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Sensitization Versus Tolerance to Haloperidol-Induced Catalepsy: Multiple Determinants

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BARNES, D. E., B. ROBINSON, J. G. CSERNANSKY AND E. P. BELLOWS. *Sensitization versus tolerance to haloperidol-induced catalepsy: Multiple determinants*. PHARMACOL BIOCHEM BEHAV **36**(4) 883–887, 1990.—The effects of dose, administration frequency, and behavioral testing conditions on the development of tolerance versus sensitization to haloperidol-induced catalepsy were tested in rats. Animals received daily or weekly injections of haloperidol (0.05–5.00 mg/kg SC) for up to 22 days. Catalepsy assessments were made either once or repeatedly using two tests: the horizontal bar and the inclined screen. Tolerance was found only in animals treated daily with haloperidol (1.5 mg/kg) and tested repeatedly on the horizontal bar. In contrast, sensitization was observed with various haloperidol doses, daily or weekly administration schedules (for most doses), either horizontal bar or inclined screen catalepsy tests, and repeated or single testing. Sensitization developed most strongly following weekly drug administration and repeated testing on the horizontal bar. No single experimental variable produced a definitive pattern of change in catalepsy over time. Dose, drug administration schedule, and behavioral test conditions all influenced the evolution of catalepsy during chronic haloperidol treatment.

Haloperidol Rat Catalepsy Sensitization Tolerance

HALOPERIDOL, a prototypical neuroleptic, induces catalepsy (maintenance of an awkward position) when administered acutely to rats. However, the ability of chronic haloperidol treatment to produce increasing or decreasing degrees of catalepsy over time remains controversial. Early researchers observed tolerance to neuroleptic-induced catalepsy during chronic drug administration using daily doses of 0.75 mg/kg haloperidol or greater (2, 3, 8). However, increased catalepsy responses (i.e., sensitization) have also been reported in rats following daily, weekly or monthly administration of 0.4 mg/kg haloperidol (1).

Differences in several elements of experimental design might account for such discrepancies. Higher drug doses may promote tolerance development (2, 3, 8). Also, Post (13) proposed that continuous drug administration independent of dose promotes tolerance while intermittent drug administration favors reverse tolerance or sensitization. Various aspects of behavioral testing procedures, such as conditioning to environmental cues (4, 7, 10, 12, 14, 15) or repeating behavioral assessments (9, 17), can also influence behavioral responses to any drug over time.

In this study, we set out to determine if definitive patterns of tolerance or sensitization to haloperidol-induced catalepsy would be produced by systematically varying drug dose, drug administration schedule, and the behavioral testing protocol. Animals received daily or weekly injections of haloperidol (0.05–5.00 mg/kg SC) for up to 22 days. Catalepsy was assessed 30–210 minutes postinjection using the horizontal bar and inclined screen tests. Some experimental groups were tested for catalepsy every week on days 1, 8, 15, and 22, while other groups received a single day of catalepsy assessment on either day 1, 8, 15, or 22.

METHOD

Drug Administration

Male Sprague-Dawley rats (175–200 g) were housed two per cage, maintained on a 12:12-hour light:dark cycle, and given unlimited access to food and water. All drug and saline injections were administered in this home environment except on days of catalepsy assessment, when injections were given in the testing

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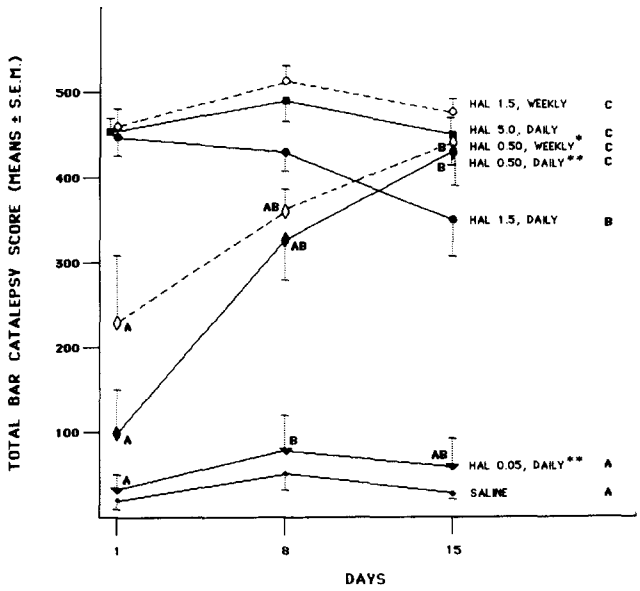

FIG. 1. Bar catalepsy scores during daily or weekly administration of haloperidol (HAL) 0.05–5.00 mg/kg SC. Catalepsy testing was performed on days 1, 8, and 15 using a horizontal bar, as described in the text. Changes in mean catalepsy scores over time were analyzed within treatment groups using Friedman's two-way ANOVA followed by a multiple comparisons test between days of treatment. Results of the post hoc testing are noted in the figure where means with different letters differ significantly (within treatment groups) at the 0.05 level. Mean values denoted by pairs of letters (e.g., AB) do not differ from values denoted by either A or B. *$F_r > 7.0$, $p < 0.05$, Friedman's two-way ANOVA by ranks; **$F_r > 9.0$, $p < 0.01$, Friedman's two-way ANOVA by ranks. Means between treatment groups were compared on day 22 using the Kruskal-Wallis one-way ANOVA (KW = 27.5, $p < 0.001$) followed by the appropriate multiple comparisons test. Results of the post hoc testing are shown in this column where different letters indicate that group means differ significantly at the 0.05 level.

environment. Animals were allowed to accommodate to the testing environment for one hour prior to the test injection.

Groups of animals received daily or weekly subcutaneous injections of haloperidol (0.05–5.00 mg/kg; McNeil Pharmaceutical) for 1–22 days in a series of experiments. Animals receiving weekly haloperidol were injected with the same volume of saline on all nondrug days. Control groups received daily saline injections.

Experimental Design

In an initial experiment, we examined haloperidol-induced catalepsy during chronic administration of a wide range of drug doses. Rats received daily or weekly injections of haloperidol (0.05–5.00 mg/kg SC) or saline for 15 days (n = 6/treatment group). Three 60-second catalepsy tests were conducted at each time point using the horizontal bar as described below. All animals were tested at 30, 90, and 150 minutes postinjection on days 1, 8, and 15. Catalepsy scores were summed for each day of testing, allowing a maximum score of 540 seconds per day.

Subsequently, we modified the behavioral protocol to decrease the number of animals achieving maximum catalepsy scores and to select two representative doses. At each time point, animals received one 5-minute trial on an inclined screen followed by one 5-minute trial on the horizontal bar. The inclined screen test was introduced because it required less handling of the animal, and in the hope it would provide a more graduated measurement of catalepsy behavior. The horizontal bar test was continued for comparison.

To examine the effects of dose and administration schedule on haloperidol-induced catalepsy, groups of animals received daily or weekly injections of haloperidol (0.15 or 1.50 mg/kg SC) or saline for 22 days (n = 10/treatment group). Catalepsy was assessed for 5 minutes on the inclined screen and the horizontal bar at 30, 90, 150, and 210 minutes postinjection on days 1, 8, 15, and 22. At each test session, animals were placed in individual plastic cages (20 × 42 cm) equipped with a horizontal wooden bar placed 8 cm high and a wire mesh screen placed at a 45-degree angle. Screen catalepsy was measured by placing the rat head-down on the screen and scoring one point for every six seconds until the rat moved two paws on the screen. Bar catalepsy was then measured by gently placing the rat's forepaws on the bar and scoring one point for every six seconds until the rat removed one paw from the bar. Animals were returned to their home cages between all test sessions. Both tests allowed a maximum score of 50 points (5 minutes) per test session. Bar and screen catalepsy scores were summed separately on each day of testing, allowing a maximum score of 200 points per day.

To examine the effects of single versus repeated behavioral assessment procedures on haloperidol-induced catalepsy, animals received daily or weekly injections of haloperidol (1.50 mg/kg SC) or saline for 1, 8, 15, or 22 days (n = 10/treatment group). Bar and screen catalepsy tests were performed as described above at 30, 90, and 150 minutes postinjection. Multiple-test groups received repeated weekly behavioral assessments (days 1, 8, 15, and 22), while single-test groups were evaluated for catalepsy on one day only (either day 1, 8, 15, or 22). Control groups received daily saline injections and a single day of behavioral assessment. Bar and screen catalepsy scores were summed separately on each day of testing, allowing a maximum score of 150 points per day.

Statistics

Data from the bar and screen catalepsy tests in each experiment were analyzed separately using catalepsy scores summed over all time points on each day of testing. For cohorts that underwent repeated behavioral assessments (related samples), differences in mean catalepsy scores across days were analyzed within treatment groups using Friedman's two-way analysis of variance (ANOVA) by ranks. Statistically significant differences then were localized by a multiple comparisons test between days of treatment (16). For single-test cohorts (independent samples), changes in mean catalepsy scores over time were tested within treatment groups using a Kruskal-Wallis one-way ANOVA, followed by the appropriate post hoc multiple comparisons test (16). Additionally, mean catalepsy scores on the final day of testing were compared across experimental treatment groups using a Kruskal-Wallis one-way ANOVA, followed by a multiple comparisons test between treatment groups.

RESULTS

The results of our initial experiment examining mean bar catalepsy scores during chronic administration (daily or weekly) of several haloperidol doses are shown in Fig. 1. Bar catalepsy scores increased significantly over time in groups treated with daily or weekly haloperidol (0.50 mg/kg). A lower daily dose of haloperidol (0.05 mg/kg) produced a statistically significant but transient increase in bar catalepsy scores on day 8 as compared to day 1. Higher doses of haloperidol elicited near-maximal catalepsy re-

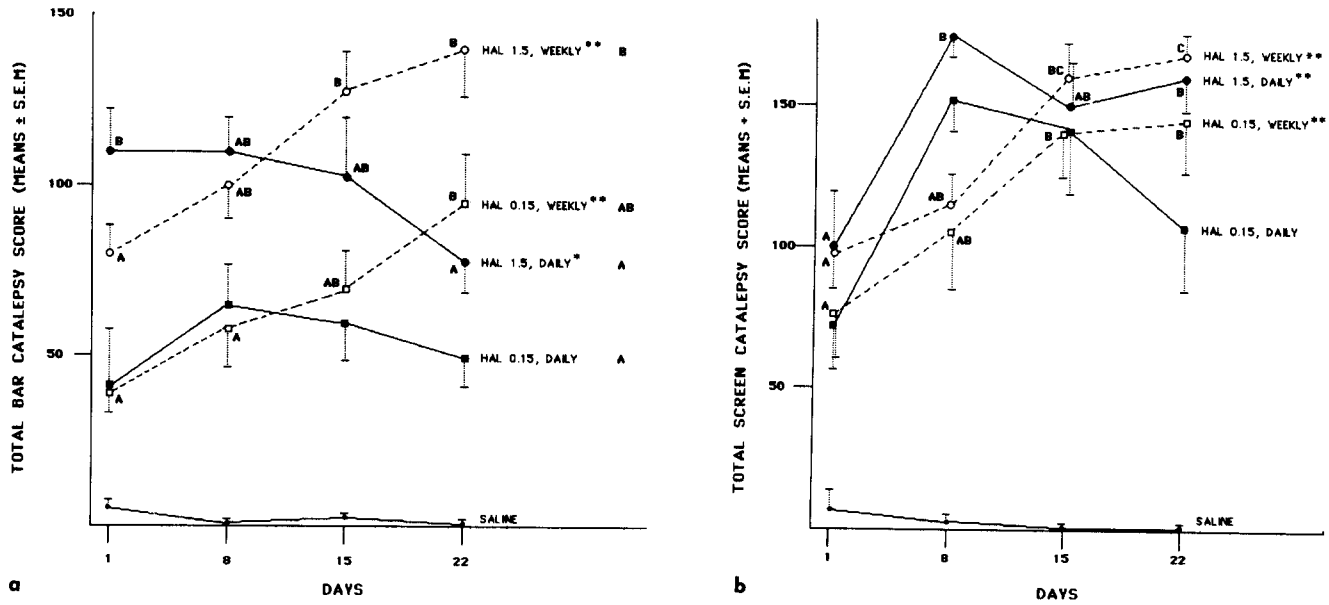

FIG. 2. Catalepsy scores during daily or weekly administration of haloperidol (HAL) 0.15 or 1.5 mg/kg SC. Animals were tested for catalepsy on days 1, 8, 15, and 22 using a horizontal bar (a) and a wire mesh screen (b), as described in the text. Changes in mean catalepsy scores over time were analyzed within treatment groups using Friedman's two-way ANOVA followed by a multiple comparisons test between days of treatment. Results of the post hoc testing are noted in the figure where means with different letters differ significantly (within treatment groups) at the 0.05 level. Mean values denoted by pairs of letters (e.g., AB) do not differ from values denoted by either A or B. *$F_r = 8.13$, $p < 0.05$, Friedman's two-way ANOVA by ranks; **$F_r > 11.3$, $p < 0.01$, Friedman's two-way ANOVA by ranks. Means between treatment groups, excluding saline controls, were compared on day 22 using the Kruskal-Wallis one-way ANOVA ($KW = 17.0$, $p < 0.001$) followed by the appropriate multiple comparisons test. Results of the post hoc testing are shown in this column where different letters indicate that group means differ significantly at the 0.05 level. Mean values denoted by pairs of letters (e.g., AB) do not differ from values denoted by either A or B.

sponses at all time points, which may explain why no changes were observed in groups treated with haloperidol 1.50 or 5.00 mg/kg. However, a comparison of all drug treatment groups on day 15 revealed that the 1.50 mg/kg daily treatment group had significantly lower bar catalepsy scores than other groups, including the 1.50 mg/kg weekly treatment group. No changes were observed in mean bar catalepsy scores in saline controls.

The results of a second experiment testing the effects of two doses of daily or weekly chronic haloperidol administration (1.50 or 0.15 mg/kg) on bar and screen catalepsy are presented in Fig. 2a and b. Mean bar and screen catalepsy scores significantly increased over time in both weekly haloperidol treatment groups (1.50 and 0.15 mg/kg). Daily administration of haloperidol (1.50 mg/kg) produced a significant decrease in mean bar catalepsy scores and a significant increase in mean screen catalepsy scores. No significant changes in bar or screen catalepsy scores were observed in the 0.15 mg/kg daily haloperidol treatment group or the saline control group. When the four drug treatment groups were compared on day 22, mean bar catalepsy scores in the weekly haloperidol (1.50 mg/kg) treatment group were significantly higher than scores in the daily haloperidol treatment groups (1.50 or 0.15 mg/kg). Mean screen catalepsy scores did not differ significantly between drug treatment groups on day 22.

The results of single versus repeated behavioral testing using the horizontal bar following daily or weekly administration of one haloperidol dose (1.50 mg/kg) are shown in Fig. 3a. Mean bar catalepsy scores in the multiple-test weekly haloperidol treatment group increased between day 8 and day 22, but no significant change was observed in bar catalepsy scores for the multiple-test daily haloperidol group. In both single-test treatment groups (daily and weekly haloperidol), mean bar catalepsy scores were significantly increased on day 15 as compared to day 8, but scores on day 22 did not differ significantly from scores on day 1 within treatment groups. The comparison of mean bar catalepsy scores on day 22 revealed significantly higher scores in the multiple-test daily haloperidol group than in the single-test weekly haloperidol group. Slightly different results were obtained when the inclined screen test was used to compare the effects of single versus repeated behavioral testing (Fig. 3b). Statistically significant changes in mean screen catalepsy scores were observed only in the multiple-test daily haloperidol treatment group, whose scores increased on day 8 as compared to day 1. On day 22, both multiple-test treatment groups appeared to have higher catalepsy scores than the single-test treatment groups. However, a multiple comparisons test between all four groups revealed a significant difference only between the daily haloperidol multiple-test group and the daily haloperidol single-test group. Again, no significant changes in bar or screen catalepsy scores were observed in the saline control groups over time.

DISCUSSION

Early researchers demonstrated that catalepsy occurs in a dose-dependent manner following acute haloperidol administration (6, 8, 11). During chronic haloperidol treatment, we also observed that higher doses of haloperidol generally produced greater catalepsy using both the horizontal bar and the inclined screen tests (see Figs. 1 and 2b). However, particular doses of haloperidol did not consistently promote definitive patterns of change in catalepsy over time. It is interesting to note that only modest tolerance (<25%) was observed during daily administration of one haloperidol dose (1.50 mg/kg). Tolerance may develop

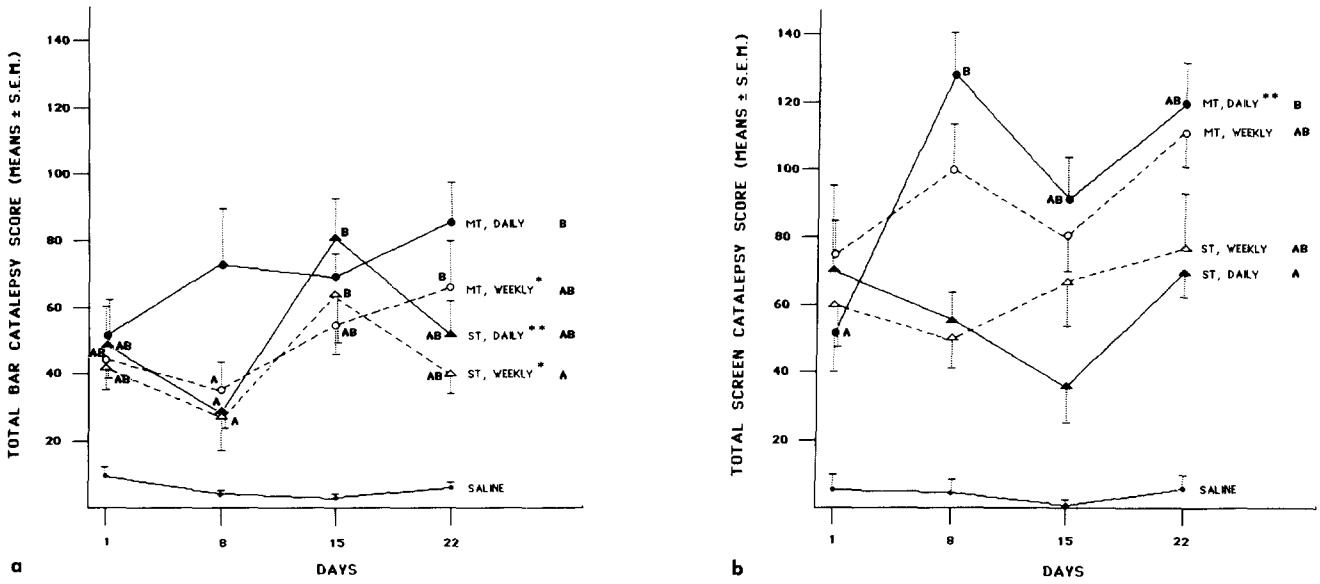

FIG. 3. Catalepsy scores during daily or weekly administration of haloperidol 1.5 mg/kg SC. Animals in the multiple-test treatment group (MT) were tested for catalepsy on days 1, 8, 15, and 22 using a horizontal bar (a) and a wire mesh screen (b), as described in the text. Animals in the single-test treatment groups (ST) were tested only once, on day 1, 8, 15, or 22. For the multiple-test treatment group, changes in mean catalepsy scores over time were analyzed using Friedman's two-way ANOVA. For the single-test treatment groups, the Kruskal-Wallis one-way ANOVA was used to analyze changes in mean catalepsy scores within treatment groups. Post hoc testing was performed using the appropriate multiple comparisons test between days of treatment. Results of the post hoc testing are noted in the figure where means with different letters differ significantly (within treatment groups) at the 0.05 level. Mean values denoted by pairs of letters (e.g., AB) do not differ from values denoted by either A or B. $*p<0.05$, Friedman's two-way ANOVA or Kruskal-Wallis one-way ANOVA. $**p<0.01$, Friedman's two-way ANOVA or Kruskal-Wallis one-way ANOVA. Means between treatment groups were compared on day 22 using the Kruskal-Wallis one-way ANOVA ($KW>7.8$, $p<0.05$) followed by the appropriate multiple comparisons test. Results of the post hoc testing are shown in this column where different letters indicate that group means differ significantly at the 0.05 level. Mean values denoted by pairs of letters (e.g., AB) do not differ from values denoted by either A or B.

more slowly during chronic administration of lower haloperidol doses, but there was no evidence of this phenomenon in our data. In contrast to the narrow conditions required for tolerance, sensitization occurred robustly and across a variety of doses.

Different schedules of drug administration may be more important in determining patterns of catalepsy over time. At each dose, weekly administration of haloperidol promoted greater degrees of sensitization. These findings lend support to Post's (13) hypothesis, but must be interpreted with caution due to certain inconsistencies in our data.

First, in our initial experiment, daily as well as weekly administration of haloperidol (0.50 mg/kg) produced increasing catalepsy scores (i.e., sensitization) on the horizontal bar (Fig. 1). This finding replicates the results of Antelman *et al.* (1) and supports his proposal that catalepsy responses to moderate haloperidol doses increase over time independent of the schedule of drug administration. However, our initial experiment used shorter catalepsy tests than the subsequent experiments (1 minute versus 5 minutes), which might also favor the demonstration of sensitization phenomena. Second, in animals receiving daily injections of haloperidol (1.50 mg/kg), catalepsy scores decreased over time when measured on the horizontal bar (Fig. 2a) but increased over time when measured on the inclined screen (Fig. 2b). This discrepancy suggests that bar and screen catalepsy tests are not analogous and cannot be used interchangeably. Finally, daily administration of haloperidol (1.50 mg/kg) produced a pattern of tolerance on the horizontal bar in the first two experiments (Figs. 1 and 2a) but not the third experiment (Fig. 3a). Inherent variability of catalepsy testing may have contributed to this inconsistency in our results.

Other studies in the literature support the hypothesis that the schedule of neuroleptic administration may influence tolerance versus sensitization development. Carey and Deveaugh-Geiss (5) reported that daily but not intermittent administration of haloperidol (0.5 mg/kg, b.i.d.) produced behavioral (recovery of locomotor activity) and biochemical tolerance (reduced homovanillic acid levels) in rats. Masuda *et al.* (11) reported that haloperidol-induced bar catalepsy scores in mice increased with intermittent administration of haloperidol (0.6, 1.2, or 4.8 mg/kg), but decreased with daily administration of haloperidol (1.2 or 4.8 mg/kg).

The data from our experiment comparing single versus repeated behavioral testing indicates that this aspect of experimental design also affects the evolution of catalepsy responses. Although there was no difference between multiple-test and single-test groups on day 1, mean screen catalepsy scores for multiple-test treatment groups were consistently higher on subsequent days of testing than scores for single-test treatment groups (see Fig. 3b). Additionally, no significant changes in mean screen catalepsy scores were observed in the single-test haloperidol treatment groups. These findings suggest that patterns of catalepsy sensitization using the inclined screen might be influenced by conditioning phenomena. Several researchers have proposed that behavioral responses to chronic drug treatment represent state-dependent conditioning not related to the pharmacological activity of the drug (4, 7, 10, 12, 14, 15). Unfortunately, our experiment was not specifically designed to study conditioning phenomena.

Our data do suggest that conditioning is neither necessary nor sufficient to induce changes in catalepsy during chronic haloperidol treatment. A transient, but statistically significant, increase in mean bar catalepsy scores was observed between day 8 and 15 in both single-test haloperidol treatment groups (see Fig. 3a). Sen-

sitization development may be weaker or more variable in the absence of repeated behavioral assessments. Alternatively, any pattern of change over time may be more difficult to demonstrate when using independent samples that introduce interanimal error variance. The effects of multiple testing may also be dependent upon the use of particular behavioral paradigms. Repeated behavioral assessments increased mean screen catalepsy scores but had no consistent effect on mean bar catalepsy scores. Similarly, Hillegaart *et al.* (9) observed increases in catalepsy following repeated testing using an inclined screen, whereas Costall *et al.* (6) reported that repeated testing on a horizontal bar had no effect on catalepsy scores. The use of repeated testing paradigms should be minimized in future studies, particularly whenever the inclined screen test is employed.

In conclusion, our results suggest that dose, drug administration schedule, frequency of behavioral testing, and choice of catalepsy test all influence patterns of change in catalepsy responses to chronic haloperidol treatment. Daily administration of one higher haloperidol dose favored tolerance while intermittent administration of lower doses favored sensitization. Repeated behavioral assessments consistently increased scores of haloperidol-treated animals tested on the inclined screen and favored sensitization, but had no significant effects on bar catalepsy scores and no effect on saline-treated animals.

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