Ionizing Radiation Decreases Veratridine-Stimulated Uptake of Sodium in Rat Brain Synaptosomes

Abstract. *Veratridine-stimulated uptake of sodium-22 in brain synaptosomes was significantly reduced by ionizing radiation over a dose range of 10 to 1000 rads. The response was dose-dependent and involved a decrease in the maximum effect of veratridine on uptake. The central nervous system may be more sensitive to ionizing radiation than generally thought, perhaps through a loss of the ability of the sodium channel to respond properly to stimulation.*

The central nervous system (CNS) is generally considered to be relatively resistant to the direct effects of ionizing radiation (*1*). For example, electrophysiological studies have suggested that ionizing radiation can induce changes in the bioelectric properties of nerve cells only at supralethal doses. Some investigators have shown an enhancement of neuroexcitability at x-ray doses less than 10,000 rads (*2*), while others have reported that radiation doses below 10,000 rads have no effect on action potentials, conduction velocity, or membrane resistance (*3*). At supralethal doses, a decrease in excitability was reported by all investigators. On the other hand, electroencephalograms and behavioral techniques provide evidence of a neural effect of radiation at doses of a few hundred rads (*4*).

In this study we explored the effects of ionizing radiation on the CNS by examining the properties of a discrete entity of neural function, the sodium channel. Excitable cell membranes are characterized, in part, by their ability to generate action potentials through time- and voltage-dependent changes in membrane conductance of sodium and potassium ions (*5*). The pathways for the two ions are distinct, that is, the membrane contains functionally defined channels that are specific for sodium and for potassium. Many investigators have approached the question of sodium channel function and structure by using radioactive sodium (^{22}Na and ^{24}Na) and naturally occurring neurotoxins, such as veratridine, that alter sodium permeability in a slow, persistent, and specific manner (*6*). We used this approach with synaptosomes as the radiation target. That synaptosomes, which are detached nerve cell endings (*7*), have functional sodium channels is shown by measurements of membrane potential, ion flux, and neurotoxin binding (*8*). Thus they provide a convenient cell-free vesicular system in which sodium channel function can be studied. Our method of measuring veratridine-stimulated uptake of sodium in synaptosomes is based on the method described by Tamkun and Catterall (*9*).

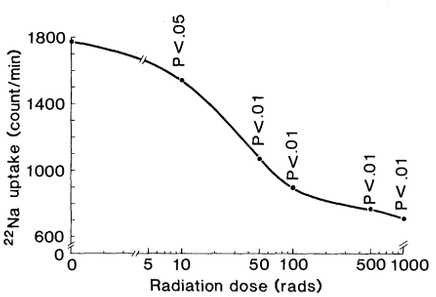

Fig. 1. Dose-response effect of high-energy electrons on veratridine-stimulated uptake of ^{22}Na. The concentration of veratridine was 10 μM. The data are means for two experiments (three observations per experiment per dose) and were subjected to a two-way analysis of variance to determine the influence of replication and dose on sodium uptake. Increasing doses of radiation significantly reduced uptake [$F(5, 24) = 70.6$, $P < .01$]. Individual comparisons were made with Dunnett's test on data pooled from both experiments since there was no significant dose × experiment interaction [$F(5, 24) = 2.17$, $P > .05$].

A crude synaptosomal suspension (*10*) was prepared from the brains of male Sprague-Dawley rats (200 to 400 g) by differential centrifugation. The material was resuspended in an ice-cold medium containing 5.4 mM KCl, 0.8 mM $MgSO_4$, 5.5 mM glucose, 50 mM Hepes-tris buffer (*p*H 7.4), 130 mM choline chloride, and bovine serum albumin (1 mg/ml). Portions of the resulting suspension were irradiated with various doses of high-energy electrons delivered from a linear accelerator (*11*). After irradiation, veratridine-stimulated uptake of ^{22}Na was determined in the synaptosomal preparation (*12*).

Synaptosomes were initially irradiated with 10 to 1000 rads of high-energy electrons. At a veratridine concentration of 10 μM, uptake of ^{22}Na was significantly reduced in a dose-dependent manner (Fig. 1). The decrease in uptake produced by irradiation ranged from 13 to 60 percent.

To further characterize the effect of ionizing radiation on veratridine-stimulated uptake of sodium, we irradiated synaptosomes with 100 to 100,000 rads and measured sodium uptake in the presence of a range of veratridine concentrations. Radiation exposure reduced the rate of ^{22}Na uptake measurably only at high concentrations of veratridine, suggesting the the maximum effect of veratridine was reduced in a dose-dependent manner by radiation (Fig. 2). No shift in the concentration-response curve was apparent. Nonspecific sodium uptake in the presence of 1 μM tetrodotoxin was not affected by radiation at the doses used.

It is not clear how ionizing radiation causes this dose-dependent decrease in veratridine-stimulated sodium uptake. Since the maximum effect of veratridine on uptake was reduced after irradiation but no induced shift in the concentration-response curve was observed, a direct disruption of the normal functioning of the sodium channel is a more likely explanation than an alteration in the affinity of sodium for the channel. Radiation may in some way reduce the number of sodium channels that can open on stimulation or may induce a conformational change that restricts the extent to which they can open.

Our results support earlier electrophysiological studies suggesting a reduction in neuroexcitability after radiation exposure, and contradict reports of radiation-induced increases in excitability. When Schwarz and Fox (*13*) used the voltage-clamp method to assess the effects of x-irradiation on nodes of Ranvier from frog nerve fibers, the peak sodium current decreased 1000 seconds after a threshold dose of about 8000 rads. While isotopic flux measurements such as were used in our experiment cannot address the time course of radiation effects, they do provide additional evidence that ionizing radiation can inactivate functional

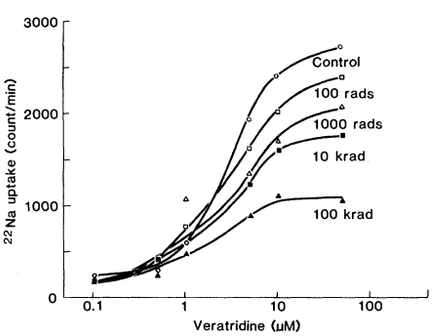

Fig. 2. Dose-response effect of high-energy electrons on the maximum uptake of ^{22}Na as stimulated by veratridine. The data are means for up to three experiments (three observations per dose of radiation and concentration of veratridine). Friedman's test (*14*) was used to determine whether radiation exposure reduced the effect of increasing concentrations of veratridine. Increasing doses of radiation significantly reduced maximum veratridine-stimulated uptake [χ^2 (4) = 103.5, $P < .001$]. Multiple comparisons of the effects of individual doses of radiation indicate that each increasing dose had a significantly greater effect ($P < .001$) than the preceding dose.

sodium channels in excitable membranes. However, our results suggest an effect at radiation doses one to two orders of magnitude lower than those used in voltage-clamp analysis. In addition to noting differences in the target tissue used, it is necessary to observe that the different types of radiation employed may vary in their relative biological effectiveness. However, preliminary studies in this laboratory with gamma-irradiation suggest a dose-dependent effect on sodium channel function similar to that produced by high-energy electrons.

A radiation effect such as we observed indicates that the mammalian CNS is more sensitive to single sublethal doses of radiation than is generally thought. If this is the case, then a radiation-induced disruption of a fundamental CNS process is possible that could affect individuals at far lower doses than previously believed, perhaps even at doses commonly used for therapeutic purposes. Further studies of discrete receptor sites in the sodium channel may provide additional information on the mechanism by which radiation exerts its effect and on the biological significance of these findings.

HENRY N. WIXON*
WALTER A. HUNT†
Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20814

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10. Use of this crude synaptosomal suspension for the sodium uptake assay led to no loss of sensitivity compared to highly purified synaptosomal preparations and was substantially more convenient.
11. Freshly prepared synaptosomes that had been suspended in the buffer solution were placed in glass test tubes (4 ml per tube). The tubes were placed in a Plexiglas holder filled with ice water for irradiation by electrons accelerated to an energy of 18 MeV at 0.55 A by the linear accelerator of the Armed Forces Radiobiology Research Institute. Pulses were delivered at a rate of 15 per second (pulse duration, 4 μsec). Dosimetry was performed with tissue-equivalent ion chambers (volume, 0.05 cm^3) placed in empty tubes. The calibration of the ion chambers is traceable to the National Bureau of Standards.
12. Various concentrations of veratridine were added to portions of the irradiated synaptosomal suspension and allowed to incubate for 10 minutes at 36°C. At this time the suspension was diluted again with a solution containing the same toxin concentrations plus 5.4 m*M* KCl, 0.8 m*M* $MgSO_4$, 5.5 m*M* glucose, 50 m*M* Hepes-tris (*p*H 7.4), 128 m*M* choline chloride, 2.66 m*M* NaCl, 5 m*M* ouabain, and 4 μCi of carrier-free [^{22}Na]Cl (New England Nuclear, 9.358 mCi/ml) per milliliter. After a 10-second incubation the reaction was terminated by adding 3 ml of an ice-cold wash solution of 163 m*M* choline chloride, 0.8 m*M* $MgSO_4$, 1.8 m*M* $CaCl_2$, 5 m*M* Hepes-tris (*p*H 7.4), and bovine serum albumin (1 mg/ml). The mixture was then rapidly filtered under vacuum through a Millipore HAWP or HAMK cellulose filter of 0.45-μm mesh. The filter was washed twice with 3-ml portions of the wash solution, and the radioactivity of the resulting sample was measured with a Packard Autogamma scintillation counter. For determination of specific veratridine-stimulated uptake, nonspecific uptake (in the presence of 1 μ*M* tetrodotoxin) was subtracted from the total uptake with veratridine. Initial results showed that the concentration of tissue used was not saturating. The time course of ^{22}Na uptake under these conditions indicated that uptake was linear until 15 seconds after the addition of the radioactive solution.
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* Present address: Laboratory of Preclinical Studies, National Institute of Alcohol Abuse and Alcoholism, Rockville, Md. 20852.
† To whom reprint requests should be sent.

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Modifying Oculomotor Activity in Awake Subjects Increases the Amplitude of Eye Movements During REM Sleep

Abstract. *The eye movements of human subjects were experimentally modified while they were awake to determine the effect of waking experience on electrooculographic activity during rapid eye movement (REM) sleep. After normal eye movements were monitored under controlled conditions, subjects spent 5 days wearing goggles that contained minification lenses and that curtailed vision to a 5° field. The amplitude and frequency of eye movements decreased when subjects were awake and increased during REM sleep; sleep stage durations and distributions were unaffected. Values returned to normal in the first 24 hours of recovery.*

Theories of the role of rapid eye movement (REM) sleep in the biological economy of mammals frequently focus principally on the spontaneous appearance of oculomotor activity (*1*) and related central nervous system (CNS) excitation (*2*) that occur in this state. Various theories share the premise that the organism benefits from the activation of the visual system during REM sleep: binocular stereopsis is improved (*3*), perceptual motor learning is integrated (*4*), or the ontogenetic development of the CNS is enhanced by endogenous neural stimulation (*5*).

Little is understood of how activity within the visual system during REM sleep is related to waking visual and oculomotor activity (*6*). Similarly, although studies of interactions between the waking state and REM dreaming have shown that meaningful waking experiences (*7*) and recent perceptual activity (*8*) can influence dream content, the regulatory mechanisms by which material is incorporated into dreams remain elusive. Does the phasic event system of the REM state discharge principally under the influence of genetotrophic factors (*9*), or might daily waking experiences affect patterns of neuromuscular activation within the REM state as they affect dreams? If REM sleep is somehow functionally related to behavior of the visual system during the waking state, it should be possible to demonstrate behavioral or physiological interactions within the visual system across these two states. Such interactions might be observed through attributes of dream content or in terms of the characteristics of REM sleep eye movements. We measured the frequency and amplitude of eye movements while subjects were awake and when they were in REM sleep; we now report that phasic events characterizing REM sleep are influenced by experimentally induced modifications of visuomotor behavior while awake.

Six volunteer subjects, 19 to 30 years old, with normal visual acuity, depth perception, and orthophoria continuously participated in a 12-day study. Each subject underwent 2 weeks of home sleep normalization and three laboratory adaptation nights prior to 12 consecutive 24-hour periods in the experiment. During the 12 live-in days (four baseline, five experimental, and three recovery), electrooculographic (EOG) activity was recorded daily with d-c amplification while the subject was awake. REM sleep eye