Pharmacology Biochemistry & Behavior, Vol. 33, pp. 633–636. Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Alprazolam But Not Diazepam Protects Hamsters With Heart Disease From the Medical Consequences of Stress

W. N. TAPP, B. H. NATELSON,[1] E. GROVER AND J. E. OTTENWELLER

Primate Neuro-Behavioral Unit, VA Medical Center, East Orange NJ 07019
and Department of Neurosciences, UMDNJ-New Jersey Medical School, Newark, NJ 07103

Received 7 December 1988

TAPP, W. N., B. H. NATELSON, E. GROVER AND J. E. OTTENWELLER. *Alprazolam but not diazepam protects hamsters with heart disease from the medical consequences of stress.* PHARMACOL BIOCHEM BEHAV **33**(3) 633–636, 1989.—We have previously shown that subjecting cardiomyopathic hamsters in the lesion-developing period of their heart disease to cold-immobilization stress had lethal consequences which could be blocked by alprazolam treatment. This experiment replicated that finding and also examined the efficacy of diazepam in this paradigm. In contrast to alprazolam, diazepam did not prevent the cardiomyopathic subjects from succumbing to the stressor. Thus, the effect of alprazolam in reducing stress-induced mortality did not reflect a generic benzodiazepine action.

Activity Benzodiazepines Cardiomyopathy Hamsters Hypothermia Stress

CARDIOMYOPATHIC hamsters (CMH) are a strain of Syrian hamsters which at 2 to 3 months of age develop cardiac microvascular spasm that leads to areas of focal myocardial necrosis (4). These lesions do not have immediate health consequences because the animal's heart adapts to them anatomically and physiologically. But over time, the heart begins to show evidence of compromised function, and finally the hamsters die with evidence of severe heart failure at about half the life expectancy of healthy hamsters (7).

Hamsters in the lesion-developing period of their disease have been treated with dibenzyline and this treatment was reported to exacerbate their pathological process and to cause unexpected death (3). Dibenzyline is an alpha-adrenergic antagonist which also increases endogenous catecholamines. Because of the unexpected effect of this treatment, we hypothesized that stress, which also increases catecholamines (6), would also be lethal in these animals. In a series of experiments (8), we collected data supporting this hypothesis. First, we found that cold-restraint stress produced lethal consequences in CMHs but not in healthy hamsters. Then, we found that stressor intensity was directly related to the degree of medical risk associated with stress: CMHs only succumbed following stress in the supine position but never following stress in the prone position; earlier work has indicated that supine restraint is a more intense stressor than prone restraint (10). Finally, we treated CMHs chronically with either alprazolam or saline before and during stress. We found that alprazolam-treated CMHs succumbed to stress less often than controls.

Because of the results of this study, we elected to compare the therapeutic effects of chronically administered alprazolam with those of the classic benzodiazepine, diazepam. This study reports that alprazolam was again successful in blocking the lethal consequences of stress in young CMHs, but diazepam was not.

METHOD

Animals

The animals were 2-month-old CMHs purchased from Canadian Hybrid Farms (Nova Scotia, Canada). Hamsters were individually housed in shoe-box cages with Sanicell bedding and were given unlimited access to Purina mouse chow and tap water. The hamsters were placed on a 12-hr light:dark schedule with lights off at 2 p.m. and were allowed to adapt to these conditions for 2 weeks; thus, hamsters were 2.5 months old at the start of the experiment. We used this light-dark schedule to allow us to stress the hamsters in their early night, a time of day when the pathological outcome of stress is increased (1). Body weights were taken three times a week during the experiment.

Determining the Behavioral Equivalence of the 2 Benzodiazepines

The dose of the 2 drugs used was determined according to a

[1]Requests for reprints should be addressed to B. H. Natelson, M.D., Department of Neurosciences, New Jersey Medical School, 88 Ross Street, East Orange, NJ 07018.

behavioral criterion. The lowest dose of each that would permit us to supine-immobilize the hamster without its making aggressive escape efforts to prevent immobilization was used. We used this criterion as an operational definition of tranquilization. The subcutaneous dose of alprazolam that produced these conditions was 16 mg/kg. In this experiment like the previous one (8), we chose to give the drug twice daily because the benzodiazepines are usually prescribed to be taken more than once daily in clinical usage. Since the tranquilizing effect of the initial injection had disappeared 4 hr after the injection, a second injection of the same dose of alprazolam was given 4 hr after the first dose. A subcutaneous injection of 96 mg/kg of diazepam produced our criterion for tranquilization 1–2 hr following injection. Several hours thereafter, the hamsters still showed some evidence of a drug effect but did not fulfill our criterion for tranquilization because they did make some escape attempts. To produce a behaviorally equivalent effect at the time when the stressor would be administered, a second diazepam dose was requisite. Testing revealed that using 48 mg/kg for this second dose produced behavioral tranquilization 1–2 hr after injection which was no longer evident the next morning. Thus, the dosing regimen of the 2 benzodiazepines produced similar behavioral tranquilization which lasted at least for the 2-hr period of time that the stressor would be applied.

Injection Regimen

Hamsters were randomly divided into three groups of 24 each. Injections were administered at 9 a.m. and 1 p.m. Since treatment took about 1 hr, completion of the second round of daily injections was timed to occur at the time of lights out—the time when stress would be administered. One group was injected with vehicle (0.25% methylcellulose), a second group with alprazolam suspended in this vehicle and a third group with diazepam suspended in the same vehicle. Injections were begun one week prior to the beginning of the stress protocol.

Stress Protocol

Hamsters within each injection group were further subdivided into stress and no-stress groups. The stress protocol was carried out on 5 consecutive days and began at 2 p.m. No-stress hamsters were removed from their cages and moved into new cages provided with bedding, but devoid of food and water. Two hr later the hamsters were returned to their home cages. Stress hamsters were removed from their cages and restrained in the supine position. Then they had their core temperature taken by digital thermometer following placement of a thermal probe into the rectum. After removal of the probe, hamsters were placed in a cold chamber with temperatures averaging about 5°C. Two hr later, the hamsters were removed from the cold, and their rectal temperatures were immediately detemined. Then they were released and returned to their home cages. The injection regimen was continued until the end of the experiment, 19 days after the last stress session.

Behavioral Observations

On our dose determination pilot study, we were impressed by the fact that alprazolam-treated hamsters seemed to show a striking hyperactivity about an hour after injection. To verify and validate that anecdotal observation, we used a four-point scale to rate the motor behavior of the hamsters: 0 = asleep; 1 = awake but immobile; 2 = eating, grooming or burrowing; 3 = moving restlessly back and forth in the cage. We scored hamsters daily 1 hr after the first injection. In addition to these measures, a second behavioral measure was made. During the course of the experiment, we anecdotally observed that hamsters in the diazepam group appeared not to show the usual behavioral repertoire of vigorous escape and vocalization displayed by hamsters in the other groups during the first injection of the day. To test this, we recorded the presence or absence of vocalization related to the hamsters' receiving their first injection of the day; we did this every 3 to 4 days beginning in the middle of the stress period for a total of 5 such recordings.

Statistical Analysis

Differences in mortality across the treatment groups were assessed for statistical significance by Fisher's Exact Test. Differences in the behavioral measures recorded before and after injection were tested for significance by Fisher's and chi-square tests. Day 1 temperatures were evaluated by one-way analysis of variance. However, hamsters in the 3 groups succumbed differentially to stress after the first day that the stressor was administered, and thus missing values occurred in the subsequent temperature and weight data that were dependent on the treatment effect (see below). This presents a problem for statistical analysis. However, in order to give the reader a sense of the trends in the data, we have treated the analyses as if the distribution of missing data was independent of treatment effects and have followed the suggestion of Edwards (2) to randomly drop animals from those groups in which more had survived in order to provide equal n's in the different treatment groups. Analyses of variance for repeated measures with Dunn's tests for individual comparisons were then performed on these data. Since these analyses are not critical to the interpretation of the results, this seems an appropriate way of dealing with the problem, but the reader must view the probabilities generated as providing only points of reference for evaluating these data.

RESULTS

Body Weight

Analysis of variance revealed a significant stress effect, $F(1,66) = 47.7$, $p<0.001$, and a significant days effect, $F(16,895) = 27.1$, $p<0.001$. Dunn's test revealed significant weight gains over the one-week prestress period (p at least <0.05). Weight then remained stable over the 5-day experimental period for the no-stress group, but showed a progressive decline for the stressed hamsters. By the fourth day after the 5-day stress regimen, weights began to increase linearly again. The interaction between drug treatment and days was significant, $F(32,895) = 3.7$, $p<0.001$. Alprazolam-treated hamsters were significantly heavier than diazepam- or vehicle-treated hamsters on the last four measurement days after the end of the stress period (means for the 3 groups respectively were 91.4, 83.8, and 83.7; $p<0.05$). There was no significant difference between the body weight of nonsurvivors as determined on the day before they died and the body weight of survivors as measured on the fifth day of stressor administration (67.4 ± 1.8 g and 71.2 ± 1.7 g respectively). Thus the body weight data do not appear to explain the differences in survival noted below.

Mortality

Figure 1 presents the survival data from the experiment. Of all 36 unstressed hamsters, only one succumbed over the course of the experiment due to the injection protocol. That animal died in the prestress injection week. In contrast, 7 of 11 vehicle-treated, stressed hamsters succumbed, as did 7 of 12 diazepam-treated, stressed hamsters, but only 2 of 12 alprazolam-treated, stressed

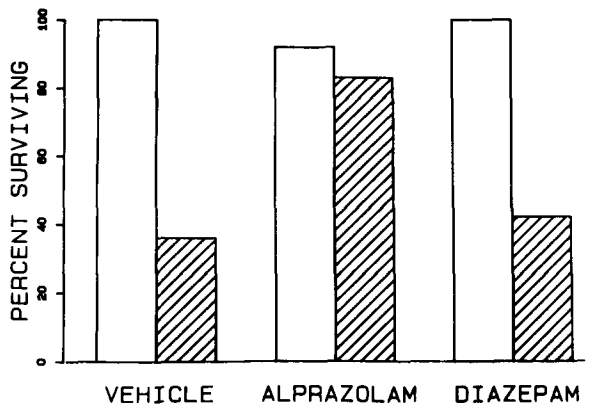

FIG. 1. Percent of cardiomyopathic hamsters surviving in the no-stress groups (open bars) and stress groups (hatched bars). Note that alprazolam selectively protects stressed CMHs from the lethal consequences of stress.

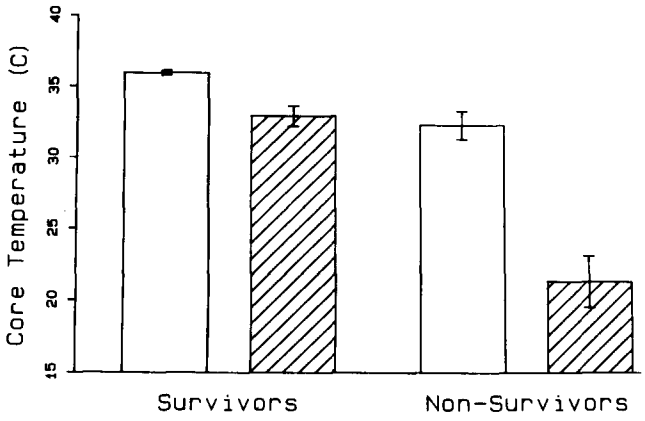

FIG. 2. Mean core temperatures (°C) ± SEM for all the hamsters that survived and did not survive stress. Data from survivors are depicted before (open bars) and after (hatched bars) the fifth stress session. Data from hamsters that succumbed to stress are depicted before (open bars) and after (hatched bars) the stress session on the day before they died.

hamsters succumbed (Fisher's exact tests between alprazolam stressed hamsters and the 2 groups respectively were 0.029 and 0.045).

Core Temperatures of Stressed CMHs

Analysis of variance of pre- and poststress temperatures collected on the first day of stress showed no differences across the treatment groups: Temperatures fell from a prestress average of 37.2°C to 33.9±0.7° (SEM), 31.7±1.1°, and 32.2±0.3° for vehicle, alprazolam and diazepam groups respectively.

A second analysis of variance was computed on data from hamsters surviving the entire study. Besides the expected temperature decline due to the stressor, the only other significant effect was a general decrease in both pre- and poststress temperatures over days for these hamsters, $F(4,72)=5.1$, $p<0.005$. Day 5 temperatures were significantly lower than temperatures found on days 1–4 (t_D's>2.73, p's<0.05). No significant effect of drug treatment was found in this analysis.

A final analysis was done on the temperatures of surviving and dying hamsters (see Fig. 2). This analysis was done independent of drug treatment because there were too few alprazolam-treated hamsters which succumbed to stress to examine drug treatments in this analysis. The analysis was based on pre- and poststress measures taken in the day prior to death for animals succumbing to stress and for the fifth day of stress for animals not succumbing to stress. There was a significant difference between temperatures found in survivors and in animals that died, $F(1,30)=32.2$, $p<0.001$, as well as a significant interaction between outcome and the expected stress-induced decline in body temperature, $F(1,30)=27.7$, $p<0.001$. Both prestress and poststress temperatures were lower in dying hamsters than in survivors (t_D's>2.74, p's<0.05). The magnitude of the stress-induced decline in core temperature was also greater in hamsters that died than in those that survived ($t_D=3.94$, $p<0.01$).

Behavioral Measures

On the first injection day, 17 of the 24 alprazolam-treated hamsters showed stereotyped walking back and forth, while only 1 of the 24 diazepam-treated hamsters and none of the vehicle-treated animals showed this ($p<0.01$). This effect was greatly attenuated by the second injection day, and it had disappeared by the third day. Thereafter, hamsters were usually asleep an hour after injection. Frequency of hamsters being awake was highest in the vehicle-treated hamsters and somewhat lower in the 2 drug-treated groups.

Vocalization data for the 5 days during which data were collected were examined. Partial habituation to the injection regimen was evident by the fact that fewer animals in each of the 6 treatment groups vocalized on the last data collection day than on the first or second. The 5 days worth of data were then summed for each of the 6 treatment groups. No significant differences were found for stress or control groups in the 2 drug-treated groups. However, vehicle-treated stressed hamsters vocalized less than vehicle-treated unstressed hamsters (no vocalizations were noted during 2 of 35 and 13 of 60 injections respectively; Fisher's test=0.04). A chi-square test then compared vehicle-treated unstressed hamsters to all alprazolam-treated hamsters (no vocalizations were detected during the injection protocol in 41 of 106 evaluations) and all diazepam-treated hamsters (no vocalizations were heard during the injection protocol in 72 of 91 evaluations) and found this distribution to be significantly different from random, $\chi^2(2)=55.5$, $p<0.001$. This analysis suggests that vehicle-treated unstressed hamsters were most aroused by the injection regimen, alprazolam-treated hamsters less so and diazepam-treated hamsters even less.

DISCUSSION

This study replicated our earlier report (8) that stress produces premature death in cardiomyopathic hamsters and that pretreatment with alprazolam prevents this from occurring. In contrast, pretreatment with diazepam was ineffective in altering the stress-induced effect. Further experiments are required to determine if other doses of diazepam are ever protective in this animal disease model.

Our earlier work using alprazolam alone and saline controls showed very similar results—both for mortality and core temperature—as we found in this experiment. In our previous paper (8), we found that alprazolam-treated hamsters had higher poststress temperatures over time than did controls. Our conclusion that this effect was neither a direct drug effect on body temperature nor an interaction between drug and stress on thermoregulation is buttressed by data reported here. Temperatures on day 1 when hamsters were all in the same state of health revealed no effect of

drug treatment. The critical variable affecting body temperature seems to be whether the hamster will survive and not which drug treatment it has received. Survivors defend body temperature better than hamsters that succumb to stress, and survivors show changes in body temperature that are independent of drug treatment. Thus, the apparent differences in core temperature reported by us previously (8) appear to reflect the presence or absence of some life-threatening state.

These data mean that alprazolam did not exert its protective effect by helping the animal thermoregulate but did so by some other mechanism. In our earlier report, we suggested that mechanism might be alprazolam's ability to reduce the stressfulness of the stressor. This experiment was designed to begin analyzing this possibility by employing a second benzodiazepine—diazepam—which like alprazolam is anxiolytic. However, in contrast to alprazolam, diazepam exerted no therapeutic effect.

Because we realized that dosage differences might prove important in understanding our results, we chose doses of the 2 drugs which produced similar degrees of behavioral tranquilization. Thus, at the doses used, hamsters treated with either diazepam or alprazolam an hr earlier allowed the experimenter to place them in the supine position with forelimbs extended. However, other behavioral observations suggested that the 2 benzodiazepines had subtler, differential effects on behavior. Only the alprazolam-treated hamsters showed hyperkinetic activity—an effect which is unlikely to explain the enhanced survival in this group because it had disappeared by the beginning of the stress procedures. Hamsters also responded to the stressor of human handling and drug/vehicle injection differentially, with vehicle animals vocalizing most often, alprazolam-treated animals vocalizing next most often, and diazepam-treated animals vocalizing the least. The fact that diazepam-treated animals were the least behaviorally aroused of all 3 groups tested and yet were not protected from the lethal effects of stress would appear to rule out the possibility that alprazolam produced its therapeutic effect by decreasing the animals' perception of the stressfulness of supine cold-immobilization.

A third possibility relates to a differential effect of the 2 benzodiazepines on the stress response, i.e., the set of all physiological changes that follow application of a stressor. Were this possibility the mechanism for the therapeutic effect of alprazolam, one would expect this drug to block stress-induced physiological changes more than diazepam. Vogel *et al.* have provided data supporting this expectation (11), and thus we consider this possibility the most likely explanation of alprazolam's protective effect at the present time. In fact, the finding of that group that alprazolam selectively depresses stress-induced epinephrine release may be the mechanism for the protective effect of the drug seen here. Thus, measuring plasma catecholamines in the stressed cardiomyopathic hamster during treatment with either alprazolam or diazepam might be an important experiment to do in the future.

In a series of prior papers, we have provided evidence to support the hypothesis that stress is particularly medically relevant for the individual with organ vulnerability (5,9). Although questions exist as to the medical consequences of stress for the healthy individual, it seems clear that stress is a particular danger for the individual with either heart disease or with the disposition to develop heart disease. This work supports that line of thought and shows that under the conditions used here, hamsters with heart disease that are subjected to stress die prematurely. Alprazolam, but not diazepam, considerably reduced the risk of this process from occurring. This finding suggests a possible role for alprazolam in the care of the ill during periods of stress.

ACKNOWLEDGEMENTS

This work was supported by VA medical research funds and by funds supplied by the Upjohn Corporation. Upjohn provided the vehicle and alprazolam used in these experiments. We are grateful to Hoffmann-La Roche for providing us with the diazepam used here.

REFERENCES

1. Ader, R. Behavioral and physiological rhythms and the develoment of gastric erosions in the rat. Psychosom. Med. 29:345–353; 1967.
2. Edwards, A. L. Experimental design in psychological research. New York: Holt Rinehart Winston; 1968:263–264.
3. Factor, S. M.; Cho, S. Alpha adrenergic blockade of the cardiomyopathic Syrian hamster: further evidence for the microvascular etiology of microencrosis. Fed. Proc. 42:920; 1983.
4. Factor, S. M.; Sonnenblick, E. H. The pathogenesis of clinical and experimental congestive cardiomyopathy: recent concepts. Prog. Cardiovasc. Dis. 27:395–420; 1985.
5. Natelson, B. H. Stress, predisposition and the onset of serious disease: Implications about psychosomatic etiology. Neurosci. Biobehav. Rev. 7:511–527; 1983.
6. Natelson, B. H.; Creighton, D.; McCarty, R.; Tapp. W. N.; Pitman, D.; Ottenweller, J. E. Adrenal hormonal indices of stress in laboratory rats. Physiol. Behav. 39:117–125; 1987.
7. Ottenweller, J. E.; Tapp. W. N.; Chen, T. S.; Natelson, B. H. Cardiovascular aging in Syrian hamsters: similarities between normal aging and disease. Exp. Aging Res. 13:73–84; 1987.
8. Tapp, W. N.; Natelson, B. H.; Creighton, D.; Khazam, C.; Ottenweller, J. E. Alprazolam reduces stress-induced mortality in cardiomyopathic hamsters. Pharmacol. Biochem. Behav. 32:331–336; 1989.
9. Tapp, W. N.; Natelson, B. H. Consequences of stress: a multiplicative function of health status. FASEB J. 2:2268–2271; 1988.
10. Vincent, G. P.; Glavin, G. B.; Rutkowski, J. L.; Paré, W. P. Body orientation, food deprivation and potentiation of restraint induced gastric lesions. Gastroenterol. Clin. Biol. 1:539–543; 1977.
11. Vogel, W. H.; Miller, J.; DeTurck, K. H.; Routzahn, B. K. Effects of psychoactive drugs on plasma catecholamines during stress in rats. Neuropharmacology 23:1105–1108; 1984.