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BRIEF COMMUNICATION

Potentiation of Cold Swim Stress Analgesia in Rats by Diazepam[1]

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LEITNER, D. S. AND D. D. KELLY. *Potentiation of cold swim stress analgesia in rats by diazepam.* PHARMACOL BIOCHEM BEHAV 21(5) 813–816, 1984.—It was hypothesized that diazepam (DZP) would attenuate the analgesia produced by cold swim stress because of its anxiolytic and biochemical anti-stress properties. In fact, it had the opposite effect. Administered alone, neither DZP nor its vehicle affected the nociceptive thresholds of rats, as assessed by a flinch-jump test. In combination with cold swim stress, DZP elevated nociceptive thresholds significantly more than did the stressor alone. This was true whether DZP was injected before or after exposure to the stressor, or whether or not DZP administration was acute or chronic.

Stress-induced analgesia Cold swim stress Diazepam Rats Flinch-jump test

ACUTE exposure to a stressor produces a wide range of biochemical and behavioral changes in an organism. These include activation of the pituitary-adrenal axis [20] with increases in blood levels of β-endorphin, adrenocorticotrophic hormone, and corticosterone [10, 13, 15].

Documented behavioral changes produced by exposure a stressor include the elevation of nociceptive thresholds a period of time which outlasts the duration of the expo-e [5,11]. Manipulation of an organism's physiological re-ɔnse to a stressor has been found to alter this analgesia [1, 8, 9, 16, 17, 18].

Other physiological manipulations which are known to ɛr the experience of stress should modulate the analgesia p ɔduced by an effective analgesic stressor, even if such m. nipulations do not interfere directly with the pituitary-adrenal axis. Diazepam (DZP), a minor tranquilizer, has been shown to attenuate the biochemical consequences of exposure to a stressor [6, 12, 21]. Because of these properties, it was hypothesized that DZP would attenuate the analgesia produced by exposure to a stressor. Specifically, the effects of DZP on the analgesia induced by a forced swim in cold water were investigated.

METHOD

Subjects

The subjects were 21 albino Sprague-Dawley male rats, approximately 250 days old at the beginning of the experiment.

Apparatus

The apparatus used for the cold swim stress was a circular steel bucket with tapered sides, 30 cm deep and 31 cm at its greatest diameter. The bucket had a thick, clear Plexiglas lid.

Flinch-jump thresholds were assessed in a small chamber (23×20×24 cm) made of aluminum and clear Plexiglas. The chamber floor was a grid consisting of 16 stainless steel rods through which a scrambled constant current footshock could be delivered.

Procedure

Subjects were first exposed to the testing procedure twice a week for two weeks to habituate them to it. This consisted of placing them individually in the testing chamber and determining their flinch and jump thresholds using a modification of the procedure developed by Evans [7]. In the present case, an ascending method of limits procedure was used; the initial footshock was 0.10 mA, incremented thereafter in 0.05 mA steps. The interval between shock presentations was 10 sec. Mean flinch and jump thresholds were computed across 6 trials for each subject.

The experiments began one week following the above procedure. Eleven of the subjects were exposed to each of six different conditions, presented in orders determined by a Latin square matrix. In conditions involving a cold water swim, subjects were placed in the bucket filled with water maintained at 2°C to a depth of 25 cm for 3.5 min. The DZP used in the study (Hoffman-LaRoche, No. R05-2807, lot

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727020) was suspended in a 25% propylene glycol solution, and injected intraperitoneally at 2 mg/ml/kg.

The conditions, which were presented with a 3 day interval between exposures, were: (1) Flinch-jump testing without swim exposure or drug injection; (2) Flinch-jump testing 30 min after DZP injection, without swim; (3) Testing 60 min after injection, without swim; (4) Testing 30 min after swim, without injection; (5) Testing 30 min after an injection which immediately followed a swim; and (6) Testing 60 min after injection, and 30 min after swim.

Two weeks after the completion of this procedure, the 11 subjects were used in a control experiment to examine the effects of propylene glycol on nociceptive thresholds. This substance is known to induce physiological and behavioral changes when administered alone [23]. Each subject was exposed to 3 conditions over 3 consecutive days. The conditions were: a flinch-jump test without injection of vehicle; testing 60 min after a vehicle injection (1 ml/kg, IP); and testing 60 min after injection of DZP (2 mg/kg, IP). The order of presentation was varied randomly for each subject.

A second control procedure was implemented 1 week after the completion of the first control procedure. Ten of the 11 subjects were used, making 2 groups of equal size. One group was injected with the vehicle (1 ml/kg) 30 min before a swim, and given a flinch-jump test 30 min after the swim; the other group was tested 30 min after a swim, without injection. These conditions were reversed for the two groups 24 hr later.

The benzodiazepenes are known to have both sedative and anxiolytic properties, and the former, but not the latter, habituate with chronic administration [19,22]. To control for the potential effects of sedation upon nociceptive thresholds, the 10 subjects who had not yet been exposed to DZP or cold swim stress were injected with DZP (2 mg/kg, IP) once daily for 20 days. The subjects were then divided into 2 groups of equal size. One group was exposed to condition 1 from the original experiment, and the other group was exposed to condition 3. Forty-eight hr later, the conditions were reversed. The chronicity of the dosage was maintained for all subjects throughout the testing period; for those exposed to condition 1, the daily dose was administered after testing was completed.

Five days after the completion of this, during which time all subjects received a daily injection of DZP, the subjects were again divided into 2 groups of equal size. One group was exposed to condition 6 from the original experiment, and the other group was exposed to condition 4. Forty-eight hr later, the conditions were reversed for the two groups. As before, chronic dosing was maintained throughout the testing period.

RESULTS

Mean flinch and jump thresholds were computed across subjects for each of the 6 conditions; these data are depicted in Fig. 1. It can be seen that DZP injected 30 or 60 min before a flinch-jump test (conditions 2 and 3) did not affect subjects' nociceptive thresholds, compared to thresholds determined without DZP injection (condition 1). As expected, exposure to a cold water swim 30 min before testing (condition 4) elevated the subjects' thresholds, compared to conditions 1, 2, and 3. DZP injected immediately after a swim and 30 min before testing (condition 5) or 30 min before a swim and 60 min before testing (condition 6) elevated thresholds above those produced by a swim alone (condition 4). No differential effect was produced by varying the injection-swim interval (condition 5 vs. condition 6).

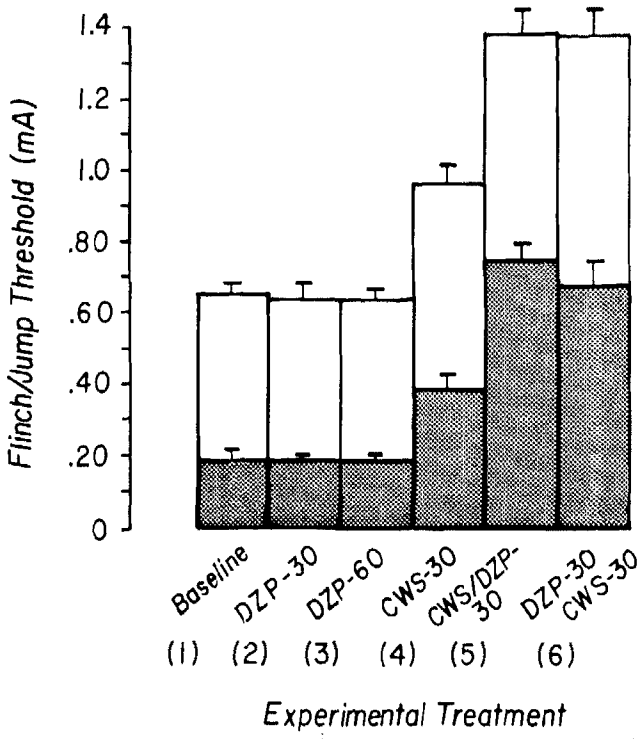

FIG. 1. Mean flinch (shaded) and jump (solid) thresholds, computed across subjects for each of the six conditions (±S.E.M.) in the initial experiment.

Separate one-way analyses of variance for repeated measures were conducted on the flinch and jump data. A significant effect of condition was found for both flinch, $F(5,50)=48.97, p<0.001$, and jump, $F(5,50)=49.68, p<0.001$. Post-hoc Newman-Keuls tests were then performed to examine individual differences between conditions. For both measures, DZP injected 30 or 60 min before testing (conditions 2 and 3) were not significantly different from the test alone (condition 1); these three conditions were all significantly different from the cold swim stress alone (condition 4); and all four of these conditions were significantly different from the two swim and DZP injection conditions (5 and 6), which were not reliably different from each other.

Means computed across subjects for the first control procedure indicated that no reliable differences among the three conditions were present for either flinch or jump. Mean flinch thresholds (mA±S.E.M.) were: no injection, 0.23±0.03; vehicle injection, 0.22±0.01; DZP injection, 0.21±0.02. Mean jump thresholds were: no injection, 0.76±0.04; vehicle injection, 0.74±0.03; DZP injection, 0.74±0.03. The lack of reliable differences was confirmed by separate one-way repeated-measures analyses of variance performed on the flinch, $F(2,20)=1.17$, NS, and jump, $F(2,20)=0.48$, NS, data.

Means computed across subjects for the second control procedure indicated that, again, no reliable differences occurred between the two conditions for either flinch or jump. Mean flinch thresholds (mA±S.E.M.) were: swim only, 0.45±0.03; vehicle and swim, 0.43±0.04. Mean jump thresholds were: swim only, 1.10±0.07; vehicle and swim,

TABLE 1

MEAN FINCH AND JUMP THRESHOLDS FOR SUBJECTS CHRONICALLY ADMINISTERED DZP WITH AND WITHOUT DZP INJECTIONS AND WITH AND WITHOUT EXPOSURE TO COLD WATER SWIM

Condition	Flinch Threshold (mA) (±S.E.M.)	Jump Threshold (mA) (±S.E.M.)
No swim; No DZP	0.27 (±0.018)	0.57 (±0.012)
No swim; DZP	0.25 (±0.018)	0.60 (±0.016)
Swim; DZP	0.40 (±0.019)	0.80 (±0.042)
Swim; No DZP	0.52 (±0.036)	0.94 (±0.055)

1.10±0.07. The lack of reliable differences was confirmed by *t*-tests for related measures performed on the flinch, $t(9)=0.68$, NS, and jump, $t(9)=0.05$, NS, data.

The results from the control procedure involving the chronic administration of DZP are depicted in Table 1, which shows mean flinch and jump thresholds, computed across subjects for the 4 conditions. The results of the first manipulation appear in the upper part of the table. No differences are apparent between flinch-jump testing alone and the testing that occurred 60 min after a DZP injection. This was confirmed by separate *t*-tests for related measures performed on the flinch, $t(9)=0.78$, NS, and jump, $t(9)=1.27$, NS, data.

The results of the second manipulation appear in the lower part of Table 1. DZP in combination with a cold water swim elevated flinch and jump thresholds above those obtained after exposure to a cold water swim alone. These differences proved to be significant when examined by *t*-tests for related measures (flinch: $t(9)=4.77$, $p<0.01$; jump: $t(9)=4.37$, $p<0.01$).

DISCUSSION

DZP is not an analgesic compound; with the dosage used in this study, the substance had no effect on nociceptive thresholds when administered alone. This is consistent with the results obtained by Kelly, Bodnar, Brutus, Woods, and Glusman [14], who, using a different analgesimetric procedure but similar drug dosages, found that DZP had no effect on either the subjects' motor behavior or perception of pain. Contrary to expectations, DZP was found to potentiate the analgesia produced by a forced swim in cold water instead of attenuating it. This synergistic interaction occurred whether or not DZP was present during actual exposure to the stressor. The vehicle solvent did not produce analgesia, nor did it potentiate the analgesia produced by cold swim stress. The potentiation of analgesia produced by DZP was present even after chronic dosing, suggesting that the general sedative effects of DZP were not the cause of the heightened analgesia.

DZP is known to produce a transient hypothermia of approximately 2°C in rats at doses of 10 mg/kg [3]; at lower doses, this hypothermia declines but is still present. The hypothermia induced by DZP may interact with the hypothermia produced by exposure to cold swim stress to produce a greater drop in body temperature than either of these alone. The subjects' body temperature remains depressed for quite some time after exposure to a cold water swim [2], and DZP would potentiate this hypothermia even if it was administered some time after exposure to the stressor. To fully explain the present data, the potentiated hypothermia would have to be present in subjects who received chronic administration of DZP as well as in subjects given acute doses; the hypothermia should not habituate with chronic dosing. Work currently in progress is exploring these possibilities.

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