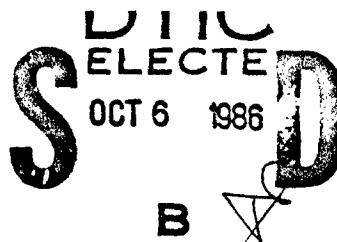


Best Available Copy

Journal of Neurochemistry
Raven Press, New York
© 1986 International Society for Neurochemistry



ARMED FORCES RADIOBIOLOGY
RESEARCH INSTITUTE
SCIENTIFIC REPORT
SR86-28

AD-A172 615

Ionizing Radiation Alters the Properties of Sodium Channels in Rat Brain Synaptosomes

Michael J. Mullin, Walter A. Hunt, and *R. Adron Harris

Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, Maryland; and
**Department of Pharmacology, School of Medicine, University of Colorado, Denver, Colorado, U.S.A.*

Abstract: The effect of ionizing radiation on neuronal membrane function was assessed by measurement of neurotoxin-stimulated $^{22}\text{Na}^+$ uptake by rat brain synaptosomes. High-energy electrons and γ photons were equally effective in reducing the maximal uptake of $^{22}\text{Na}^+$ with no significant change in the affinity of veratridine for its binding site in the channel. Ionizing radiation reduced the veratridine-stimulated uptake at the earliest times measured (3 and 5 s), when the rate of uptake was greatest. Batrachotoxin-stimulated $^{22}\text{Na}^+$ uptake was less sensitive to inhibition by radiation. The binding of [^3H]saxitoxin to its receptor in the sodium channel was unaffected by exposure to ionizing radiation. The effect of ionizing radiation on the lipid order of rat brain synaptic plasma membranes was measured by the fluores-

cence polarization of the molecular probes 1,6-diphenyl-1,3,5-hexatriene and 1-[4-(trimethylammonium)phenyl]-6-phenyl-1,3,5-hexatriene. A dose of radiation that reduced the veratridine-stimulated uptake of $^{22}\text{Na}^+$ had no effect on the fluorescence polarization of either probe. These results demonstrate an inhibitory effect of ionizing radiation on the voltage-sensitive sodium channels in rat brain synaptosomes. This effect of radiation is not dependent on changes in the order of membrane lipids. **Key Words:** Ionizing radiation—Sodium channels—Membrane fluidity—Fluorescent probes—Neuronal membranes. Mullin M. J. et al. Ionizing radiation alters the properties of sodium channels in rat brain synaptosomes. *J. Neurochem.* 47, 489–495 (1986).

Exposure to ionizing radiation has a complex effect on the CNS, an effect that depends on the dose delivered and time elapsed after radiation exposure. In the whole animal, exposure to low to moderate doses (50–400 rad) of radiation produces a conditioned taste aversion in rats (Smith, 1971; Rabin et al., 1982), a reduction in the threshold for electrically induced seizures (Rosenthal and Timiras, 1961; Pollack and Timiras, 1964; Sherwood et al., 1967), and changes in the electroencephalogram of rabbits (Minamisawa and Tsuchiza, 1977). Doses of radiation in the range of 1,000–1,500 rad produce arousal in rats (Kimeldorf and Hunt, 1965) and increased locomotor activity in mice (Mickley et al., 1983a,b). Radiation doses of >10,000 rad depress the CNS, and the irradiated animals demonstrate lethargy, disorientation, ataxia, reduced locomotor activity, and an inability to avoid shock

(Casarett and Comar, 1973; Mickley and Teitelbaum, 1978; Bogo, 1984).

The mechanisms of radiation-induced changes in CNS function are largely unknown. Neurochemical studies have demonstrated a reduction in brain cyclic nucleotide levels (Hunt and Dalton, 1980) and increases in high-affinity choline uptake and K^+ -stimulated dopamine release (Hunt et al., 1979) after exposure to 5,000–10,000 rad. In addition, β -endorphin like-immunoreactivity is reduced in the brain after 1,500 rad (Mickley et al., 1983a,b).

Electrophysiological studies of the effects of ionizing radiation have yielded conflicting results. Several studies have reported a temporary enhancement of neuronal excitability at doses of <10,000 rad (Bachofer and Gautereaux, 1960; Bachofer, 1962), whereas others have reported no change in the amplitude or rate of rise of the action potential,

Received October 18, 1985; accepted February 14, 1986.

Address correspondence and reprint requests to Dr. M. J. Mullin at Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145, U.S.A.

Abbreviations used: BTX, batrachotoxin; DPH, 1,6-diphenyl-1,3,5-hexatriene; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; SPM-2, synaptic plasma membranes; STX, saxitoxin; SV, scorpion venom; TMA-DPH, 1-[4-(trimethylammonium)phenyl]-6-phenyl-1,3,5-hexatriene; TTX, tetrodotoxin.

DTIC FILE COPY

20030121065

conduction velocity, or membrane resistance at radiation doses of <math> < 10,000 \text{ rad}</math> (Gerstner and Orth, 1950; Gasteiger and Campbell, 1962). Higher doses of radiation have usually been reported to reduce neuronal excitability.

In many cells, DNA has been shown to be the primary target for ionizing radiation. However, in the mature, postmitotic CNS, radiation-induced changes in the structure and function of neuronal membranes may be the site of action. The interaction of ionizing radiation with water is known to produce a variety of reactive products, including hydroxyl and hydrogen radicals, hydrated electrons, hydrogen peroxide, and superoxide radicals (Okada, 1970). The reactive products of water can then interact with membrane lipids or proteins, resulting in lipid peroxidation or oxidation of sulfhydryl groups (Edwards et al., 1984). Structural modification of membrane components would be expected to cause changes in the functional properties of membranes.

We have examined the effect of ionizing radiation on the properties of voltage-sensitive sodium channels in rat brain synaptosomes. Neuronal sodium channels were studied, because they are believed to be involved in the control of neuronal excitability (Catterall, 1984) and because the functional properties of these channels are sensitive to perturbations of the lipid and protein components in the membrane microenvironment (Baumgold, 1980; Saum et al., 1981; Harris and Bruno, 1985a).

MATERIALS AND METHODS

Materials

Scorpion venom (SV; *Leiurus quinquestriatus*), tetrodotoxin (TTX), and veratridine were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Batrachotoxin (BTX) was kindly supplied by Dr. John Daly (Laboratory of Bioorganic Chemistry, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, MD, U.S.A.). Carrier-free $^{22}\text{NaCl}$ was obtained from New England Nuclear (Boston, MA, U.S.A.); [^3H]saxitoxin (STX) with a specific activity of 9.3 Ci/mmol and a radiochemical purity of 50% was a generous gift from Dr. Stephen Davio (Pathophysiology Division, U.S. Army Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD, U.S.A.). Fluorescent probes were obtained from Molecular Probes, Inc. (Junction City, OR, U.S.A.). All other chemicals were obtained from commercial sources and were of analytical grade.

Animals

Male Sprague-Dawley rats weighing 200–300 g (Charles River Breeding Laboratories, Inc., Wilmington, MA, U.S.A.) were housed two per cage with free access to water and standard laboratory chow.

Measurement of synaptosomal sodium uptake

A crude synaptosomal (P_2) fraction was prepared from rat brain after removal of the cerebellum and brainstem

by a modification of the method of Gray and Whittaker (1962). The final pellet was resuspended in ice-cold incubation buffer (8–10 ml/brain) containing 5.4 mM KCl, 0.8 mM MgSO_4 , 5.5 mM glucose, 130 mM choline chloride, and 50 mM *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (HEPES), with the pH adjusted to 7.4 with Tris base. The uptake of $^{22}\text{Na}^+$ was measured by a slight modification of the method of Tamkun and Catterall (1981). Aliquots (50 μl) of the synaptosomal suspension were incubated for 2 min at 36°C. BTX or veratridine was then added, and the incubation was continued for an additional 10 min. The samples were then diluted with 300 μl of uptake solution containing 5.4 mM KCl, 0.8 mM MgSO_4 , 5.5 mM glucose, 128 mM choline chloride, 5 mM ouabain, 2 mM NaCl, 1.3 μCi of $^{22}\text{NaCl}/\text{ml}$, the indicated concentration of BTX or veratridine, and 50 mM HEPES (pH adjusted to 7.4 with Tris). After a 5-s incubation (except where noted), uptake was terminated by addition of 3 ml of ice-cold wash solution containing 163 mM choline chloride, 0.8 mM MgSO_4 , 1.7 mM CaCl_2 , 1 mg/ml of bovine serum albumin, and 5 mM HEPES (pH adjusted to 7.4 with Tris). The mixture was rapidly filtered under vacuum through a 0.45 μm cellulose filter with 0.45- μm pores (041255; Amicon), and the filters were washed twice with 3 ml of wash solution. Radioactivity was determined by liquid scintillation spectrometry. The data are presented as corrected specific uptake (Mullin and Hunt, 1984, 1985) after subtraction of nonspecific uptake (BTX or veratridine plus TTX, 1 μM present in incubation and uptake buffers).

[^3H]STX binding assay

[^3H]STX binding was measured using a slight modification of the method of Krueger et al. (1979). A synaptosomal (P_2) pellet was prepared as described above, but the final pellet was resuspended in an incubation buffer containing 140 mM NaCl and 20 mM HEPES (pH 7.5 at 4°C). An aliquot (200–300 μg of protein) of synaptosomes was mixed with various concentrations of [^3H]STX (0.10–10 nM) in a final volume of 1.5 ml, and the samples were incubated for 60 min at 0–2°C. Following incubation, the samples were diluted with 5 ml of ice-cold incubation buffer and rapidly filtered through glass fiber filters (GF/F; Whatman) under vacuum. The filters were washed twice with 5 ml of ice-cold incubation buffer, and the radioactivity remaining on the filter was determined by liquid scintillation spectrometry. Nonspecific binding was measured in the presence of 10 μM TTX. The K_D and B_{max} values were calculated according to the procedure of Scatchard (1949).

Fluorescence measurements

A HH-1 T-format polarization spectrofluorimeter (BHL Associates, Burlingame, CA, U.S.A.) with fixed excitation and emission polarization filters was used to measure fluorescence intensity parallel and perpendicular to the polarization phase of the exciting light (Harris and Schroeder, 1982). Polarization of fluorescence and intensity of fluorescence were calculated by an on-line microprocessor. Similar instrumentation is presented in more detail by Johnson et al. (1979). The fluorescent probes 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-[4-(trimethylammonium)phenyl]-6-phenyl-1,3,5-hexatriene (TMA-DPH) were used. The excitation wavelength was 362 nm, a 03FGG001 filter (Melles Griot, Irvine, CA,

U.S.A.) was used in the excitation beam, and KV389 filters (Schott Optical, Duryea, PA, U.S.A.) were used for emission. Cuvette temperature was maintained by a circulating water bath and monitored continuously by a thermocouple inserted into the cuvette to a level just above the light beam.

Synaptic plasma membrane (SPM-2) preparations were used for all fluorescence measurements. The cerebellum and brainstem were removed from the brain, and SPM-2 preparations were separated by Ficoll and sucrose density centrifugation as described by Harris and Schroeder (1982). Membranes were resuspended in phosphate-buffered saline (8 g/L of NaCl, 0.2 g/L of KCl, 0.2 g/L of KH_2PO_4 , 1.15 g/L of $\text{Na}_2\text{HPO}_4 \cdot 7 \text{H}_2\text{O}$, and 0.48 g/L of HEPES, pH 7.4) at a concentration of 1–3 mg of protein/ml and were frozen and kept at -80°C before analysis. SPM-2 preparations were diluted to 0.05 mg of protein/ml, and fluorescent probes were incorporated at 35°C for 15 min with frequent vortex mixing. DPH was dissolved in tetrahydrofuran, and TMA-DPH was dissolved in tetrahydrofuran/water (1:1 vol/vol). The probes were added in a volume of 0.3–0.5 $\mu\text{l}/\text{ml}$ to give a probe concentration of 40–80 ng/ml. Control levels of fluorescence (baseline) were determined at 20 or 35°C . In certain cases, an aliquot (1–10 μl) of ethanol was added to the cuvette, and fluorescence was determined 3–5 min later.

Irradiation procedures

The synaptosomes or SPM-2 preparations were placed in glass test tubes in a Plexiglas ice bath and were irradiated with 18.5 MeV electrons from the Armed Forces Radiobiology Research Institute linear accelerator. Pulse duration was 4 μs , and pulses were delivered at a rate of 15/s. Dose rate was ~ 12 rad/pulse. Synaptosomes were exposed to γ radiation using a ^{60}Co source at a dose rate of 100 rad/min (for the 100-rad dose only) or 7,600 rad/min. Dosimetry was performed using a 0.05- cm^3 tissue equivalent ion chamber whose calibration is traceable to the National Bureau of Standards. The ion chamber was placed in a glass test tube inside the Plexiglas ice bath during dosimetry measurements.

Miscellaneous methods

Protein content was determined by the method of Lowry et al. (1951), using bovine serum albumin as the standard. Data are presented as mean \pm SEM values. In each experiment, triplicate samples were prepared for each concentration of toxin. The number of experiments is given in the legend of each table and figure. Statistical analysis was performed using Student's *t* test. Multiple comparisons with a control were done by analysis of variance and Dunnett's test (Dunnett, 1964).

RESULTS

Ion flux studies

Incubation of synaptosomes with the alkaloid toxin veratridine caused a concentration-dependent increase in synaptosomal $^{22}\text{Na}^+$ uptake. The effects of exposure to high-energy electrons on $^{22}\text{Na}^+$ uptake are shown in Fig. 1. Irradiation with doses of 100, 1,000, and 10,000 rad caused a dose-dependent inhibition of veratridine-stimulated $^{22}\text{Na}^+$ uptake. The uptake of $^{22}\text{Na}^+$ uptake into irradiated synap-

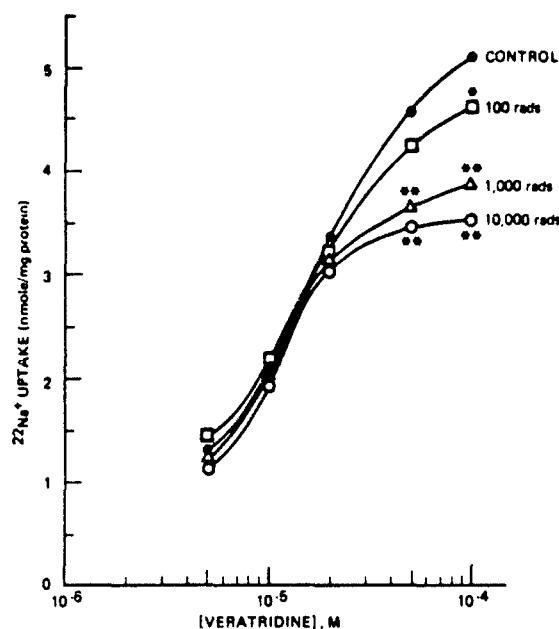


FIG. 1. Effect of high-energy electrons on veratridine-stimulated $^{22}\text{Na}^+$ uptake by rat brain synaptosomes. In each experiment, triplicate samples were incubated with the indicated concentration of veratridine, and $^{22}\text{Na}^+$ uptake was measured during a 5-s uptake phase. Data are mean values from four experiments; the SEM was 5–10% of the mean. Significant effects of radiation are indicated as follows: * $p < 0.05$, ** $p < 0.01$.

tosomes was significantly different from control values only at the higher concentrations of veratridine. A double-reciprocal analysis of these data indicated that the maximal uptake was reduced with no change in the affinity of veratridine for its binding site in the channel (data not shown). In addition, nonspecific sodium uptake in the presence of veratridine and TTX and the uptake in the absence of any added toxins were unaffected by ionizing radiation (data not shown). These findings are in agreement with previous work on ion channels in our laboratory (Wixon and Hunt, 1983).

To determine if other types of ionizing radiation had a similar effect on the sodium channel, we irradiated synaptosomes with γ rays and measured the uptake of $^{22}\text{Na}^+$ over a range of concentrations of veratridine (Fig. 2). Exposure to γ rays reduced the maximal effect of veratridine with no apparent shift in the concentration-effect curve. The magnitude of the radiation-induced inhibition of veratridine-stimulated $^{22}\text{Na}^+$ uptake was similar in synaptosomes exposed to high-energy electrons or γ radiation.

When sodium channels were activated by BTX, the effect of radiation was less pronounced at a given dose of radiation (Fig. 3). One thousand rad of high-energy electrons reduced the maximal effect of veratridine by 25%, whereas the maximal effect of BTX was reduced by only 11%. At 3 μM

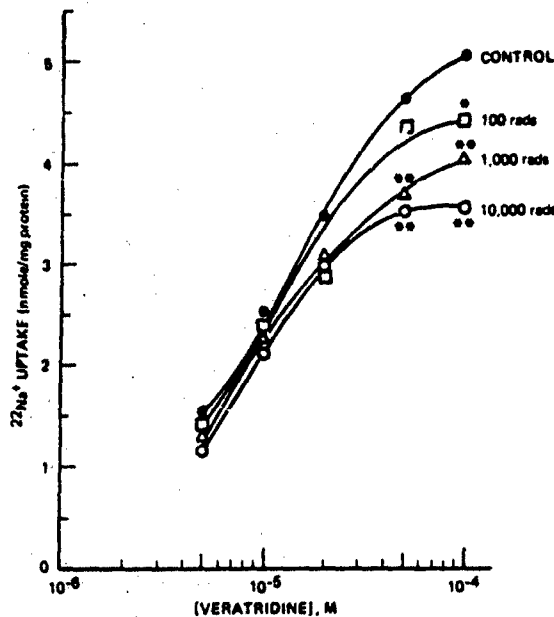


FIG. 2. Effect of γ radiation on veratridine-stimulated $^{22}\text{Na}^+$ uptake by rat brain synaptosomes. In each experiment, triplicate samples were incubated with the indicated concentration of veratridine, and $^{22}\text{Na}^+$ uptake was measured during a 5-s period. Data are mean values from three to five experiments; the SEM was 5–10% of the mean. Significant effects of radiation are indicated as follows: * $p < 0.05$, ** $p < 0.01$.

BTX, the higher dose of radiation (10,000 rad) caused a significant ($p < 0.05$) reduction in $^{22}\text{Na}^+$ uptake. This difference in the potency of radiation may be related to the fact that BTX is considered to be a full agonist, whereas veratridine is a partial agonist in stimulating $^{22}\text{Na}^+$ uptake (Catterall, 1980). In addition, the mechanism by which veratridine and BTX stimulate sodium uptake may be slightly

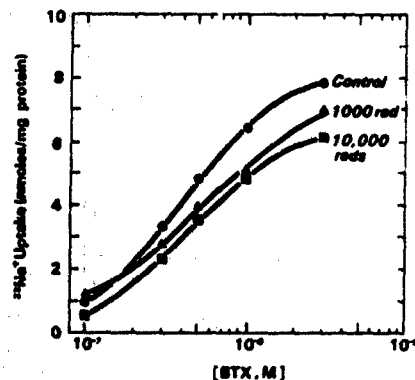


FIG. 3. Effect of high-energy electrons on BTX-stimulated $^{22}\text{Na}^+$ uptake by rat brain synaptosomes. Triplicate samples were incubated with the indicated concentration of BTX, and $^{22}\text{Na}^+$ uptake was measured during a 5-s period. Data are mean values from three experiments.

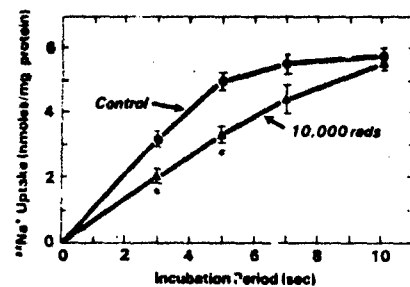


FIG. 4. Time course of veratridine-stimulated $^{22}\text{Na}^+$ uptake. In each experiment, triplicate samples were incubated with veratridine ($200 \mu\text{M}$) for 10 min, and $^{22}\text{Na}^+$ uptake was measured at the indicated interval. Synaptosomes were irradiated with 10,000 rad of high-energy electrons. Data are mean \pm SEM (bars) values from three to five experiments. A significant effect of ionizing radiation is indicated as follows: * $p < 0.05$.

different because of other properties of the toxins (Miller, 1983; Tanaka et al., 1983).

The time course of veratridine-stimulated $^{22}\text{Na}^+$ uptake in control and irradiated synaptosomes is shown in Fig. 4. Exposure to 10,000 rad of high-energy electrons significantly reduced veratridine-stimulated $^{22}\text{Na}^+$ uptake at incubation times of 3 and 5 s but not at later time points. Thus, ionizing radiation reduced the initial rate of neurotoxin-dependent $^{22}\text{Na}^+$ uptake when the influx was unidirectional and the rates of uptake were greatest.

In synaptosomes and cultured neuroblastoma cells, the small polypeptide toxins present in certain SVs enhance the neurotoxin-dependent influx of sodium through an allosteric mechanism (Catterall, 1980). The data in Table 1 illustrate that the interaction of SV and veratridine remains intact in irradiated synaptosomes.

^3H STX binding

In addition to the receptor sites for veratridine and SV, sodium channels in synaptosomes also

TABLE 1. Effect of high-energy electrons on SV-induced enhancement of veratridine-stimulated $^{22}\text{Na}^+$ uptake

Dose (rad)	$^{22}\text{Na}^+$ uptake (nmol/mg of protein/5 s)	
	Veratridine ($100 \mu\text{M}$)	Veratridine ($100 \mu\text{M}$) + SV ($100 \mu\text{g/ml}$)
0	4.97 ± 0.10	6.92 ± 0.22^a
1,000	3.91 ± 0.25^b	6.03 ± 0.36^a
10,000	3.51 ± 0.18^c	5.79 ± 0.27^a

After irradiation, synaptosomes were preincubated with SV ($100 \mu\text{g/ml}$) for 2 min followed by a 10-min incubation with veratridine, and $^{22}\text{Na}^+$ uptake was measured after a 5-s uptake phase. Data are mean \pm SEM values from three experiments.

^a Denotes a significant effect of SV, $p < 0.01$.

^b Denotes a significant effect of radiation, $p < 0.05$.

^c Denotes a significant effect of radiation, $p < 0.01$.

TABLE 2. Effect of ionizing radiation on binding of [³H]STX to rat brain synaptosomes

Group	B_{max} (pmol/mg of protein)	K_D (nM)
Control	2.80 ± 0.15	1.64 ± 0.10
Irradiated*	2.65 ± 0.23	1.76 ± 0.20

Synaptosomes were incubated at 0–2°C for 60 min with [³H]STX (0.10–10 nM) in the absence and presence of 10 μM TTX, and binding was measured using a rapid filtration assay. Data are mean ± SEM values from four experiments.

* Synaptosomes were irradiated with 10,000 rad of high-energy electrons.

contain a receptor site for TTX and STX. The binding of [³H]STX is sensitive to changes in the lipid and protein components of the membrane (Baumgold, 1980). However, exposure to 10,000 rad of high-energy electrons did not alter the equilibrium binding parameters of [³H]STX (Table 2). In addition, exposure to 25,000 rad of γ radiation did not affect the concentration of TTX required to inhibit veratridine-stimulated ²²Na⁺ uptake by 50% (data not shown).

Fluorescence polarization studies

Ionizing radiation in doses of ≥500 rad has been shown to increase the fluidity of erythrocyte membranes (Edwards et al., 1984; Yonei et al., 1984). In addition, there is a strong correlation between inhibition of veratridine-stimulated sodium influx and increased membrane fluidity in mouse brain synaptic membranes (Harris and Bruno, 1985a). The fluorescence polarization of the probes DPH and TMA-DPH in rat brain synaptic plasma membranes after 10,000 rad of ionizing radiation is shown in Table 3. There was no significant difference in the baseline fluorescence polarization of DPH or TMA-DPH in the irradiated membranes compared with the corresponding control. The response of irradiated membranes to the lipid-disordering effect of ethanol was identical to the response in control membranes (Table 4). We conclude that a 10,000-rad dose of ionizing radiation has no effect on the baseline fluidity of synaptic plasma membranes

and, in addition, the response of irradiated membranes to the fluidity-inducing effect of ethanol is also unchanged. Thus, the changes in synaptosomal sodium uptake we observed are not due to changes in the order or arrangement of lipids in the neuronal membranes.

DISCUSSION

The CNS has traditionally been considered to be somewhat resistant to the effects of ionizing radiation. However, there is a growing body of evidence that low to moderate doses of radiation cause a wide variety of behavioral effects. In addition, the state of neuronal excitability, as assessed by changes in seizure thresholds, appears to be quite sensitive to radiation (Pollack and Timiras, 1964; Sherwood et al., 1967). Only recently have the possible mechanisms underlying these effects been studied with the methods available to membrane biologists and neurochemists.

The results of the present study confirm and extend our earlier observation that ionizing radiation reduced the veratridine-stimulated uptake of ²²Na⁺ by rat brain synaptosomes (Wixon and Hunt, 1983). In addition, we determined that high-energy electrons and γ radiation were equally effective in reducing veratridine-stimulated ²²Na⁺ uptake. In this regard, it is interesting to note that high-energy electrons are nearly twice as potent as γ photons in degrading performance in certain behavioral paradigms (Hunt, 1983). Ionizing radiation did not affect the interaction of SV with veratridine or the binding of [³H]STX to its receptor site in the sodium channel. Thus, only the receptor site for veratridine was affected by exposure of the membrane to ionizing radiation. Ionizing radiation reduced the maximal effect of veratridine and BTX without affecting the affinity of these toxins for the receptor site in the channel. We are currently studying the effect directly by measuring the effect of radiation on the binding of radiolabeled BTX.

There are several similarities in the effects of ionizing radiation and intoxicant-anesthetic drugs on the function of rat brain sodium channels. These

TABLE 3. Effect of ionizing radiation on baseline fluorescence polarization of DPH and TMA-DPH

Probe	Temperature	Baseline fluorescence polarization	
		Control	Irradiated
DPH	20°C	0.313 ± 0.002	0.315 ± 0.004
	35°C	0.253 ± 0.002	0.253 ± 0.004
TMA-DPH	20°C	0.335 ± 0.002	0.339 ± 0.003
	35°C	0.303 ± 0.002	0.305 ± 0.003

SPM-2 preparations were irradiated with 10,000 rad of high-energy electrons, and fluorescence polarization was measured as described in Materials and Methods. Data are mean ± SEM values (n = 6).

TABLE 4. Effect of ethanol *in vitro* on fluorescence polarization of DPH

Ethanol concentration (mM)	Change in polarization of DPH	
	Control	Irradiated*
75	-0.003 ± 0.001	-0.002 ± 0.001
150	-0.004 ± 0.001	-0.004 ± 0.001
300	-0.007 ± 0.001	-0.008 ± 0.001
600	-0.013 ± 0.001	-0.013 ± 0.002

The indicated concentration of ethanol was added to each cuvette, and the change in the baseline fluorescence polarization of DPH was measured at 35°C. Data are mean ± SEM values (n = 6).

* SPM-2 preparations were irradiated with 10,000 rad of high-energy electrons.

drugs and radiation reduce the initial rate of uptake and reduce the maximal effect of the toxins with little or no effect on affinity, and neither the SV-veratridine interaction nor the action of TTX is affected appreciably (Mullin and Hunt, 1984, 1985; Harris and Bruno, 1985b). Also, the effect of BTX is less sensitive than the effect of veratridine to inhibition by these agents (Mullin and Hunt, 1985; Harris and Bruno, 1985b).

Because ionizing radiation has been shown to increase the fluidity of erythrocyte membranes (Yonei and Kato, 1978; Yonei et al., 1984) and because there is a good correlation between increased membrane fluidity and reduced neurotoxin-stimulated sodium influx (Harris and Bruno, 1985a), we investigated the effects of ionizing radiation on the fluidity of synaptic plasma membranes and no difference in the response of these membranes to the lipid-disordering effect of ethanol at radiation doses that reduce the neurotoxin-stimulated uptake of $^{22}\text{Na}^+$ by synaptosomes. As DPH is a probe of the lower (methyl terminal) portions of lipid acyl groups and TMA-DPH is a probe of the glycerol backbone and upper (carboxyl) portions of the acyl groups in membranes (Harris et al., 1984), we conclude that a 10,000-rad dose of ionizing radiation does not affect the order or packing of lipids in the neuronal membranes. Although alterations in the order of packing of membrane lipids may be an important determinant of radiation damage in certain biological membranes (Edwards et al., 1984; Yonei et al., 1984), this does not appear to be the case for membranes derived from the CNS. Perturbation of other membrane constituents such as oxidation of sulfhydryl groups and/or changes in protein conformation may be related to the observed effect of ionizing radiation on the functional properties of neuronal sodium channels.

Acknowledgment: We thank Mr. Tom Dalton for expert technical assistance and Mrs. Marion Golightly for excellent secretarial assistance in preparing the manuscript.

REFERENCES

- Bachofner C. S. and Gautereaux M. E. (1960) Bioelectric responses *in situ* of mammalian nerves exposed to x-rays. *Am. J. Physiol.* 198, 715-717.
- Bachofner C. S. (1962) Radiation effects on bioelectric activity of nerves, in *Response of the Nervous System to Ionizing Radiation* (Haley T. J. and Snider R. S., eds), pp. 573-583. Academic Press, New York.
- Baumgold, J. (1980) ^3H -Saxitoxin binding to nerve membranes: inhibition by phospholipase A_2 and by unsaturated fatty acids. *J. Neurochem.* 34, 327-334.
- Bogo V. (1984) Effects of bremsstrahlung and electron radiation on rat motor performance. *Radiat. Res.* 100, 313-320.
- Casarett A. P. and Comar C. L. (1973) Incapacitation and performance decrement in rats following split doses of fission spectrum radiation. *Radiat. Res.* 53, 455-461.
- Catterall W. A. (1980) Neurotoxins that act on voltage-sensitive sodium channels in excitable membranes. *Annu. Rev. Pharmacol. Toxicol.* 20, 15-43.
- Catterall W. A. (1984) The molecular basis of neuronal excitability. *Science* 223, 653-661.
- Dunnett C. W. (1964) New tables of multiple comparisons with a control. *Biometrics* 20, 482-491.
- Edwards J. C., Chapman D., Cramp W. A., and Yatvin M. B. (1984) The effects of ionizing radiation on biomembrane structure and function. *Prog. Biophys. Mol. Biol.* 43, 71-93.
- Gasteiger E. L. and Campbell B. (1962) Alteration of mammalian nerve compound action potentials by beta irradiation, in *Response of the Nervous System to Ionizing Radiation* (Haley T. J. and Snider R. S., eds), pp. 597-606. Academic Press, New York.
- Gerstner H. B. and Orth J. S. (1950) Effects of high intensity x-ray irradiation on the velocity of nerve conduction. *Am. J. Physiol.* 130, 232-286.
- Gray E. G. and Whittaker V. P. (1962) The isolation of nerve endings from brain: an electron-microscopic study of cell fragments derived by homogenization and centrifugation. *J. Anat.* 96, 79-87.
- Harris R. A. and Bruno P. (1985a) Membrane disordering by anesthetic drugs: relationship to synaptosomal sodium and calcium fluxes. *J. Neurochem.* 44, 1274-1281.
- Harris R. A. and Bruno P. (1985b) Effects of ethanol and other intoxicant-anesthetics on voltage-dependent sodium channels of brain synaptosomes. *J. Pharmacol. Exp. Ther.* 232, 401-406.
- Harris R. A. and Schroeder T. (1982) Effects of barbiturates and ethanol on the physical properties of brain membranes. *J. Pharmacol. Exp. Ther.* 223, 424-431.
- Harris R. A., Baxter D. M., Mitchell M. A., and Hitzemann R. J. (1984) Physical properties and lipid composition of brain membranes from ethanol tolerant-dependent mice. *Mol. Pharmacol.* 25, 401-409.
- Hunt W. A. (1983) Comparative effects of exposure to high-energy electrons and gamma radiation on active avoidance behaviour. *Int. J. Radiat. Biol.* 44, 257-260.
- Hunt W. A., and Dalton T. K. (1980) Reduction in cyclic nucleotide levels in the brain after a high dose of ionizing radiation. *Radiat. Res.* 83, 210-215.
- Hunt W. A., Dalton T. K., and Darden J. H. (1979) Transient alterations in neurotransmitter activity in the caudate nucleus of rat brain after a high dose of ionizing radiation. *Radiat. Res.* 80, 556-562.
- Johnson D. A., Lee N. M., Cooke R., and Loh H. H. (1979) Ethanol-induced fluidization of brain lipid bilayers: required presence of cholesterol in membranes for the expression of tolerance. *Mol. Pharmacol.* 15, 739-746.
- Kimeldorf D. J. and Hunt E. L. (1965) Radiation effects on performance capacity, in *Ionizing Radiation: Neural Function and Behavior* (Kimeldorf D. J. and Hunt E. L., eds), pp. 166-213. Academic Press, New York.

- Krueger B. K., Ratzlaff R. W., Strichartz G. R., and Blaustein M. P. (1979) Saxitoxin binding to synaptosomes, membranes and solubilized binding sites from rat brain. *J. Membr. Biol.* 50, 287-310.
- Lowry O. H., Rosebrough N. J., Farr A. L., and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265-275.
- Mickley G. A. and Teitelbaum H. (1978) Persistence of lateral hypothalamic-mediated behaviors after a supralethal dose of ionizing radiation. *Aviat. Space Environ. Med.* 49, 868-873.
- Mickley G. A., Stevens K. E., White G. A., and Gibbs G. L. (1983a) Endogenous opiates mediate radiogenic behavioral change. *Science* 220, 1185-1187.
- Mickley G. A., Stevens K. E., Moore G. H., Deere W., White G. A., Gibbs G. L., and Mueller G. P. (1983b) Ionizing radiation alters beta-endorphin-like immunoreactivity in brain but not blood. *Pharmacol. Biochem. Behavior* 19, 979-983.
- Miller C. (1983) Integral membrane channels: studies in model membranes. *Physiol. Rev.* 63, 1209-1242.
- Minamisawa T. and Tsucuzi T. (1977) Long-term changes in the averaged evoked potentials of the rabbit after irradiation with moderate x-ray doses. *Electroencephalogr. Clin. Neurophysiol.* 43, 416-424.
- Mullin M. J. and Hunt W. A. (1984) Ethanol inhibits veratridine-stimulated sodium uptake in synaptosomes. *Life Sci.* 34, 287-292.
- Mullin M. J. and Hunt W. A. (1985) Actions of ethanol on voltage-sensitive sodium channels: effects on neurotoxin-stimulated sodium uptake in synaptosomes. *J. Pharmacol. Exp. Ther.* 232, 413-419.
- Okada S. (1970) Cells. in *Radiation Biochemistry, Vol. 1*. (Altman K. L., Gerber G. B., and Okada S., eds), pp. 3-76. Academic Press, New York.
- Pollack M. and Timiras P. S. (1964) X-ray dose and electroconvulsive responses in adult rats. *Radiat. Res.* 21, 111-119.
- Rabin B. M., Hunt W. A., and Lee J. (1982) Studies on the role of central histamine in the acquisition of a radiation-induced conditioned taste aversion. *Radiat. Res.* 90, 609-620.
- Rosenthal E. and Timiras P. S. (1961) Changes in brain excitability after whole-body x-irradiation in the rat. *Radiat. Res.* 15, 648-657.
- Saum W. R., McGee R. Jr., and Love J. (1981) Alteration of the action potential of tissue cultured neuronal cells by growth in the presence of a polyunsaturated fatty acid. *Cell Mol. Neurobiol.* 1, 319-324.
- Scatchard G. (1949) The attraction of proteins for small molecules and ions, in *Annals of the New York Academy of Sciences, Vol. 51: Molecular Interactions* (Fuoss R. M., ed), pp. 660-672. New York Academy of Sciences, New York.
- Sheiwood N. M., Welch G. P., and Timiras P. S. (1967) Changes in electroconvulsive thresholds and patterns in rats after x-rays and high-energy proton irradiation. *Radiat. Res.* 30, 374-390.
- Smith J. C. (1971) Radiation: its detection and its effects on taste preferences, in *Progress in Physiological Psychology, Vol. 4* (Stellar E. and Sprague J. M., eds), pp. 53-117. Academic Press, New York.
- Tamkun M. M. and Catterall W. A. (1981) Ion flux studies of voltage-sensitive sodium channels in synaptic nerve-ending particles. *Mol. Pharmacol.* 19, 78-86.
- Tanaka J. C., Eccleston J. F., and Barchi R. L. (1983) Cation selectivity characteristics of the reconstituted voltage-dependent sodium channel purified from rat skeletal muscle sarcolemma. *J. Biol. Chem.* 258, 7519-7526.
- Wixon H. N. and Hunt W. A. (1983) Ionizing radiation decreases veratridine-stimulated uptake of sodium in rat brain synaptosomes. *Science* 220, 1073-1074.
- Yonei S. and Kato M. (1978) X-ray induced structural changes in erythrocyte membranes studied by use of fluorescent probes. *Radiat. Res.* 75, 31-45.
- Yonei S., Akasaka S., and Kato M. (1984) Studies on the mechanism of radiation-induced structural disorganization of human erythrocyte membranes. *Int. J. Radiat. Biol.* 46, 463-471.



Accession For	
NTIS GRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	21