The Effect of Sympathomimetic Amines on Radiation-Induced Tolerance to Pentobarbital in the Rat (34800)

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Sublethal doses of radiation have been shown to increase the electrical background activity of the central nervous system (CNS) (1–3). Barnes reported that 102 R of X rays enhanced pentobarbital tolerance in mice (4). He suggested that this radiation-induced increase in tolerance to pentobarbital is due to facilitated CNS electrical activity antagonizing barbiturate depression.

Under normal physiologic conditions facilitated CNS electrical transmissions would result in an increased release of central neurotransmitter. If radiation increases tolerance to pentobarbital by facilitating electrical activity, then drugs which increase the release rate of or deplete stored quantities of central neurotransmitter should modify radiation-induced changes in pentobarbital toxicity. The ability of irradiated rats to tolerate pentobarbital was measured after treatment with combinations of amphetamine and reserpine to test this hypothesis.

Materials and Methods. Male Sprague-Dawley rats[2] (250–300 g) were housed one per cage in an environment-controlled room and acclimated for a minimum of 1 week.

Rats were bilaterally irradiated using an X-ray generator and the following exposure factors: 300 kVp, 20 mA, inherent filtration 4.8 mm beryllium, added filtration 2 mm copper and 1 mm aluminum, HVL 1.5 mm copper, and source-to-animal center line distance of 50 cm. The animals were placed in rectangular Plexiglas[3] boxes, and two rats were irradiated at the same time. Depth-dose measurements made in cylindrical Plexiglas rat phantoms gave a dose rate at the midline of 129 rads/min. Groups of rats received midline tissue doses of 250, 500, or 1000 rads.

Sodium pentobarbital acute toxicity was determined 24 hr after the animals were irradiated. Control, fasted control and irradiated animals were lightly anesthetized with ether, and the tail vein was catheterized using a 23-gauge needle attached to polyethylene tubing (i.d. 0.011 in., o.d. 0.024 in.). Catheterization was performed approximately 15 min before pentobarbital infusion was initiated. The rats were allowed to recover from the anesthesia (recovery was considered complete when the rat was able to walk without ataxia), then were confined in Plexiglas cylinders for infusion. Pentobarbital was infused via the polyethylene tubing at a constant rate of 2.95 mg/min (0.116 ml of solution per min) using an infusion pump. The end point of acute toxicity for pentobarbital in the animal was the cessation of respiration. The amount of pentobarbital required to achieve this end point was calculated as milligrams per kilogram of body weight.

The effect of amphetamine sulfate on pentobartibal toxicity was determined in irradiated (24 hr after 1000 rads) and in unirradiated rats. Amphetamine (8 mg/kg) was administered intraperitoneally ½ hr prior to pentobarbital infusion (a time sufficient to insure the onset of drug activity as indicated by restlessness, agitation, and increased mo-

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tor activity of the animals). The effect of preirradiation reserpine treatment (0.1 mg/kg/day intramuscularly for 4 days) on pentobarbital acute toxicity was tested in irradiated (24 hr after 1000 rads) and unirradiated rats. The same regimen of reserpine treatment was tested in conjunction with amphetamine given ½ hr prior to pentobarbital infusion in irradiated (24 hr after 1000 rads) and in unirradiated rats.

Differences between the mean values of the test groups were evaluated for statistical significance using Student's *t* test.

Results. Table I summarizes the results. Rats demonstrated an increased pentobarbital tolerance 24 hr after receiving 500 and 1000 rads, but not after 250 rads. Fasting unirradiated rats for 48 hr had no effect on pentobarbital tolerance.

Unirradiated, amphetamine-treated rats demonstrated an increased ability to tolerate pentobarbital. When amphetamine was administered to animals 24 hr after a 1000-rad dose a decrease in pentobarbital tolerance was observed; this decrease was not significant at the 0.05 level of probability.

TABLE I. The Effects of X-Ray, Amphetamine, and Reserpine Administration on Pentobarbital Acute Toxicity in the Rat.

Regimen	Number of rats	Toxic dose of pentobarbital mg $kg^{-1} \pm$ SE
Control	29	87.2 ± 5.5
Control, fasted 48 hr	12	85.7 ± 5.2
Irradiated (24 hr after 250 rads)	15	85.6 ± 4.8
Irradiated (24 hr after 500 rads)	12	[a]107.6 ± 5.4
Irradiated (24 hr after 1000 rads)	12	[a]106.3 ± 3.5
Amphetamine-treated	21	[a]105.7 ± 4.8
Amphetamine-treated (24 hr after 1000 rads)	18	76.5 ± 5.0
Reserpine-treated	7	85.9 ± 7.1
Reserpine- and Amphetamine-treated	9	91.2 ± 4.8
Reserpine-treated (24 hr after 1000 rads)	10	91.1 ± 5.9
Reserpine- and Amphetamine-treated (24 hr after 1000 rads)	11	84.7 ± 2.9

[a] $p < 0.05$ (treatment group compared with control group).

Four days of reserpine treatment abolished the increased tolerance to pentobarbital in unirradiated amphetamine-treated rats as well as in irradiated rats.

Discussion. Five hundred- and 1000-rad whole-body doses of X rays were antagonistic to the depressant action of pentobarbital on the CNS of the rat; however, no such effect resulted from a dose of 250 rads. The increased ability to tolerate pentobarbital was not due to inappetence of the animals since unirradiated rats fasted for 48 hr did not show any change in pentobarbital tolerance as compared to fed control animals. The increased central neuronal activity suggested by the data from this study appears to confirm the reported increase in CNS electrical activity after exposure to radiation.

In situations of stress, the immediate physiologic response of the animal is attempted restoration of homeostasis. A large facilitatory input from the peripheral organs is transmitted to the reticular activating system which, in turn, increases the electrical activity of nearly all parts of the CNS to accomplish this restoration (5). Increased bioelectrical activity would result in an increased release of central neurotransmitters, particularly in the important respiratory and vasomotor centers located in the midbrain. Chief of these neurotransmitters is norepinephrine which is normally found in high concentrations throughout this region of the CNS (6).

Reserpine and amphetamine were studied in various combinations with pentobarbital and irradiation. Both drugs act centrally by increasing the release of norepinephrine (6). Amphetamine's ability to reverse the depressant effect of barbiturates (7) was observed as a decreased pentobarbital toxicity in unirradiated animals. This decreased pentobarbital toxicity appeared similar to that observed after irradiation. However, irradiated rats treated with amphetamine appeared to have a decreased tolerance to pentobarbi-

tal, 24 hr postexposure (1000 rads), as compared to control animals (the decrease was not statistically significant in this experiment). From these data, it appears that when X radiation and amphetamine treatment are combined the ability of each to counteract CNS pentobarbital depression is lost. It is difficult to explain why the combination of amphetamine and irradiation does not increase pentobarbital tolerance when each used singly does.

Reserpine is an intraneuronal depletor of norepinephrine (8). Reserpinization abolished the increased tolerance of irradiated animals to pentobarbital. This observation further implicated central norepinephrine in the decreased pentobarbital toxicity caused by X irradiation. Similar effects of reserpinization on pentobarbital toxicity were observed in amphetamine-treated rats.

Summary. Male Sprague-Dawley rats were given 250-, 500-, or 1000- rad midline tissue doses of 300 kVp X rays. Twenty-four hours postirradiation the acute toxicity of sodium pentobarbital was determined in these animals. Rats receiving 500 and 1000 rads demonstrated an increased tolerance to sodium pentobarbital, but no change in toxicity was observed in animals given 250 rads, indicating a threshold dose of radiation is necessary to elicit this response. This radiation-induced increase in pentobarbital tolerance was similar in degree to that observed in unirradiated rats treated with amphetamine, a known barbiturate antagonist. However, when rats received amphetamine as well as 1000 rads of X rays no increased tolerance to sodium pentobarbital was observed. Four days of reserpine treatment prior to irradiation also abolished the radiation-induced increase in sodium pentobarbital tolerance. These results suggest that increased central norepinephrine levels are responsible for the radiation-induced increase in tolerance to pentobarbital.

1. Cooper, G. P., and Kimeldorf, D. J., Science **143**, 1040 (1964).

2. Garcia, J., Buchwald, N. A., Bach-y-Rita, G., Feder, B. H., and Koelling, R. A., Science **140**, 289 (1963).

3. Hunt, E. L., and Kimeldorf, D. J., Nature **200**, 536 (1963).

4. Barnes, C. D., Radiat. Res. 30, 351 (1967).

5. Guyton, A. C., "Textbook of Medical Physiology," p. 786. Saunders, Philadelphia (1966).

6. Iverson, L., and Glowinski, J., J. Neurochem. **13**, 671 (1966).

7. Goodman, L. S., and Gilman, A., "The Pharmacological Basis of Therapeutics," 3rd ed., p. 501. Macmillan, New York (1965).

8. Axelrod, J., Physiologist **11**, 63 (1968).

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