown in Figures 1 and 2, its activity was greatly enhanced ($p > 0.001$ to $p < 0.01$). Minor changes were found to occur in the activities of the other three brain enzymes.
Figure 3. Brain enzyme activity changes in rats exposed to radiation rich in gamma rays

Figure 4. Brain enzyme activity changes in rats exposed to radiation rich in gamma rays
studied \((p > 0.01 \text{ to } p < 0.05)\). No significant changes were observed in the activity of acetylcholinesterase.

IV. DISCUSSION

In a previous study \(^3\) we had shown that, when rats were exposed to incapacitating doses of mixed gamma-neutron radiation with a gamma to neutron ratio of approximately 1.5 in the form of a pulse, monoamine oxidase appeared to be the most radiosensitive of the enzymes studied and its activity was markedly decreased. We observed that the pattern of incapacitation of rats during the first few minutes after pulse irradiation differed depending on whether the radiation spectrum was rich in neutrons or in gamma rays. When rats were exposed to radiation rich in neutrons the incapacitation was manifested as lack of coordination or collapse of the animal, often accompanied by convulsions or muscle spasms. In contrast, when the animals were exposed to the same dose of a radiation rich in gamma rays, they appeared to be overexcited, hyperactive and showed a high degree of discomfort. These observations prompted us to investigate possible causes. From the results presented in the present study it is obvious that monoamine oxidase, which is responsible for the oxidative deamination of catecholamines and indoleamines, is the enzyme whose activity is most affected and that the direction of this activity change (decrease or enhancement) depends on the quality of the radiation the animals received. Furthermore, the magnitude of this activity change was found to be higher in the cerebellum of the irradiated rats. A similar pattern of activity changes has been observed with liver enzymes involved in fatty acid synthesis. \(^8\) Significant changes in the activity of acetylcholinesterase have been reported in brain regions of developing rats after prenatal x irradiation \(^6\) or x irradiation.
in infancy. However, in contrast to the results of these investigators, under our experimental conditions no appreciable changes were observed in the activity of this enzyme, whether the animals received irradiation rich in neutrons or in gamma radiation. It should be emphasized, however, that our irradiation conditions, radiation dose, age of the rats and time of sacrifice following exposure were very different from those of others. A possible interpretation of this discrepancy could be that in addition to the entirely different experimental conditions, an age-dependent radiosensitivity may exist in specific cells in the brain of the irradiated animal.

The effect of ionizing radiation on the activities of tyrosine hydroxylase and tryptophan hydroxylase, the rate-limiting enzymes in catecholamine and indoleamine biosynthesis, was not determined in this series of experiments. Also, the actual levels of neurotransmitters in the various brain regions of the irradiated animal were not measured. It remains therefore to be determined to what extent these levels of neurotransmitters are involved in the manifestation of the behavioral differences observed during early transient incapacity.
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