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Radiation-Induced Impairment of Neuronal Excitability

Radiation causes a decrease in the synaptically evoked activity of CA1 hippocampal pyramidal cells. This effect is dose and dose-rate dependent. Hydrogen peroxide, which produces hydroxyl free radicals when combined with FE^{+3}, produces similar damage. In contrast, the radioprotectant, dithiothreitol, increases the excitability of hippocampal neurons. These studies indicate that radiation can directly affect the function of central neurons.

INTRODUCTION

For the past thirty years, ionizing radiation has been known to acutely alter the electrical activity of the brain[1] as well as to produce behavioral deficits.[2] Recording from a variety of brain areas, the most striking change in electrical activity following X-radiation is the appearance of high frequency activity (spiking) in the hippocampus.[3,4] This appears within 30 minutes of exposure to 4 Gy whole body exposure and reaches a maximum after 5 to 7 hours. The spiking is sustained for almost 2 weeks.[3] Very little concomitant change in cortical activity is seen,[3,4] but the arousal threshold for the midbrain reticular formation decreases with approximately the same time course.[4] Evoked potential studies also show an alteration in brain activity following radiation exposure. Rosenthal and Timiras[5] observed that 2.5 Gy X-rays decreased the latency and reduced the size of evoked potentials in the olfactory cortex. Their observations led them to suggest that radiation reduced synaptic inhibition. Exposure to ionizing radiation tends to increase

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susceptibility to seizures. The frequency of audiogenic seizures in mice is enhanced following chronic exposure to low levels of radiation.[6] The threshold for electroconvulsive seizures is reduced in adult rats[7,8] after as little as 0.25 Gy X-irradiation.[7] Acute studies on the spinal cord of the cat[9-11] reveal that 5-50 Gy X-radiation enhances the spinal monosynaptic reflex, augments the intracellularly recorded excitatory postsynaptic potential but does not change the inhibitory postsynaptic potential.[11,12] Single unit studies in olfactory cortex[13] and in hippocampus[14] show a change in patterns of action potential generation, predominantly a depression of spontaneous and evoked activity.

Nervous system damage produced by radiation *in vivo*, as in the studies described above, is likely to be influenced by systemic effects. Exposure to ionizing radiation is accompanied by decreased blood pressure and reduced blood flow in a variety of brain regions[15-17] including the hippocampus.[18] The decrease in brain blood flow is likely to produce ischemic damage, especially in the hippocampus which is a particularly vulnerable area.[19,20] Ischemia, produced by a transient occlusion of the arterial blood supply, causes an increase in potassium and a decrease in calcium concentrations in the extracellular neuronal environment.[20] Simultaneously, neuronal activity is inhibited. Seven hours after an ischemic episode of this kind, spontaneous action potentials appear much more frequently than normal in the hippocampus but not in the cortex.[20,21] Reperfusion following ischemia is thought to produce excessive free radicals that could exacerbate the damage caused directly by radiation.[22,23]

Because the blood brain barrier is disrupted by ionizing radiation,[24,25] radiation-released mediators[26] as well as normal plasma constituents may have abnormal access to neurons. Levels of histamine, prostaglandins, β-endorphins, and neurotensin as well as other compounds are elevated by ionizing radiation.[18,26,27] Histamine is a powerful neuromodulator that increases the excitability of hippocampal neurons.[28-30] While prostaglandins do not directly alter the excitability of hippocampal pyramidal cells,[31] they may have actions in other areas of the central nervous system or even indirect actions on the metabolic or glial activity.[32] Many of the other radiation-released mediators also are known to be neuromodulators. In understanding the changes of neuronal

excitability that occur with exposure to ionizing radiation, one cannot ignore the influence of these circulating factors which can affect both neurons and glial cells. The neuronal environment is finely tuned. A disruption of this environment, as caused by an altered blood brain barrier, is likely to produce abnormal neuronal activity.

APPROACH

Analysis of the effects of radiation on the nervous system is very complex. Are the changes due to ischemia? Are they caused by higher than normal concentrations of histamine or prostaglandins? Or is radiation acting direclty on neurons in their local environment? Since radiation-induced neuronal death occurs only at doses greater than 100 Gy, classically, neurons have not been considered radiation sensitive.[32] Most of the nervous system damage has been attributed to glial cell death and vascular damage.[33,34] This analysis, however, fails to consider the complex physiological functions of neurons that are likely to be impaired by radiation and free radical attack of membrane lipids and proteins.

To study the damage produced by radiation without the complicating factors caused by systemic injury, hippocampal brain slices were used as an experimental model.[35–37] Not only has previous literature indicated a particular sensitivity of this brain region to radiation, but this model provides a complex network of neurons, accessible to analysis, that allows assessment of integrated neuronal function. Because of the laminar structure of the hippocampus, organization of the tissue is maintained in a thin slice (400–500 μm). The input and output pathways to the CA1 region can be clearly identified and selectively stimulated with metal bipolar electrodes (0.02-2 mA). Glass microelectrodes are used to record the evoked activity from the population of neurons in a defined cell body layer (orthodromic population spike, top of Fig. 1) and simultaneously from the corresponding dendritic region (dendritic response, bottom Fig. 1). Analysis of radiation- and drug-induced changes in the somatic and dendritic recordings can provide information on the efficacy of synaptic transmission (synaptic damage) and the ability of the synaptic potential to generate an action

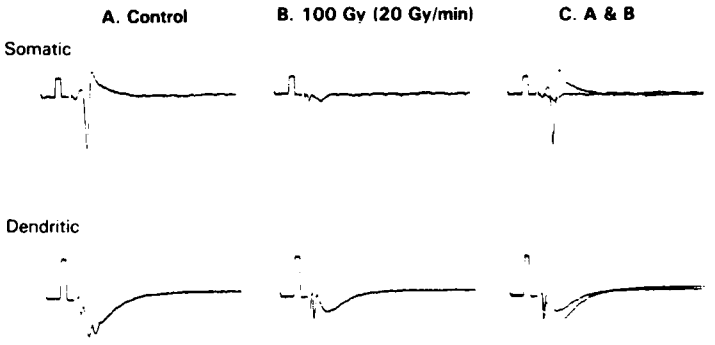

FIGURE 1 Effects of γ-radiation (100 Gy at 20 Gy/min) on population spike (somatic) and population synaptic response (dendritic) of CA1 neurons of guinea pig hippocampus. A: Recordings from a sham-irradiated slice. B: Recordings from an exposed slice. C: Superimposition of A and B. Square wave preceding all traces is a calibration pulse of 1 mV, 2 ms.

potential (postsynaptic damage). Slices can be studied before, during, and after application of radioprotectants and free radical producing agents. Radiation damage can only be tested in our system by comparing paired exposed and control slices some time after ^{60}Co radiation and sham radiation.

RADIATION DAMAGE IN AN ISOLATED NEURONAL SYSTEM

Gamma radiation (^{60}Co) can cause both synaptic and postsynaptic damage in hippocampal brain slices. The orthodromic population spike is greatly reduced by exposure. The dendritic response, the summated synaptic potential, also is decreased (Fig. 1). The decrease in the population spike is not proportionate to the decrease in the dendritic response. Even when the stimulus intensity is increased sufficiently to elicit a dendritic response in the irradiation slice comparable to that in the control slice, the resulting population spike is smaller. This indicates that while synaptic function is impaired, postsynaptic damage also is present. In contrast, however, even very high doses (200 Gy) do not alter the antidromically evoked spike. The damage to the orthodromic spike, therefore, is

not to the action potential *per se* but rather to the synaptic generation of the action potential.

The effects of radiation are dose and dose-rate dependent (Fig. 2). Increasing the dose increases the damage to the orthodromic population spike. Likewise, increasing the dose rate increases the damage to the orthodromic population spike. A dose rate of 20 Gy/min shifts to the left the dose response curve for radiation at 5 Gy/min. At 5 Gy/min, significant deficits are seen only at 75 Gy and greater. At the higher dose rate, the damage caused by 50 Gy radiation is significantly greater than at the lower dose rate. Synaptic damage and postsynaptic damage differ in their dose-rate dependence. Impairment of synaptic efficacy is more severe at higher dose rates. At 5 Gy/min it significantly contributes to the damage only at doses of 150 Gy and greater. At 20 Gy/min, however, synaptic damage is significant at 75 Gy. This dose-rate dependence suggests the existence of repair mechanisms. Lipid peroxidation, which varies inversely with dose rate, is an unlikely mechanism for synaptic damage. Impairment of postsynaptic spike generation, however, is not dose-rate dependent. It makes a significant contribution to the population spike damage at doses of 75 Gy and above, regardless of dose rate. Because of the distinction from synaptic damage, postsynaptic damage is likely to result from a different molecular mechanism.

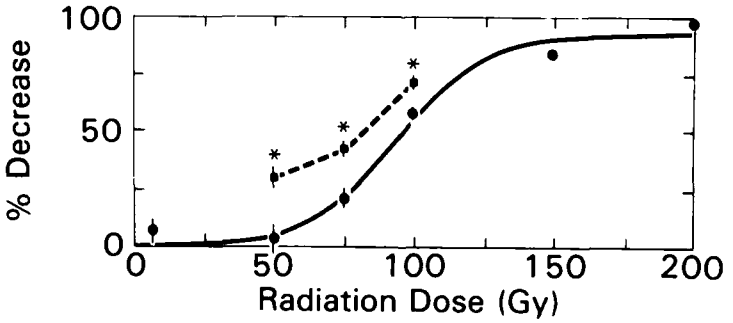

FIGURE 2 Dose response curve of effect of γ-radiation on the population spike recorded from the CA1 region of hippocampal slices. Solid line (Circles) shows effect of radiation at 5 Gy/min while dashed line (squares) shows effects of radiation at 20 Gy/min. Asterisks indicate that the effects of the two dose rates are significantly different from one another.

PEROXIDE MODEL OF FREE RADICAL DAMAGE

In an *in vitro* system, free radicals are likely to mediate the damage caused by ionizing radiation. Hydrogen peroxide or peroxide with ferrous ions can be used as a model system to generate the very reactive, hydroxyl free radicals through the Fenton reaction. Damage produced can be compared with radiation damage. Peroxide and peroxide/iron produce very similar deficits to ionizing radiation (Fig. 4A). Both synaptic efficacy and postsynaptic action potential generation are impaired following exposure to peroxide.[35] Figure

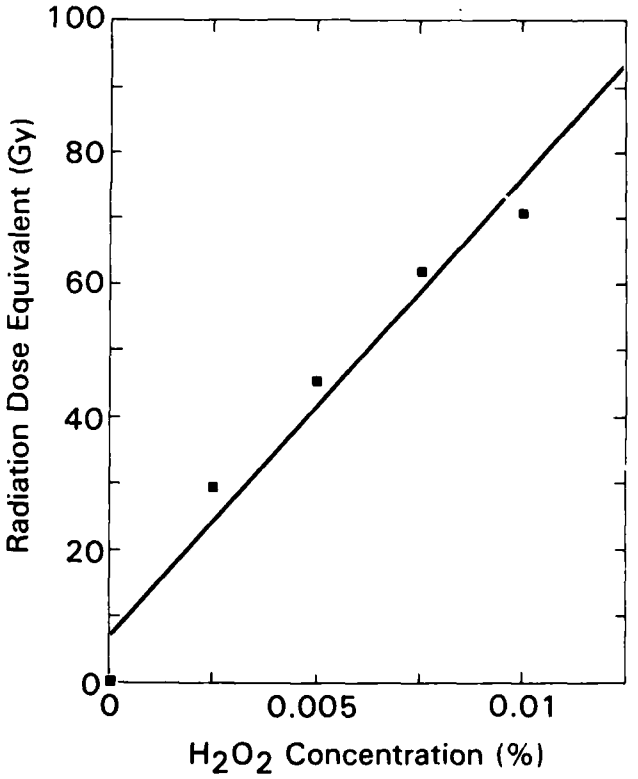

FIGURE 3 Radiation dose equivalent for peroxide damage. Concentration of hydrogen peroxide that causes a decrease in the orthodromic population spike is plotted against the radiation dose at 5 Gy/min required to elicit a similar deficit. Straight line is computer fit by linear regression.

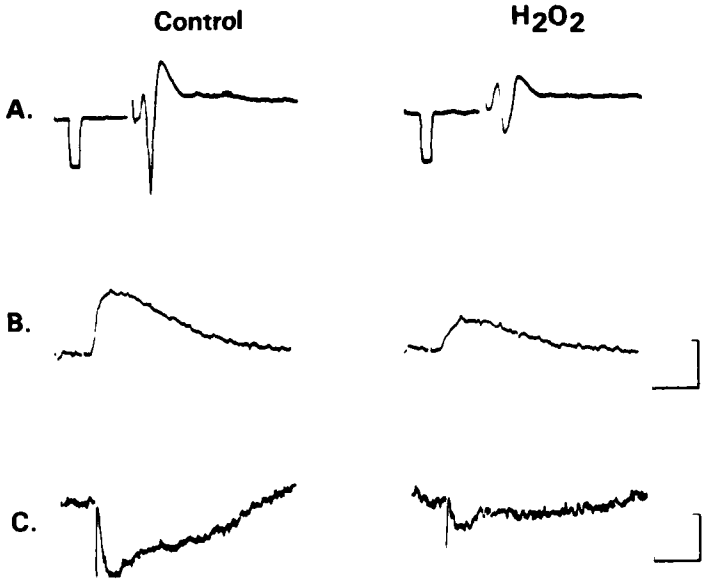

FIGURE 4 Effects of hydrogen peroxide (0.01%) on CA1 pyramidal cells of the hippocampus. A: Peroxide causes a decrease in the orthodromic population spike. Preceding calibration pulse is 1 mV, 2ms. B: Excitatory postsynaptic potential recorded intracellularly from pyramidal cell is reduced by peroxide. Calibration: 10 mV, 10 ms. C: Antidromic inhibitory postsynaptic potential is reduced by peroxide. Calibration 1 mV, 100 ms.

3 shows a curve relating peroxide concentration to a radiation dose producing similar degree of damage. A concentration of 0.005% (1.8 mM) produces the same decrease in the orthodromic population spike as approximately 45 Gy γ radiation at 5 Gy/min. The distinction should be made that peroxide damage is evaluated during exposure while radiation damage is evaluated at least 15 minutes following exposure. Peroxide damage is, at least with the lower doses, reversible. Within a half hour of removal, the effects of 0.005% peroxide or less are not functionally apparent. Higher doses of peroxide do produce decreases in the population spike that are not completely reversible within the experimental time frame. With this in mind, the peroxidative damage may actually be equivalent to much lower radiation doses.

An intracellular analysis[38] is possible using the peroxide model

for generation of free radicals. As predicted by field potential experiments, intracellularly recorded synaptic potentials are reduced by peroxide (Fig. 4B, C). Both the excitatory postsynaptic potential and the inhibitory postsynaptic potential are attenuated by peroxide. This does not result from a change in membrane resistance or membrane potential; neither is significantly affected by peroxide. Directly evoked action potentials are not altered. However, the neurons cannot sustain a train of action potentials as well as in control; that is, spike frequency adaptation is increased by exposure to peroxide.[38]

ACTIONS OF A RADIOPROTECTANT

Dithiothreitol (DTT) is a sulfhydryl reducing agent that acts as a radioprotectant.[39,40] It was tested in the hippocampal brain slice in the hope of finding an antagonist to radiation and peroxide damage. DTT has direct effects on the neural tissue.[37] Interestingly, it appears to produce damage that is the inverse of that caused by peroxide and radiation. The orthodromic population spike is increased in amplitude following a 30-minute exposure to 500 μM DTT (Fig. 5A). In addition, DTT causes the appearance of additional spikes in the field potential suggesting abnormal repetitive firing of the cells following exposure to the radioprotectant. Spontaneous burst firing also appears.[37] Analysis of the dendritic and somatic field potential recordings suggests that the synaptic potentials become much more efficient in eliciting spikes. Intracellular recordings[41] reveal that the excitatory postsynaptic potential is prolonged but the inhibitory postsynaptic potential is unaffected by DTT (Fig. 5B,C). Excitatory inputs, that normally produce only one action potential, evoke a train of spikes following exposure to DTT. Membrane resistance is not significantly altered, but membrane potential is slightly (2-8 mV) depolarized. In contrast to peroxide, DTT reduces spike frequency adaptation; the cells are more capable of sustaining a train of action potentials.

It is likely that the damage caused by DTT is a consequence of its sulfhydryl reducing properties since the oxidized form of DTT is without similar effects.[37] The hydroxyl free radical is a strong oxidizing agent and both peroxidative and radiation damage might be a consequence of oxidation of membrane constituents. Al-

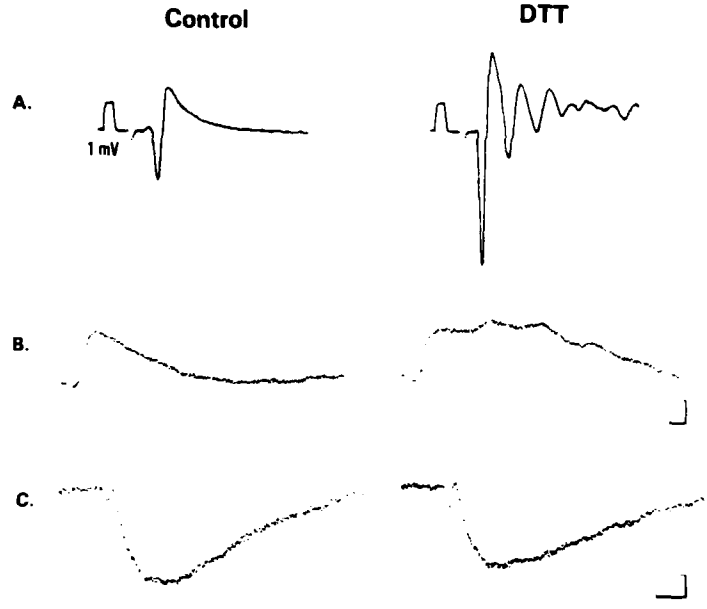

FIGURE 5 Effect of 30-minute exposure to dithiothreitol (500 μM) in CA1 neurons of hippocampus. A: DTT increases the amplitude of the population spike and elicits multiple peaks in the extracellularly recorded somatic potential. Calibration pulse preceding trace is 1 mV, 2 ms. B: Intracellularly recorded excitatory postsynaptic potential is prolonged following DTT treatment. Calibration: 2 mV, 4 ms. C: Inhibitory postsynaptic potential is unaffected by DTT. Calibration: 1 mV, 25 ms.

though DTT might not be a drug of choice to protect against radiation damage, it might be valuable as a tool to reverse the effects of free radical attack. The feasibility of this possibility needs to be further evaluated experimentally.

CONCLUSIONS

Nervous system damage caused by ionizing radiation is quite complex. Electrical activity in the brain is disrupted *in vivo* by doses as low as 200 rads. The experimental deficits described here, although experimentally elicited with higher doses, are likely to contribute to this nervous system damage. Only very slight changes

in neural properties are sufficient to disrupt the intricately balanced neuronal networks of the brain. *In vivo* the damage is compounded by ischemia and radiation-released mediators. Repair mechanisms, which *in vitro* might be adequate to functionally restore neurons, might be rendered inadequate *in vivo*. In evaluating the effects of radiation on the central nervous system, it is important to recognize the variety of contributing factors; not only is the environment changing due to altered blood flow and a damaged blood brain barrier, neuronal excitability is altered.

Disclaimer

Views presented in this paper are those of the authors; no endorsement by the Defense Nuclear Agency has been given or should be inferred. Research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council.

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