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Benzodiazepines Decrease Grooming in Response to Novelty but not ACTH or β-Endorphin

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DUNN, A. J., A. L. GUILD, N. R. KRAMARCY AND M. D. WARE. *Benzodiazepines decrease grooming in response to novelty but not ACTH or β-endorphin.* PHARMAC. BIOCHEM. BEHAV. 15(4) 605–608, 1981.—Excessive grooming in response to intracerebroventricular (ICV) $ACTH_{1-24}$ was assayed following various doses of diazepam, chlordiazepoxide and flurazepam. Grooming scores were only affected by doses of the benzodiazepines higher than those that depressed locomotor activity. Similarly, diazepam did not affect excessive grooming induced by ICV β-endorphin, nor did chronic chlordiazepoxide affect ACTH-induced grooming. By contrast similar doses of the benzodiazepines decreased the increased grooming score observed when mice were observed in a novel as opposed to the home cage. This result is consistent with the hypothesis that novel cage-induced grooming is caused by an increase in the ventricular content of ACTH or β-endorphin, and that the benzodiazepines decrease or prevent this increase. It is not consistent with hypotheses of a functional antagonism between ACTH and benzodiazepines, at least insofar as the mechanisms involved in the production of grooming are concerned.

ACTH β-endorphin Excessive grooming Benzodiazepines Novelty

INTRACEREBROVENTRICULAR (ICV) injection of ACTH causes excessive grooming in rats [8] and mice [18]. Increased grooming has long been known to occur in certain stressful situations [5] and has been considered to be a displacement behavior [13]. Recently, increased grooming has been observed following transfer of rats to a novel environment [1, 3, 15] and other mild stressors (see [4] and [10] for reviews). It has been argued that this grooming induced by mild stress may be due to intracerebral secretion of ACTH. In support of this, novelty-induced grooming was impaired in hypophysectomized rats [3], although another group failed to replicate this result [15]. However, ICV administration of a specific antiserum to ACTH impaired novelty-induced grooming, whereas peripheral injections of ACTH antiserum or ICV injections of control serum were ineffective [3]. Furthermore, naloxone and haloperidol, both of which impair ACTH-induced grooming [9,21], impaired novelty-induced grooming at doses that did not alter locomotor or exploratory activities [11].

An extensive literature suggests that benzodiazepines reduce stress responses, in particular by decreasing ACTH and glucocorticoid secretion [16]. If novelty-induced grooming is indeed caused by secretion of ACTH, then benzodiazepines should decrease the grooming response to novelty. Because others have suggested a functional antagonism between ACTH and the benzodiazepines [6,7], it is first necessary to test whether benzodiazepines affect ACTH-induced grooming.

In the present study, we investigated the effects of benzodiazepines on ACTH-, β-endorphin- and novelty-induced grooming. In addition to further substantiation of the involvement of ACTH and/or β-endorphin in novelty-induced grooming, we hoped also to determine whether the functional antagonism between ACTH and the benzodiazepines extends to the grooming-eliciting properties of ACTH.

EXPERIMENT 1: EFFECTS OF VARIOUS DOSES OF DIAZEPAM AND CHLORDIAZEPOXIDE ON ACTH-INDUCED GROOMING

METHOD

Animals

Male albino mice 19–24 g at surgery were used. Animals were prepared for intracerebroventricular (ICV) injections with injection ports overlying each lateral ventricle as previously described [2]. After surgery and for the duration of the experiment, the animals were housed singly and maintained on a 12:12 light/dark cycle (lights on 7 a.m.). The average room temperature was 24°C. The animals were allowed free access to food and water.

Materials

$ACTH_{1-24}$ (Cosyntropin, Organon) was dissolved in 0.9% saline to a concentration of 0.5 mg/ml. Diazepam (DZP): the supplied 5 mg/ml DZP solution (Roche) was diluted to 0.125 mg/ml, 0.05 mg/ml and 0.025 mg/ml with a vehicle solution

composed of 40% propylene glycol, 10% ethyl alcohol and 50% saline (0.9%). Chlordiazepoxide-HCl (CDP, Roche) was dissolved in 0.9% saline at 1.0 mg/ml, 0.5 mg/ml and 0.25 mg/ml.

ICV injections (1 μl each side) of either 0.9% saline or $ACTH_{1-24}$ were made as previously described [2] with a microsyringe specially fitted with a needle guard so as to place the needle tip in the lateral ventricle. Intraperitoneal (IP) injections of either a vehicle or drug solution were administered with a constant injection volume of 0.1 ml.

Procedure

The screws were removed from the injection ports and each animal was placed in an individual Plexiglas observation box (16.5×29×15 cm). The ICV injections were administered and the animals replaced into the observation boxes, at which time behavioral observations began. Fifteen min later, the animals were injected IP with DZP, CDP or saline. The animals were replaced and behavioral observations continued for 30 min. Behaviors scored were grooming, locomotor activity, stretching and yawning, and quiescence, using a time-sampling technique as previously described [2] and previously validated [8]. On Days 1–4 each of twelve animals received the three doses of DZP and the vehicle treatment in a randomly assigned sequence in a Latin Square design, such that each animal received each dose on one of the four days. On Days 7–10, each animal received the three doses of CDP and the vehicle treatment according to the same design. On Day 13, 6 animals received both ICV and IP injections of saline.

TABLE 1

EFFECTS OF BENZODIAZEPINES ON $ACTH_{1-24}$-INDUCED GROOMING

Treatment		Median Percentage Scores*		
ICV	IP	G	M	Q
$ACTH_{1-24}$	DZP vehicle	46	31	11
$ACTH_{1-24}$	0.1 mg/kg DZP	48	30	18
$ACTH_{1-24}$	0.2 mg/kg DZP	47	14‡	36‡
$ACTH_{1-24}$	0.5 mg/kg DZP	33	12§	48§
$ACTH_{1-24}$	CDP vehicle (saline)	52	38	4
$ACTH_{1-24}$	1 mg/kg CDP	43	47	6
$ACTH_{1-24}$	2 mg/kg CDP	56	24	10
$ACTH_{1-24}$	5 mg/kg CDP	59	21†	19‡
Saline	Saline	12	83	3

Behavioral scores for the period 15 to 45 min following ICV injection (0 or 30 min following benzodiazepines).

*Median percentage score on: G, grooming; M, locomotor activity; Q, quiet. Twelve mice were used for this experiment, each animal being exposed to each dose of each drug for one day, in a Latin Square design.

†Significantly different from vehicle, $p<0.05$, ‡$p<0.02$, §$p<0.01$ (Wilcoxon matched-pairs signed-ranks test).

RESULTS

The results are presented as median percentage scores for each dose of drug over all days (Table 1). Although the behavioral observations began immediately after the ICV injections, only the scores during the 30-min time period immediately following the IP injections (15–45 min) were used for comparison. The scores during the first 15 min were to ensure that the ACTH effectively induced grooming. In all conditions tested, benzodiazepines did not significantly alter the grooming scores displayed in ACTH-treated animals. However, significant decreases in locomotor activity and significant increases in quiescence were observed with increasing drug doses (Table 1).

Since behavioral differences between acute and chronic administrations of benzodiazepines have been reported both from animal and clinical studies [7], comparisons between the days of treatment were made using single factor ANOVA for repeated measures for each behavior. These comparisons failed to find any significant difference between the days, i.e. the number of benzodiazepine administrations received did not significantly alter the behavioral responses (for each behavior, $F(3,33) < 1$).

EXPERIMENT 2: EFFECTS OF BENZODIAZEPINES ON ACTH- AND β-ENDORPHIN-INDUCED GROOMING

METHOD

Materials

ACTH, DZP and CDP were used as before. β-endorphin (Peninsula Laboratories) was dissolved at 0.15 mg/ml in saline. Flurazepam capsules (Roche) were dissolved in 0.9% saline (0.15 mg/ml) and filtered to remove the filler.

Procedure

Methods were essentially the same as in the first experiment, but a more convenient cannulation procedure was used. Rather than implanting jewellers' screws, small plastic cannulas (PE-50, Clay-Adams) were implanted in an identical position to a depth of 2 mm. Observations were performed in a large Lexan box 14×41×55 cm, divided into 12 equal-sized cubic compartments 13×13×13 cm. Each compartment was sealed by a Lexan door held in place by a magnetic door catch. The walls between compartments were painted opaque with gray paint, but the front and back walls were left transparent.

Mice were injected with either ACTH or β-endorphin (ICV) followed immediately by benzodiazepines or saline (IP). On the following day drug and saline treatments were interchanged. For the chronic CDP treatment, mice were injected once daily for 5 days with CDP (5 mg/kg, IP) as described by File [7]. ACTH was administered ICV 15 min before CDP on Days 1 and 5. Behaviors were scored every 30 sec from 15 through 60 min after the ICV injection.

RESULTS

Treatment with benzodiazepines at the same time as ACTH also failed to affect ACTH-induced grooming (Table 2). Flurazepam (0.5 mg/kg, IP) did not alter ACTH-induced grooming. And DZP (0.2 mg/kg, IP) did not affect β-endorphin-induced grooming. Neither drug affected the moving or quiet scores in these tests.

Also, since File [6] had found opposite effects of CDP

TABLE 2

EFFECTS OF TREATMENT WITH BENZODIAZEPINES ON $ACTH_{1-24}$- OR β-ENDORPHIN-INDUCED GROOMING

Treatment			Median Percentage Score		
ICV	IP	n	G	M	Q
$ACTH_{1-24}$	Saline	12	53	38	8
	Flurazepam (0.5 mg/kg)	12	53	35	7
β-Endorphin	Saline	12	44	41	11
	DZP (0.2 mg/kg)	12	49	41	7

Mice were injected ICV with either $ACTH_{1-24}$ (1 μg) or β-endorphin (0.3 μg) immediately followed by a benzodiazepine or saline. Behaviors were scored every 30 sec from 15 to 60 min following injection as described in the text. Saline- and benzodiazepine-treatments were reversed on the following day in each experiment. Combined data are presented from both days. The benzodiazepine treatments did not significantly alter any of the behavioral scores.

TABLE 3

EFFECTS OF CHRONIC CHLORDIAZEPOXIDE ON ACTH-INDUCED GROOMING

	Median Percentage Grooming Score		
	15–30 min	30–45 min	45–60 min
Day 1	53	47	53
Day 5	57	53	60
Day 6	43	43	50

Chlordiazepoxide (CDP) was administered (5 mg/kg, IP) on Days 1 through 5 but not Day 6, 30 min after ICV $ACTH_{1-24}$ on Days 1 and 5. No significant differences in grooming scores were observed (n=7).

administered acutely or chronically in the social interaction test, we studied the effect on ACTH-induced grooming of chronic CDP, in the dose and schedule used by File. There were no significant differences (Table 3) in the grooming, moving and quiet scores between Days 1, 5 and 6.

These results indicate that benzodiazepines do not affect ACTH- or β-endorphin-induced grooming, at least at doses that do not decrease locomotor activity.

EXPERIMENT 3: EFFECTS OF BENZODIAZEPINES ON NOVELTY-INDUCED GROOMING

METHOD

No surgery was performed but mice were housed individually. Behaviors were scored in the multicompartmented Lexan box described above, in a quiet room with a dim light (50 W light bulb, at a distance of 1.5 m) and a "white-noise" generator (75 dB).

TABLE 4

EFFECTS OF BENZODIAZEPINES ON NOVELTY-INDUCED GROOMING

Experiment	Median Percentage Score		
	Grooming	Moving	Quiet
1. Saline	45	43	11
CDP (2 mg/kg)	27‡	59‡	11
2. Home-cage	2‡	88‡	3
Saline	48	34	16
CDP (5 mg/kg)	29‡	36	34‡
3. Saline	56	36	12
DZP (0.5 mg/kg)	26‡	27†	41‡
4. Day 0 (No injection)	57	41	9
Day 1 (CDP)	14†	28	47‡
Day 5 (CDP)	22*	27	40
Day 6 (No injection)	30‡	39	38

Experiments 1–3: Mice (n=12) were scored for 60 min following placement in the novel box. Chlordiazepoxide, diazepam or saline was administered 10 min before this. All grooming scores in the novel box were significantly greater than in the home cage.

*Significantly different from saline ($p<0.05$, †$p<0.02$, ‡$p<0.01$, Wilcoxon matched-pairs signed ranks test).

Experiment 4: Mice (n=9) were scored from 60 min following placement in the novel box. CDP was injected on Days 1 to 5 but not Day 6 10 min before behavioral testing. Animals were not placed in the novel box on Days 2–4.

*Significantly different from Day 0, $p<0.05$, †$p<0.02$, ‡$p<0.01$.

Procedure

In Experiments 1–3, benzodiazepines or vehicle were injected 10 min before the mice were placed in the 'novel box.' On the next day, the drug and vehicle groups were interchanged. In Experiment 4, CDP (5 mg/kg) was injected for five successive days, starting on Day 1. Behaviors were scored on Days 0, 1, 5 and 6, starting 10 min after the IP injection on Days 1 and 5. In all experiments, behaviors were scored for 45 min, commencing immediately after the animals were placed in the 'novel box.'

RESULTS

The results (Table 4) clearly showed that whereas mice treated with saline showed a marked increase in grooming in the novel box over that observed in the home cage, the increased grooming was significantly depressed by pretreatment with CDP or DZP. At 2 mg/kg CDP, locomotor activity was increased, while grooming was decreased, whereas at 5 mg/kg grooming was decreased and quiescence increased while locomotor activity was unchanged. These data indicate a selective effect of CDP on grooming. Similarly 0.5 mg/kg DZP depressed grooming and increased both quiet and moving scores. Lower doses of DZP (0.2 mg/kg) gave variable results.

The results obtained with chronic CDP (Experiment 4) were very similar to those observed with acute CDP. Day 1 is equivalent to an acute experiment, and grooming scores were decreased as before. On Day 5 the data were almost identical. On Day 6, when the mice did not receive CDP,

grooming scores were increased relative to Days 1 and 5. The grooming score on Day 6 is lower than Day 0, probably because the animals had habituated to the novel box [1,3]. These results are consistent with the idea that benzodiazepines decrease stress-induced release of ACTH, and presumably β-endorphin.

GENERAL DISCUSSION

Whereas benzodiazepines increase interaction in a social interaction test of anxiety, ACTH decreases interactions and this effect can be prevented by chronic treatment with chlordiazepoxide [6,7]. A functional antagonism is also suggested by the report that CDP attenuated the ability of ACTH to inhibit spontaneous neural activity in the corticomedial amygdala [14]. And, that, whereas chronic CDP treatment elevated the content of serotonin (5-HT) and decreased the content of 5-hydroxyindoleacetic acid (5-HIAA, the major 5-HT metabolite) in rat hypothalamus, midbrain and cerebral cortex, ACTH increased the 5-HIAA content of midbrain and hypothalamus [7]. ACTH also antagonized the flurazepam-induced increases in spontaneous and decreases in K^+-induced [^{3}H]5-HT release from slices of amygdala [14].

The inability of chlordiazepoxide, diazepam or flurazepam to antagonize ACTH- or β-endorphin-induced grooming indicates that benzodiazepines have little or no affinity for whatever receptor the peptides act upon to elicit grooming. It also suggests that the functional antagonism between the benzodiazepines and ACTH is not universal. There is ample evidence for the existence in the brain of multiple receptors for ACTH [4, 12, 20].

Benzodiazepines have been suggested to act as anxiolytic agents by antagonizing the anxiogenic actions of ACTH [6,7]. Grooming is often displayed in stressful or conflict situations [5, 10, 13] and may thus reflect the release of ACTH [3]. The failure of the benzodiazepines to antagonize the grooming-eliciting action of ACTH suggests either, that grooming is not a manifestation of the anxiogenic actions of ACTH, or that the benzodiazepines act selectively to antagonize only certain components of the anxiogenic actions of ACTH.

Although we hypothesize that ACTH and/or β-endorphin released into the brain in response to novel stimulation activates the increased grooming observed in this situation [3], we do not know the origin of the peptides. They could arise either from central opiocortin neurons [19], or from the anterior pituitary by retrograde transport [17]. The data on the effects of hypophysectomy on novelty-induced grooming are conflicting [3,15] but, even were they not, hypophysectomy produces so many changes in an animal that the results could not be conclusive. Because benzodiazepines reduce the pituitary secretion of ACTH in response to stress [16], and it is likely that free cerebral β-endorphin increases during stress [4], we expect that the appearance of ACTH and β-endorphin in the cerebral extracellular fluid will increase during stress, and that the increase will be attenuated by benzodiazepines. Thus, benzodiazepines would decrease the appearance of ACTH and β-endorphin in the brain, and hence the grooming response. Our data are consistent with this interpretation so that they provide some support for the hypothesis that, during mild stress, ACTH and β-endorphin are released into the brain, and act on it to induce grooming.

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