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Comparison of Behavioral and Radioprotective Effects of WR-2721 and WR-3689

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McDONOUGH, J. H., P. C. MELE AND C. G. FRANZ. *Comparison of behavioral and radioprotective effects of WR-2721 and WR-3689.* PHARMACOL BIOCHEM BEHAV **42**(2) 233–243, 1992.—The behavioral effects of the radioprotectant agents ethiofos, *S*-2-(3-aminopropylamino)ethylphosphorothioic acid (WR-2721) and *S*-2-(3-methylaminopropyl)aminoethylphosphorothioic acid (WR-3689) were evaluated in rats trained to respond under a multiple fixed-interval 120-s, fixed-ratio 50-response (mult FI FR) schedule of milk reinforcement. Each compound produced dose-dependent reductions in responding under both schedules over the same dose range (100–180 mg/kg, IP); ED_{50}s indicated that WR-3689 was slightly more potent than WR-2721. On several performance measures, WR-3689 produced greater decrements during a second dose-effect determination, whereas WR-2721 had more pronounced effects during the initial one. In a second series of studies, low (56 mg/kg) and high (180 mg/kg) doses of both drugs were tested for radioprotective effects in rats responding under an FR-50 schedule of milk reinforcement and exposed to a nonlethal (5 gray, Gy) or lethal (10 Gy) dose of ionizing radiation (^{60}Co gamma rays). Neither dose of radiation altered FR response rates on the day of exposure (day 1). Five Gy of gamma radiation produced a 25–40% reduction in response rates on days 2–5 (24–72 h) after exposure. Neither dose of WR-2721 or WR-3689 provided significant protection against these performance decrements. All groups exposed to 10 Gy experienced a progressive decline in FR responding on days 2–5 after exposure. Performance of groups that received pretreatment with the 180-mg/kg dose of either drug or the 56-mg/kg dose of WR-3689 was maintained at significantly higher levels than saline-treated controls on days 4–5 after exposure to 10 Gy; however, even at these higher levels of performance response rates remained below 50% of preirradiation control levels. Subsequently, 56 and 180 mg/kg WR-3689 and 180 mg/kg WR-2721 were found to provide protection against the lethal consequences of the 10-Gy exposure. Thus, neither WR-2721 nor WR-3689 afforded any significant short-term protection against radiation-induced performance decrements when these drugs were administered at either behaviorally ineffective or behaviorally disruptive doses. Rather, the beneficial effects of these drugs paralleled their ability to antagonize radiation-induced lethality.

Ionizing radiation Radioprotection WR-2721 WR-3689 Operant behavior Performance decrement Rats

THE radioprotective compound ethiofos [*S*-2-(3-aminopropylamino)ethylphosphorothioic acid] (WR-2721) is generally considered the most efficacious drug for protection against the lethal effects of ionizing radiation (6,9,10,26). Under optimal conditions, animals treated with WR-2721 survive exposure to almost twice the lethal dose of radiation (3,6,26), and WR-2721 is currently used clinically as an adjunct to both radiotherapy and chemotherapy treatment for cancer (10,25). However, WR-2721 has several drawbacks for use in other than clinical settings. First, WR-2721 is ineffective when administered orally. Second, it produces significant behavioral side effects at doses that provide maximal protection against radiation-induced lethality (1,2,13,15,16). For example, in animal studies, doses from 200–400 mg/kg WR-2721 provide progressively greater levels of radioprotection, but these doses also produce progressively greater and prolonged reductions in trained or spontaneous motor activity (2,13,16). In humans, hypotension, gastrointestinal disturbances, and hypocalcemia are the most serious and most frequently reported side effects (9).

S-2-(3-Methylaminopropyl) aminoethylphosphorothioic acid (WR-3689) is another radioprotectant drug that is a close structural analog of WR-2721. Although the radioprotectant capabilities of WR-3689 are reported to be less than those achieved with WR-2721, WR-3689 is less toxic and retains its radioprotective effect when administered orally (3,7,9,10). For these reasons, WR-3689 may have use in a wider variety of situations than WR-2721. The behavioral side effects of WR-3689, however, have not been systematically evaluated. Therefore, the first goal of this investigation was to evaluate the behaviorally disrupting effects of these two radioprotectant drugs on a multiple fixed-interval 120-s, fixed-ratio 50-response (mult FI FR) schedule of reinforcement. This operant conditioning procedure has been used routinely to char-

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acterize the behavioral effects of a wide variety of compounds (14).

Exposure to ionizing radiation produces disruptions of schedule-controlled behavior that are both dose and time dependent (4,5,11,18–20). The ability of radioprotectant agents to moderate these radiation-induced performance decrements has not been extensively investigated; only four studies have been reported that evaluated the ability of radioprotectants such as WR-2721 to counteract the effects of radiation exposure on performance (1,2,13,21). Sharp et al. (21) reported that pretreatment of rhesus monkeys with *n*-decylaminoethanethio-sulfuric acid (WR-1607) prevented the immediate but temporary degradation in performance that occurs following exposure to rapidly delivered, supralethal doses of radiation (10–40 gray, Gy). (The Gy is a unit of absorbed dose of ionizing radiation.) However, WR-1607 provided no protection against the lethal effects of these high levels of exposure. Bogo et al. (1,2) studied the ability of WR-2721 to protect rodents and nonhuman primates from the early performance decrements produced by exposure to high, supralethal doses of radiation. Unlike WR-1607, which protected against such decrements, the combined effects of WR-2721 and radiation exposure produced more severe performance decrements than either treatment alone. In these three studies, the radiation challenge doses that were used were well in excess of levels against which lethality protection can be provided with these compounds. In contrast, Landauer et al. (13) reported that mice protected with WR-2721 against the lethal effects of lower doses of radiation displayed suppressed levels of spontaneous activity for up to 6 months after exposure. Thus, a second goal of the present work was to determine whether either WR-2721 or WR-3689 could provide protection against the behavioral as well as lethal effects of radiation exposure. The approach taken, however, differed from that used in previous studies. First, both low as well as high doses of the radioprotectants were used to determine whether drug doses that have minimal behavioral effects may afford any protection. Second, two radiation doses were used: a low (5 Gy) dose that produces moderate performance decrements but is not lethal, and a high (10 Gy) dose that produces pronounced performance decrements and is ultimately lethal but is still within the protective range of these drugs.

EXPERIMENT 1

METHOD

Animals

Seven adult, male Sprague-Dawley rats, weighing 275–350 g at the start of the study, were used. They were quarantined on arrival and screened for evidence of disease. They were housed individually in plastic Micro-isolator cages containing sterilized woodchip bedding. Acidified water (pH = 2.5–3.0), commonly used to reduce the possibility of infection in irradiated organisms, was provided ad lib. Access to food was restricted to maintain animals at 80% of their free-feeding body weights established prior to behavioral testing. Animal holding rooms were maintained at 21 ± 1°C with 50 ± 10% relative humidity using at least 10 air changes per hour of 100% conditioned fresh air. A 12-h lighting cycle was in effect with full-spectrum lights on from 0600–1800.

Apparatus

Identical operant conditioning chambers (Coulbourn Instruments, Inc., Lehigh Valley, PA) were used. The front wall of each chamber contained a response lever, three cue lights mounted above the lever, a house light, a Sonalert speaker, and an opening that allowed access to a dipper that presented 0.06 ml sweetened condensed milk (a 1:1, v:v, mixture of Bordens Eagle Brand and tapwater). Each chamber was enclosed in a sound-attenuating cubicle that was equipped with an exhaust fan for frequent air exchange. The testing room containing the cubicles had masking noise present continuously. Control of experimental stations and recording of data were accomplished with a PDP 11/73 computer using SKED-11 software (State Systems, Inc., Kalamazoo, MI) and cumulative recorders (Gerbrands Corp., Arlington, MA) located in a separate room.

Behavioral Procedure

Rats were trained to lever press using an automated procedure consisting of two schedules of milk delivery that were in effect simultaneously. When the house light was illuminated, a variable-time (VT) schedule presented the dipper automatically on the average of once every 60 s, while a fixed-ratio 1 (FR 1) schedule presented the dipper after each lever press. The dipper was presented for 4 s and was signalled by illuminating a light over the dipper; the house light was extinguished during dipper presentation. The VT schedule was discontinued after 10 lever-press responses had been made in a single daily session. Sessions lasted 60 min or until 100 responses had been made, whichever occurred first. Rats that did not acquire the lever-press response after five to seven training sessions were shaped by the method of successive approximations.

After an additional one or two sessions under FR 1, rats were exposed to a series of increasing fixed-interval (FI) schedules over the next 10–15 training sessions until an FI 120-s schedule was in effect. Training on the FI 120-s schedule was continued for another 10–15 sessions, and then the FR component of the multiple schedule was introduced. The FR value was gradually raised over 10–15 training sessions until the terminal FR 50 schedule was reached. Only the house light was illuminated during the FI component. During the FR component, the three cue lights were illuminated and a 60-dB, 2.8-KHz tone was sounded. Each component schedule was presented three times during a daily session; the FI schedule component was always presented first, and the two schedules alternated throughout the session. Components ended with the first reinforcer delivered after 10 min or automatically if a reinforcer was not obtained after 12.5 min (2.5 min limited hold on component duration). There was a 10-s time out (TO) between components when all environmental cues were extinguished. Total session time was approximately 1 h. Rats were trained for approximately three months on the final schedule to establish stable baselines before drug testing was begun.

Drugs

WR-2721 (lot BL20103) and WR-3689 (lot BL08385) were obtained from the Department of Experimental Therapeutics, Walter Reed Army Institute of Research. The drugs were dissolved in saline immediately before injection. Injection volume was 1 ml/kg. Drugs were injected IP 15 min before behavioral testing. All rats were tested at each drug dose and each dose was tested twice. Doses were given in an ascending and then a descending order. Drugs were usually administered on Tuesdays and Fridays. Dose-effect curves were determined first for WR-2721 and then for WR-3689.

Data Analysis

Overall response rate, postreinforcement pause duration, and running response rate were calculated for both FI and FR responding for each session. Overall response rate was calculated by dividing the total number of responses in the three FI or FR components by the total duration of the respective components (excluding the time the dipper was raised). Postreinforcement pause duration was defined as the time from the end of a dipper presentation until the first response of the next ratio or interval. Running response rate was the response rate calculated with the postreinforcement pause time omitted. Index of curvature was also calculated for the FI data (8) to provide a measure of the temporal pattern of responding, often positively accelerated, that typically occurs under this schedule of reinforcement. For each behavioral measure, the data were analyzed using a within-group design with both drug dose and replication considered repeated-measures factors. The criterion level for significance was set at $p < 0.05$. In addition, dose estimations for drug effects on performance were determined using the following procedure. For each rat, the FI and FR response rate data from each baseline session prior to each drug session were used to calculate 95% confidence limits for nondrug performance. Then, for each drug session the performance of each rat was dichotomously categorized as "not decremented" (within the 95% confidence limits) or "decremented" (below the lower 95% confidence limit); performance after drug administration never exceeded the upper 95% confidence limit. These data were then used to determine ED_{50} using standard probit analysis procedures.

RESULTS

WR-2721 decreased FI response rates in a dose-dependent manner (Fig. 1, top); significant decreases occurred at the 133- and 180-mg/kg doses, $F(6, 36) = 6.20$, $p < 0.01$. There was a significant dose × replication interaction on FI responding, $F(6, 36) = 3.23$, $p < 0.05$. This was due to a greater decrease in FI response rates the second time rats received 56 mg/kg and less of a decrease in FI rates the second time they received 180 mg/kg.

There was also a significant dose × replication interaction for the effect of WR-2721 on the number of FI reinforcers earned, $F(6, 36) = 4.03$, $p < 0.01$. On the ascending series, there was a reduction in the number of FI reinforcers earned after the 133- or 180-mg/kg doses, while almost all possible reinforcers were earned during the descending drug series (Fig. 1, middle). Analysis of the index of curvature data revealed that there were no dose-related changes in this measure of FI performance (not shown).

The 133- and 180-mg/kg doses of WR-2721 also produced significant decreases in FR response rates [$F(6, 36) = 7.46$, $p < 0.01$; Fig. 1, bottom]; the replication and dose × replication factors were not significant. The decrease in FR responding produced by WR-2721 was due to a reduction in FR running response rate, $F(6, 36) = 7.15$, $p < 0.01$, and a concurrent increase in postreinforcement pause time [$F(6, 36) = 4.59$, $p < 0.01$; not shown].In addition, there was a significant dose × replication effect for FR running response rates $F(6, 36) = 2.64$, $p < 0.05$; these rates were decreased less the second time rats received the 180-mg/kg WR-2721 dose.

Like WR-2721, WR-3689 significantly decreased FI response rates, $F(5, 30) = 4.09$, $p < 0.05$, at doses of 133 and 180 mg/kg (Fig. 1, top). However, unlike WR-2721, WR-3689 did not reduce response rates in a manner that varied significantly across replications of the dose-effect function, even though response rates tended to be decreased to a greater degree during the second (descending) than the first (ascending) determination.

WR-3689 also decreased the number of FI reinforcers earned [$F(5, 30) = 10.57$, $p < 0.01$; Fig. 1, middle]. This effect was significantly greater on the descending-dose series than on the ascending series, $F(1, 6) = 6.45$, $p < 0.05$, which was the reverse of effects observed with WR-2721. On the FI index of curvature measure, WR-3689 produced dose-dependent decreases that paralleled the effect on overall FI responding [$F(5, 30) = 7.45$, $p < 0.01$; not shown]. However, because reductions in the index of curvature occurred only when response rates were severely suppressed this effect cannot be considered particularly meaningful.

WR-3689, at 133 and 180 mg/kg, produced significant reductions in overall responding in the FR component [$F(5, 30) = 9.42$, $p < 0.01$; Fig. 1, bottom]; this effect did not vary significantly between the ascending- and descending-dose series. The reduction in overall FR responding was due to a reduction in running response rate, $F(5, 30) = 11.67$, $p < 0.01$, and an increase in postreinforcement pause time [$F(5, 30) = 7.65$, $p < 0.01$; not shown]. Moreover, there was a significantly greater ($p < 0.05$) increase in total FR postreinforcement pause time on the descending-dose series mean = 221.2 s) compared to the ascending series (mean = 74.4 s).

A comparison of the effects of each drug on rates of responding expressed as a percentage of baseline control rates is presented in Fig. 2. Analysis of these data showed that WR-2721 produced equivalent rate-decreasing effects under both the FI and FR schedules, whereas WR-3689 produced significantly greater decreases in overall FR than FI response rates ($p < 0.01$). Probit analysis estimates of the median effective doses (and the lower and upper confidence limits) for producing rate-decreasing effects on each schedule for each drug were: FI: WR-2721 ED_{50} = 99.5 mg/kg (74.3–144.2 mg/kg), WR-3689 ED_{50} = 80.6 mg/kg (59.0–105.9 mg/kg); FR: WR-2721 ED_{50} = 78.2 mg/kg (57.3–108.9 mg/kg), WR-3689 ED_{50} = 55.7 mg/kg (31.3–76.7 mg/kg). Probits indicate that WR-3689 was slightly more potent than WR-2721 in reducing response rates.

EXPERIMENT 2

The first experiment demonstrated that both WR-2721 and WR-3689 reduced schedule-controlled performance at doses (133–180 mg/kg) lower than those reported to provide maximal protection against radiation-induced lethality [200–400 mg/kg; (3,6,22,24)]. Although high doses of these drugs appear to provide maximal protection, lower drug doses that produce minimal behavioral effects may also provide some protection against radiation-induced lethality. This may be especially relevant because dose-effect curves for radiation-induced lethality are typically very steep, and even small shifts in these curves may result in protection of significant numbers of animals. In addition, radiation exposure produces decrements in the performance of trained behaviors. At sub- and near-lethal exposure levels, these performance decrements become most evident in the days immediately following exposure (11,18–20). Most previous behavioral studies of radioprotectants in conjunction with radiation exposure have dealt primarily with the ability of the drug to antagonize performance

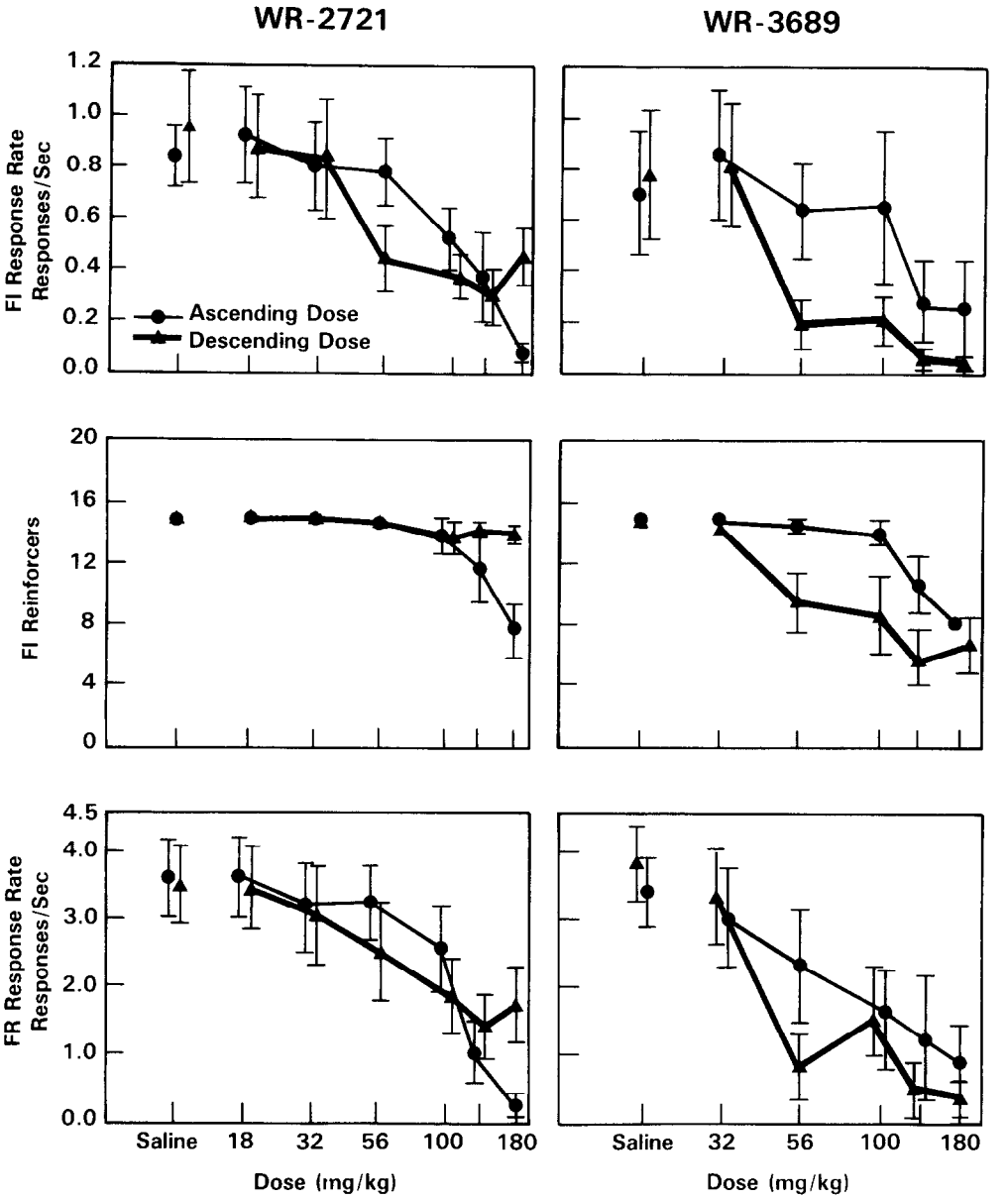

FIG. 1. Effects of WR-2721 (left) and WR-3689 (right) on selected measures of performance under a multiple FI 120-s, FR 50-response schedule of milk reinforcement. Top: FI response rate; center: FI reinforcers; bottom: FR response rate. (●), ascending dose-effect determination; (▲), descending dose-effect determination. Each point represents the mean ± SEM of seven rats.

decrements that occur within the first hour following rapidly delivered, supralethal levels of exposure (1,2,21). These high levels of exposure are well in excess of the protective capabilities of these drugs against radiation-induced lethality, and the performance decrements they produce are distinctly different from the types of behavioral decrements that occur following sub- or near-lethal levels of exposure. Only one study has investigated the ability of a radioprotectant to moderate radiation-induced behavioral decrements following lethal (yet survivable when given the radioprotectant) exposure. Landauer et al. (13) reported that mice protected with WR-2721 from the lethal effects of a 14-Gy exposure displayed significantly reduced levels of spontaneous locomotor activity for almost 6 months following exposure.

Experiment 2 followed a similar approach and was designed to extend these findings by directly comparing WR-2721 and WR-3689 for radioprotective efficacy against radiation-induced performance decrements and lethality. In this experiment, rats responded under an FR 50 schedule of milk reinforcement, and both high and low doses of the two radio-

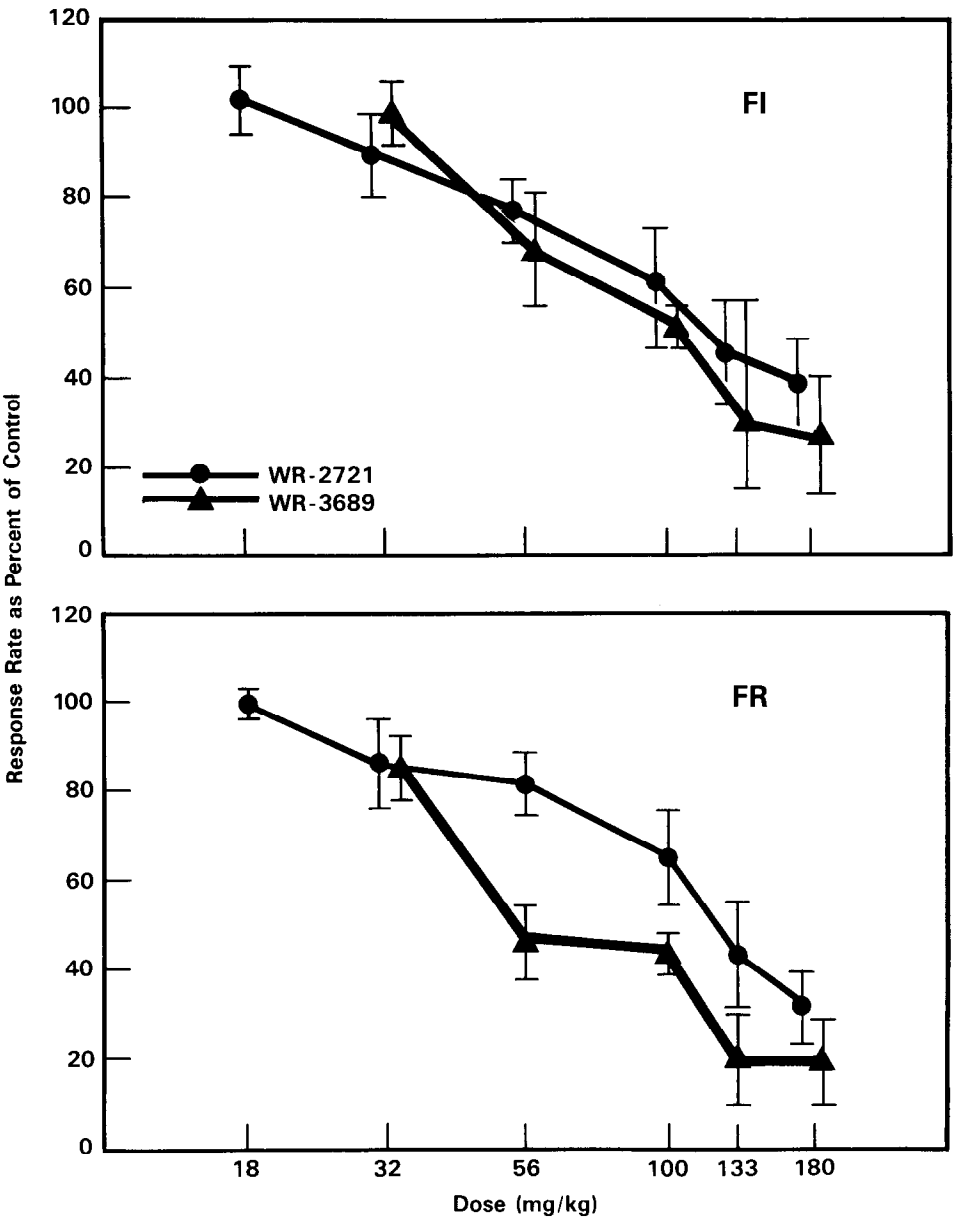

FIG. 2. Comparison of the rate-decreasing effects of (●) WR-2721 and (▲) WR-3689 under the multiple FI 120-s (top), FR 50-response (bottom) schedule of milk reinforcement. Response rates are expressed as a percentage of baseline control rates. Each point represents the mean ± SEM of seven rats.

protectant drugs were tested for their ability to moderate performance decrements that occur following either a nonlethal or lethal exposure to ionizing radiation.

METHOD

Animals

Adult, male Sprague-Dawley rats were used. Animals were maintained under identical conditions as described for the first experiment.

Behavioral Procedures

Rats were trained to lever press as described above. After one or two sessions under FR 1, the FR value was gradually increased over 15–20 sessions until an FR 50 schedule was in effect. Sessions ended with the first milk delivery after 30 min or automatically if a reinforcer was not obtained after 30.5 min (0.5 min limited hold on session duration). Only the house light was illuminated during the session. Training on the FR 50 schedule continued for 30–40 sessions to

stabilize responding before experimental treatments were begun.

Radiation Exposure

Rats were placed in well-ventilated, clear-plastic, restraining tubes for irradiation. Rats were habituated to the tube-restraint procedure over several weeks. They were restrained and transported to the exposure room on at least five occasions before either the sham test or the actual exposure day. Approximately 1 week before radiation exposure, rats were assigned to test groups (n = 6–7/group) and a sham-exposure test was conducted the next day. For the sham-exposure test, each rat was injected with its assigned drug dose, immediately placed in a restraining tube, and then transported to the exposure facility where, 15 min after injection, a sham exposure took place (rats were placed in the exposure room but were not irradiated). Rats were then returned immediately to the behavioral laboratory for testing. This series of manipulations was performed to determine the combined effects of drug injection, restraint, and transportation on performance of the FR 50 task. The procedure on the day of irradiation was identical to that just described with the exception that 15 min after drug injection rats were given a bilateral, whole-body exposure to gamma rays from a ^{60}Co source at a rate of 2.5 Gy/min to a total dose of either 5 or 10 Gy. Prior to irradiation, the dose rate at the midline of an acrylic rat phantom was measured using a 0.5-cc tissue-equivalent ionization chamber (Exradin, Inc., Lisle, IL). The dose rate at the same location with the phantom removed was measured using a 50-cc ionization chamber fabricated in-house. The ratio of these two dose rates, the tissue-air ratio (TAR), was used to determine the doses for irradiated rats. In these experiments, the TAR was 0.93. All ionization chambers have calibration factors traceable to the National Institute of Standards and Technology. Dosimetry measurements were performed following the AAPM Task Group 21 *Protocol for the Determination of the Absorbed Dose from High-Energy Photon and Electron Beams* (22). In each experiment, three doses of each radioprotectant drug (saline control, 56 and 180 mg/kg) were tested against one of two radiation challenge doses (nonlethal dose = 5 Gy; lethal dose = 10 Gy). Therefore, in each study there were six experimental groups. The first FR test session began approximately 5 min after exposure ceased, and testing continued 5 days per week for 30 days, the standard duration used to assess rodent survival following radiation exposure.

Data Analysis

Overall FR response rate was considered the best indicator of performance and was the only measure analyzed in detail. The last 10 days of preirradiation baseline performance (exclusive of the days rats were placed in restraining tubes and the sham-exposure day) were averaged for each rat; these individual means were used to calculate baseline performance for each group and determine change from baseline data. For the 5-Gy exposures, separate analyses were performed for the first 5 days after exposure and for the entire 30-day period after exposure. Because of deaths within the various 10-Gy treatment groups, formal statistical analysis was only performed on the performance data from the days when within-group ns were constant. Data were analyzed using two-way repeated measures analyses of variance (ANOVAs), with drug dose being the between-groups factor and days the repeated measure. Significant effects were further evaluated using Dunnett's multiple comparison test or t-tests with Bonferroni corrections. The criterion for significance was set at $p < 0.05$.

RESULTS

WR-2721

There were no between-group differences in response rates on baseline or "tube-restraint" days before exposure. Response rates on tube-restraint days did not differ significantly from baseline control days for individual groups. Baseline control response rates (mean ± SEM in responses per second) for the groups exposed to 5 Gy gamma radiation were 2.13 ± 0.52 (saline), 1.80 ± 0.26 (56 mg/kg), and 1.89 ± 0.27 (180 mg/kg). Baseline rates for the groups exposed to 10 Gy were 1.64 ± 0.31 (saline), 2.08 ± 0.30 (56 mg/kg), and 1.57 ± 0.32 (180 mg/kg). On the sham-exposure day, only the performance of the group given 180 mg/kg WR-2721 was significantly affected; responding was reduced to less than 50% of the preirradiation baseline rate (Fig. 3). Saline and 56 mg/kg WR-2721-treated groups performed at levels that were not significantly different from their baseline response rates.

WR-2721 + 5-Gy Exposure

Drug dose did not differentially affect performance, nor were there any significant dose × day interactions after the 5-Gy exposure. However,there were significant within-group changes in response rates. The performance of the saline-treated group was normal on the day of exposure, but was reduced significantly relative to baseline on days 2–5 following exposure. Performance of the saline-treated group had recovered by day 8 and did not vary significantly from baseline for the remainder of the study. Performance of the 56-mg/kg WR-2721 group was reduced significantly relative to its baseline on days 1–3 following exposure, and then recovered to baseline levels for the remainder of the study. The group treated with 180 mg/kg WR-2721 showed only a slightly different pattern of results: a nonsignificant reduction in responding on the day of exposure, significant reductions in performance on days 2–5 following exposure relative to baseline, and then recovery to preirradiation baseline performance levels for the remainder of the study.

WR-2721 + 10-Gy Exposure

The pattern of results was markedly different in groups that received the 10-Gy radiation exposure. On the day of the 10-Gy radiation exposure, there were no statistically significant decrements in performance from baseline within each drug treatment group, nor were there any significant differences between groups. On days 2–5 postexposure, the performance of all treatment groups deteriorated progressively from their preirradiation baselines, reaching a nadir on day 5. There were no significant differences between groups in the magnitude of the performance decrement or the rate of decline over these days.

Throughout the week after the 10-Gy exposure, all rats maintained their body weights within ± 15 g of target weight. Two rats in the 180-mg/kg WR-2721 ± 10-Gy dose group left a small amount (mean = 4.8 g) of food over the first 24 h (days 1–2) after exposure. In all groups, entire food rations were consumed on days 2–3 and only small amounts (mean = 3.1 g) were left on days 3–4; in contrast, performance had declined 65–72% from baseline over this time. On days 4–5,

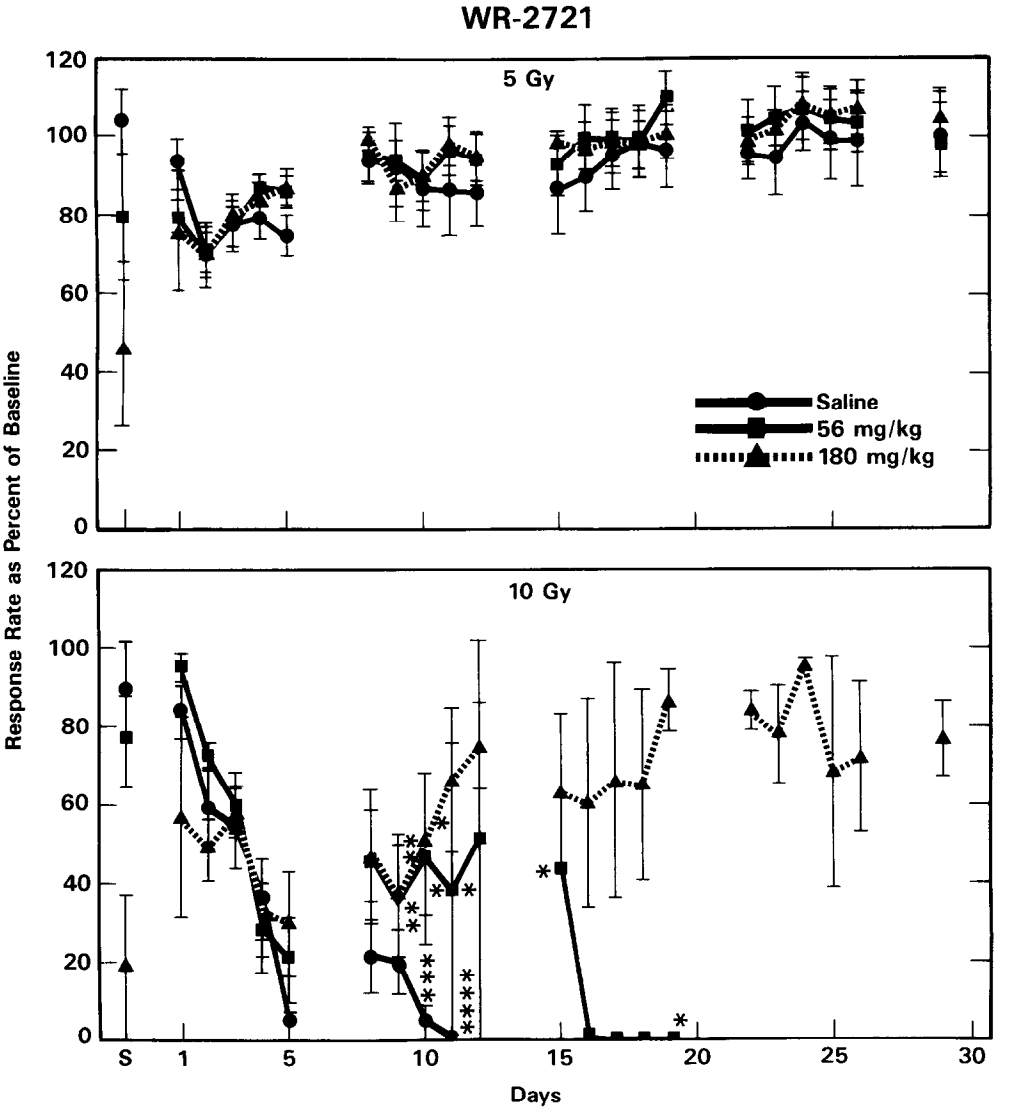

FIG. 3. Effects of (●) saline, (■) 56 mg/kg WR-2721, or (▲) or 180 mg/kg WR-2721 on FR 50 response rates and survival following exposure to 5 Gy (top) or 10 Gy (bottom) of [^{60}Co] gamma radiation. Response rates are expressed as a percentage of baseline control rates. Each point represents the group mean ± 1 SEM. Points at S represent the performance of the respective treatment groups on the day of sham exposure (n = 6–7 per group). Radiation exposure occurred on day 1 for all groups 15 min prior to the behavioral testing. Asterisks that accompany curves on the 10-Gy exposure condition indicate deaths in these groups.

the time of maximal performance decrement in the first week, substantial amounts of food were left by all three 10-Gy treatment groups (saline: mean = 9.9 g; 56 mg/kg: mean = 12.8 g; 180 mg/kg: mean = 9.5 g).

On test sessions 8 and 9, performance of all treatment groups recovered somewhat relative to their performance on day 5. However, analysis of recovery was confounded after this point by varying mortality rates across groups. Saline-treated rats began dying between days 10–11, and all rats in this group died by day 12. Rats that received 56 mg/kg WR-2721 began dying after day 9; five of the six rats died between days 9 and 14, and the last rat died on day 23. In contrast, only three of the six rats that received 180 mg/kg WR-2721 died, and these deaths occurred between days 10–11. As might be expected, the performance of all three treatment groups became quite variable during the time that rats were showing signs of radiation toxicity. Three rats that received 180 mg/kg WR-2721 survived; their performance recovered to 80–90% of preirradiation baseline levels by the end of the third week after exposure. Subsequently, daily response rates of one rat were quite variable, while response rates of the other two rats generally remained near preirradiation baseline levels for the remainder of the study.

WR-3689

There were no significant between-group differences in response rates on baseline or tube-restraint days before expo-

sure. Baseline control response rates (mean ± 1 SEM in responses per second) for the groups exposed to 5 Gy gamma radiation were 2.15 ± 0.25 (saline), 2.22 ± 0.52 (56 mg/kg), and 2.07 ± 0.39 (180 mg/kg). Baseline rates for the groups exposed to 10 Gy were 2.32 ± 0.39 (saline), 1.97 ± 0.23 (56 mg/kg), and 1.69 ± 0.16 (180 mg/kg). On the sham-exposure test, the 180 mg/kg dose of WR-3689 decreased FR responding significantly relative to this groups's own baseline performance, the performance of the saline-treated group, and the performance of the group given 56 mg/kg WR-3689 (Fig. 4). The 56-mg/kg dose of WR-3689 produced small but nonsignificant reductions in responding.

WR-3689 + 5-Gy Exposure

The only significant between-groups effect occurred on the day of exposure when the 180-mg/kg WR-3689 treatment group responded less than either the saline or 56-mg/kg WR-3689 treatment groups (Fig. 4, top). Moreover, the saline, $F(5, 75) = 4.69$, $p < 0.01$, and 180-mg/kg WR-3689, $F(5, 75) = 9.25$, $p < 0.01$, treatment groups showed significant changes in response rates over the 5 days following the 5-Gy exposure. Relative to their baseline levels of performance, saline-treated rats showed significant decreases in FR performance on days 2, 3, and 5 postexposure, while the group treated with 180 mg/kg WR-3689 displayed significant decreases in performance on postexposure days 1, 2, and 3. In contrast, response rates were reduced to a small but nonsignificant degree (the largest reduction occurred on day 2) from baseline control values in the group given 56 mg/kg WR-3689 prior to irradiation. By the beginning of the second week after exposure, the performance of each group had recovered to within its preexposure baseline range, where it remained for the duration of the study.

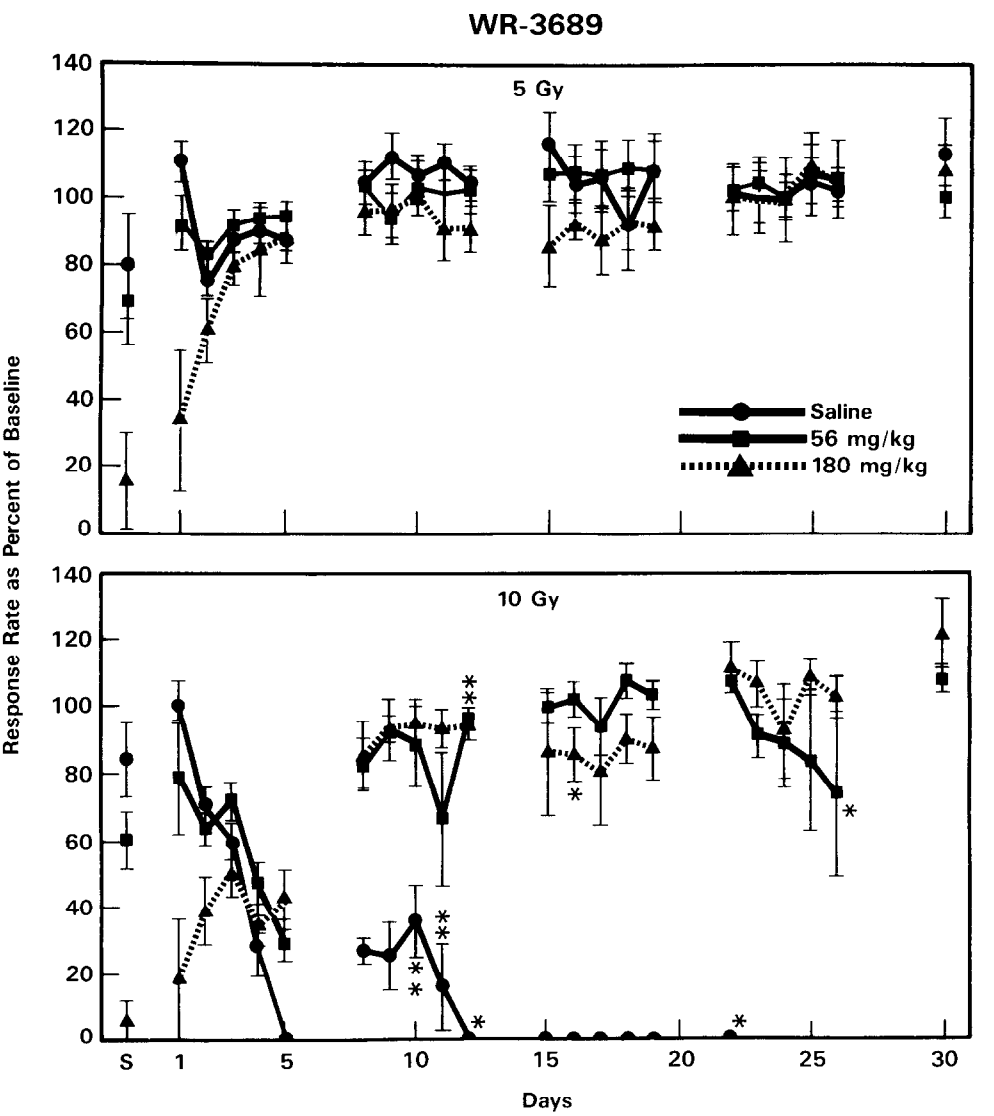

FIG. 4. Effects of (●) saline, (■) 56 mg/kg WR-3689, or (▲) 180 mg/kg WR-3689 on FR 50 response rates and survival following exposure to 5 Gy (top) or 10 Gy (bottom) [^{60}Co] gamma radiation. Presentation format is identical to that of Fig. 3. Asterisks that accompany curves on the 10-Gy exposure condition indicate deaths in these groups.

WR-3689 + 10-Gy Exposure

Following the 10-Gy exposure, each treatment group showed a distinct pattern of performance changes (Fig. 4, bottom). Saline-treated rats performed normally on the day of irradiation; performance then declined progressively to virtually negligible levels on day 5. On days 8 and 9 postirradiation, the performance of this group showed a modest recovery. However, performance rapidly declined again over the next two sessions as rats in this group began to die. Five of the rats in this group died between days 10 and 12 after exposure and one rat died on day 23 (this rat never performed more than 1–3 ratios per session after day 11).

Rats receiving the 56-mg/kg WR-3689 treatment also performed within their baseline range on the day of exposure to 10 Gy. Their performance progressively declined over days 2–5 to a level that was 30% of their preexposure baseline. Their performance recovered to levels not significantly different from their preexposure baseline on day 8. The "recovered" performance of this treatment group was maintained throughout the remainder of the study, except during the sessions that immediately preceded death for two rats (days 12 and 27).

For rats that received the 180-mg/kg WR-3689 treatment, performance during the test session immediately following the 10-Gy exposure was suppressed to the same extent that had occurred on the sham-exposure test. Performance improved somewhat but remained significantly below baseline over days 2–5. On day 5, the performance of this treatment group was 34% of baseline, a level not significantly different from the 56-mg/kg WR-3689 treatment group but significantly greater than the saline-treated group. On day 8, the performance of the 180-mg/kg WR-3689 group recovered to levels not significantly different from their preirradiation baseline values; performance was maintained at this level throughout the remainder of the study. Only one rat in this group died; this death occurred on day 16.

Rats in various treatment groups left different amounts of their daily food ration over the first 5 days after irradiation with 10 Gy. Five rats in the 180-mg/kg WR-3689 group left substantial amounts of chow (9–12 g) on the day after exposure and small amounts of food (<3–4 g) on each of the following 3 days. Similarly small amounts of food were left by three rats in the 56-mg/kg WR-3689 treatment group and by one saline-treated rat during the first week (days 4–5) after exposure. These results are the inverse of the performance data: Saline-treated subjects left the least food yet experienced the most pronounced performance decrement over this period, while the group that received 180 mg/kg WR-3689 left substantial amounts of food but continued to perform (especially on day 5).

One unexpected finding of these studies was the failure of 56 mg/kg WR-2721 to provide any protection against the lethal effects of 10 Gy radiation, whereas the same dose of WR-3689 protected 50% of 10 Gy-exposed rats. It has been reported that WR-2721 provides a greater shift to the right in the radiation-induced lethality function than WR-3689 (3). Thus, the failure of 56 mg/kg WR-2721 to provide any protection against this level of radiation challenge, while WR-3689 did, was not anticipated and raised the question whether some procedural variable may have contributed to this finding. To address this possibility, a replication of the WR-2721 + 10 Gy study was performed (n = 6/group: saline, 56 and 180 mg/kg WR-2721). Operant training, drug dosing, and radiation exposures were conducted in an identical fashion to that already described. The results, both the survival data and the operant-performance data, were virtually identical to those of the initial WR-2721 experiment.

Saline-pretreated animals performed normally on the day of exposure, then showed a progressive decline in responding over the next several test sessions to near-zero performance on day 5 postexposure. This was followed by minimal improvement in performance on days 8–9 before a decline associated with mortality occurred; all saline-pretreated subjects died between days 8–11. Similarly, the group treated with 56 mg/kg WR-2721 performed at baseline levels on the test immediately after exposure, and their performance declined to near-zero levels on day 5 postexposure. The 56 mg/kg WR-2721-treated group did show a more pronounced recovery of performance than saline-treated rats over sessions 8–12, but performance then declined again and all rats died within the 30-day postexposure testing period. Rats in this group began dying on day 10, five of six of them had died by day 14, and the final rat died on day 24. The performance of the group treated with 180 mg/kg WR-2721 was significantly decremented on the day of exposure, primarily because of the high dose of the drug. Their performance improved toward baseline on day 2 to a level not different from the other two treatment groups. Over days 3–5 their performance declined, but on day 5 their performance began to recover and was significantly greater than either of the other two treatment groups. By day 8, the performance of the 180-mg/kg WR-2721 pretreatment group had recovered to preirradiation baseline levels, and despite one death (day 10) it remained in a range not significantly different from baseline for the remainder of the 30-day testing period. This mortality rate (1/6) was not substantially different from the 3/6 deaths experienced by the comparable treatment group in the initial study. Thus, in terms of protection against radiation-induced lethality and both the magnitude and duration of the postexposure performance decrements the results of this second experiment with WR-2721 replicated the findings of the initial study.

GENERAL DISCUSSION

The major finding of the present study was that neither WR-2721 nor WR-3689, at either a low or high dose, produced a substantial blockade of the decrements in FR performance that occurred following radiation exposure, regardless of whether the radiation exposure was lethal or not. However, several interesting differences between WR-2721 and WR-3689 were revealed. First, although high doses of both compounds were equally efficacious in protecting against the lethal effects of radiation exposure, only WR-3689 offered any protection at low drug doses. Second, the calculated ED_{50}s indicated that WR-3689 was somewhat more potent than WR-2721 in producing behavioral decrements as indexed by disruption of mult FI FR performance.

The results of the mult FI FR experiment clearly indicated that both WR-2721 and WR-3689 produced dose-dependent decreases in response rates during each of the component schedules. The rate-decreasing effects of WR-2721 were similar for both component schedules, while for WR-3689 the FR dose-effect curve was shifted to the left of that for FI when rates were expressed as percentage of control. Unlike the recent report by Liu et al. (15), the effects of neither radioprotectant appeared to be characterized by an "all or none" suppression of responding. In that study, WR-2721 was tested in rats performing under an FR 20 schedule of water reinforce-

ment; doses of 75 or 100 mg/kg WR-2721 produced almost total cessation of responding (ED_{50} = 58.5 mg/kg). In the present study, in contrast, animals continued to perform under both component schedules at doses up to 180 mg/kg of either radioprotectant. Although total suppression of performance did occur in selected cases, this was not a consistent finding. More typically, animals continued to perform throughout the session, albeit at very low response rates. This is most readily demonstrated by the continued acquisition of reinforcers under the FI component of the schedule at the high doses of both drugs. This difference between these results and those of Liu et al. (15) could be due to one or more procedural differences such as the type of reinforcer used to maintain behavior, the use of a multiple vs. a simple schedule of reinforcement, or the FR value.

Although there were no major differences in the character of the performance decrements produced by each compound in the present study, there were several interesting differences between the two drugs in the pattern in which effects were manifested on the dose-effect replications. WR-2721 produced significantly less of an effect on several indices of FI performance and on FR running response rate on the descending-dose series relative to the ascending-dose series. In contrast, WR-3689 produced significantly greater effects on the number of FI reinforcers acquired and on FR postreinforcement pause duration on the descending-dose series. There is no evidence to indicate an enhanced metabolism of WR-2721 with repeated dosing at the intervals and dose ranges used in this study (17). Moreover, based on physiochemical properties, WR-3689 is very similar to WR-2721 and should be metabolized in the same fashion (7). Thus, it is unlikely that these differential behavioral effects on the ascending and descending dose-effect determinations were due to pharmacokinetic differences between the two drugs. In general, though, pronounced decreases in performance occurred with either compound at doses $\geq$ 100 mg/kg. Most studies of the radioprotective efficacy of these compounds have focused on drug doses substantially higher than those used here (e.g., >200 mg/kg), and several authors have ascribed at least part of the radioprotective effects of these drugs to the general behavioral depression that is produced (12,13,16,23).

The second series of experiments addressed the question of whether relatively low doses of these drugs provide any protection against the lethal and behavioral effects of exposure to gamma radiation. The results show that neither compound, at either the low or the high dose, was effective in totally protecting animals against the decreases in FR performance that occur in the initial days following either nonlethal or lethal radiation exposure. At the lower radiation dose (5 Gy), the magnitude and time course of the depression in performance was essentially equivalent for all treatment groups. One notable finding was that the performance of the 56-mg/kg WR-3689 group never dropped significantly below its preirradiation baseline in the week following exposure. At the lethal radiation dose, the radioprotective effects of each drug were clearly evident, yet again there was no clear drug-induced antagonism of the pronounced behavioral decrement. At best, treatment with a radioprotectant attenuated only the relative degree of the performance decrement. This pattern of performance change over days after the lethal 10-Gy exposure (i.e., a progressive decline in responding to near-zero levels on day 5, followed by a modest recovery on days 8–10) mirrors the performance changes that were reported after exposure to a high but generally nonlethal dose (i.e., a gamma-ray dose of 9 Gy produced 14% lethality over 30 days) (20). The most striking difference between the two patterns is that nearly total recovery of performance occurs in the second week of testing following exposure to the 9-Gy dose, while after 10 Gy performance remains severely disrupted. Behavioral recovery during the second week after exposure was observed in the present study in rats that survived the 10-Gy exposure when pretreated with either WR-2721 or WR-3689.

Both WR-2721 and WR-3689 were equally efficacious at high doses in protecting rats against the lethal effects of radiation exposure. All rats that received saline as a pretreatment died, and all but one of the deaths occurred on days 10–12 postexposure. This is consistent with the time frame associated with radiation-induced hematopoietic failure (9,25). The 180-mg/kg dose of either drug protected 50% (WR-2721, replication 1) to 86% (WR-3689; WR-2721, replication 2) of the rats, but only WR-3689 showed any protective effects (50%) at the lower 56-mg/kg dose. Rats that had received a radioprotectant and died did so primarily over the same time frame as unprotected animals (70% died on days 10–12), although there were four animals that survived 16, 22, 24, and 27 days, respectively. Thus, drug treatment did not substantially alter survival time in these animals. Perhaps the most interesting finding of the study was that a low dose (56 mg/kg) of WR-3689 provided significant protection against the lethal 10-Gy radiation challenge, whereas an equivalent dose of WR-2721 was ineffective. This result was not anticipated, but the replication of the WR-2721 study confirmed that 56 mg/kg of this drug fails to provide protection under these conditions.

In summary, the two radioprotectant drugs WR-2721 and WR-3689 produced highly similar, dose-dependent behavioral decrements. Neither drug was capable of effectively antagonizing behavioral decrements that occurred following radiation exposure, which indicates that the mechanisms responsible for the behavioral decrements may be independent from those responsible for radiation-induced lethality. WR-3689 was capable of providing significant radioprotective effects at a low dose that produced no behavioral disruption by itself; WR-2721 did not provide protection at the same dose. This finding, in conjunction with its reported oral effectiveness, suggests that WR-3689 is a compound that possesses a number of desirable features as a radioprotectant that WR-2721 does not.

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