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BRIEF COMMUNICATION

Endothelin-C-Terminal Hexapeptide Increases Grooming in Mice

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SCHERRER, J. F., J. E. MORLEY AND J. F. FLOOD. *Endothelin-C-terminal hexapeptide increases grooming in mice.* PHARMACOL BIOCHEM BEHAV **48**(4) 1031-1035, 1994.—Grooming behavior has been considered a response to stress, and a number of stress-related peptides have been demonstrated to modulate grooming behavior. In the experiments reported here, endothelin-C-terminal hexapeptide containing amino acid residues 16-21, ET[16-21], increased grooming with a maximum effect at 0.75 μg. ET[16-21] did not significantly alter eating or locomotor behavior. Both α-helical CRF (10 μg) and neuropeptide Y (1 μg) inhibited the grooming produced by ET[16-21].

Dopamine CRF Edothethelin Grooming Mice NPY

IN 1988, Yanagisawa et al. (26) isolated a long-acting vasoconstrictor peptide from endothelial cells. This 21 amino-acid peptide with two intrachain disulphide bridges linking paired cysteine amino acid residues was named endothelin. Subsequently, related endothelin peptides have been identified (7). Endothelin-1 (ET1) binds to sites in the thalamus, hypothalamus, and basal ganglia (20). Endothelin-3 (ET3), the dominant form in the brain, inhibits water intake in fluid deprived rats (23) and activates the release of corticotropin-releasing hormone (6). ET1 and ET3 share a common endothelin-C-terminal hexapeptide of amino acid residues 16-21,ET[16-21].

Corticotropin-releasing hormone (CRF) increases grooming and decreases feeding (15). CRF appears to enhance grooming due to a specific effect within the paraventricular nucleus of the hypothalamus (11,14). In view of the activating effects of ET3 on CRF, we investigated whether ET[16-21] alone would enhance feeding and grooming behaviors and whether CRF antagonist would block ET[16-21] enhanced grooming. Neuropeptide Y (NPY) has been demonstrated to have a variety of behavioral effects (5), one of which is inhibition of grooming (18). We, therefore, studied whether NPY could inhibit the grooming induced by ET[16-21].

METHOD

Subjects

Male TAC (SW) mice 2-3 months of age obtained from Taconic Farms, Inc., Germantown, NY, served as subjects. They were individually housed in plastic cages and maintained on a 12 L : 12 D schedule (lights off at 1800 h) under controlled temperature (21-23°C). Water and food (Purina Rodent Laboratory Chow 5001) were available ad lib except where noted. ET[16-21], α-helical CRF antagonist and NPY were obtained from Peninsula Laboratories, Inc., Belmont, CA. Each was prepared in physiological saline at pH of 6.5.

Surgery

Mice were prepared for an intracerebroventricular (ICV) injection 48 h before the experiments. In brief, mice were anesthetized with methoxyflurane, placed in a stereotaxic instrument, and a hole was drilled over the lateral ventricle where it joins the third ventricle (−0.5 mm relative to bregma, 0.5 right of the central suture). All mice were experimentally naive and only served as subjects on one occasion.

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Experiment 1

On the day of the experiments, the mice were injected ICV at a depth of 2.8 mm (relative to the skull surface) over a 30 s period with a 2 μl volume of 0.50, 0.75, 1.0 μg of ET[16-21] or saline delivered through a 30 gauge needle cut to 2.8 mm and attached to a 10 μl syringe. To avoid anesthetic interference with drug or food consumption, mice were wrapped in a towel and injected free-hand through the predrilled hole. The injection procedure required less than 1 min with the mouse being restrained about 30 s. The technicians who performed these procedures had less than a 1% failure rate for injection accuracy tested by injecting thionin ICV and determining the location of dye in frozen brain sections. Following the injections, mice were given up to 10 min to recover full normal motor behavior before intiating behavioral ratings. Mice that did not recover activity within 10 min were removed from the study. A preweighed food pellet was placed in the cage immediately after the injection and removed 1 h later so as not to disturb the mice during the behavioral observation period.

All behavioral testing was conducted in the home cage of each mouse (12 cm by 22 cm clear plastic). Grooming, eating,

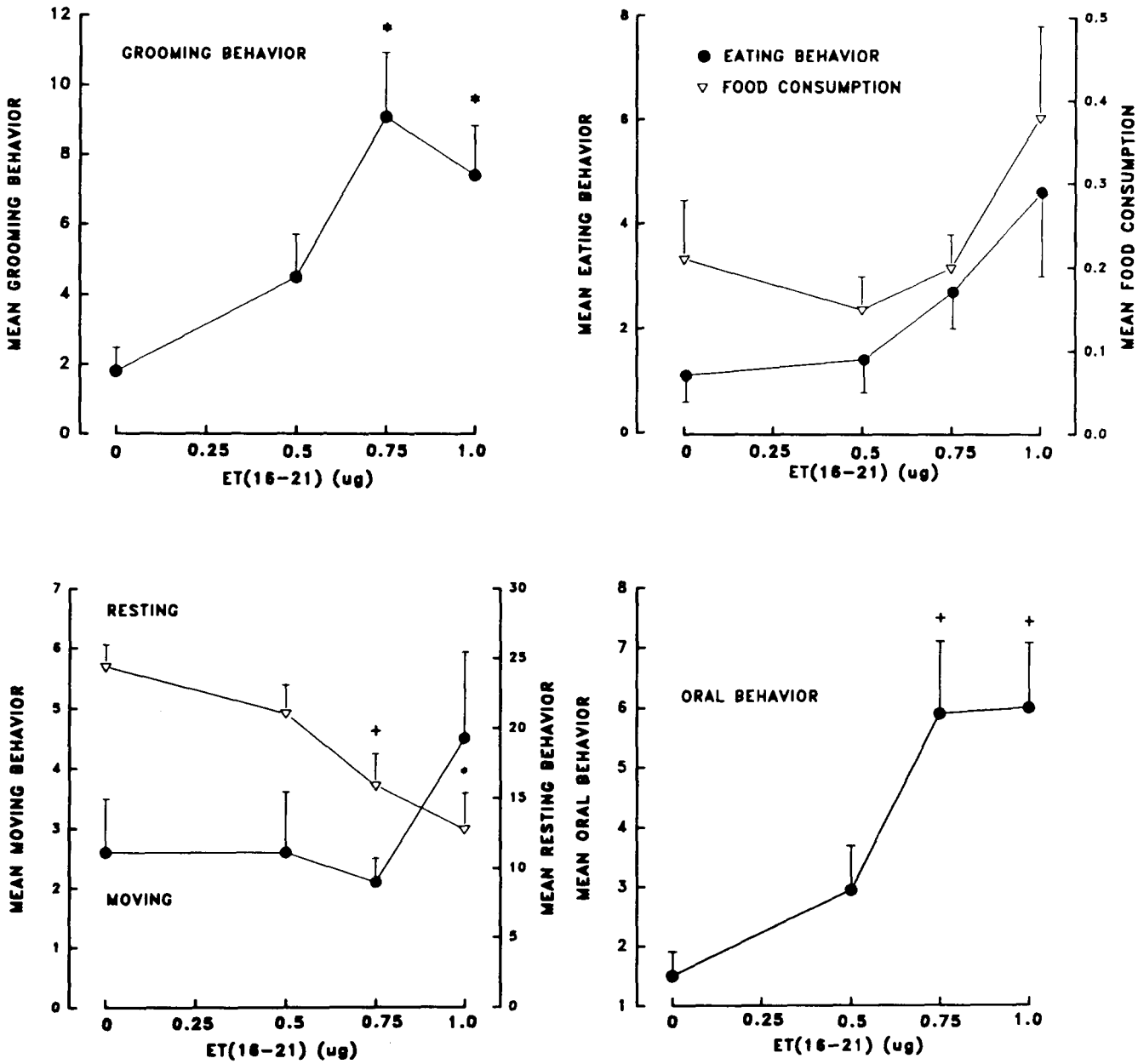

FIG. 1. Effects of ET[16-21] on grooming, eating and food consumption, moving and resting, and oral behaviors. Overall, ET[16-21] increased grooming and oral behavior, decreased resting and was without a significant effect on eating, food consumption, or locomotion. Error bar represents the standard error of the mean. Means differing from that of the control group (0 μg) at $p < 0.05$ are indicated by a + or at $p < 0.01$ by an asterisk (*).

moving, and resting behaviors were recorded at 1-min intervals for 30 min by trained observers unaware of the treatment administered. Each subject was observed sequentially for 10 s within each 1 min interval. The dominant behavior displayed during the 10-s period was recorded as either a grooming, eating, moving, or resting behavior. Grooming behaviors consisted of face, feet, tail or fur washing, scratching, or stroking of the fur. Eating behavior consisted of gnawing on the food pellet or chewing. Moving was scored if mice were perambulating, rearing, or pushing bedding about the cage. Resting behavior consisted of remaining immobile and awake or sleeping. Only one behavior that occurred for the majority of each 10 s period was scored. Food pellet weight change was recorded in grams at the end of the observation period.

Experiment 2

All procedures were the same as in Experiment 1, except mice received two injections 30 s apart: saline-saline, saline-CRF antagonist at 10 μg, saline-ET[16–21] at 0.75 μg, or CRF antagonist-ET[16–21]. In a second part of the Experiment, 1 μg of NPY was administered in place of CRF antagonist.

Statistics

Scores for each type of behavior were summed over the 30 min testing period for each subject. The number of observations for grooming, moving, and resting behaviors were analyzed by a parametric one-way analysis of variance (ANOVA). Dunnett's *t*-test was used to determine if the means of each drug group differed significantly from the mean of the control group (9). Tukey's tests were used to determine if means differed significantly among experimental groups (3,9). Previous research by Johansson et al. (8) indicated that grooming behavior may be an extension of general oral behaviors, including eating, which were increased following administration of low doses of SKF-38393. Therefore, we combined the data from grooming and eating and created a new variable, oral behavior. Due to a lack of homogenity of variance, a nonparametric Kruskal–Wallis one-way ANOVA was used to evaluate a treatment effect for eating behavior, food intake, and oral behavior (25). Dunn's multiple comparison test, run on rank differences from the Kruskal–Wallis ANOVA, was used to test for a significant difference in the rank means of the drug groups and the control group.

RESULTS

ET[16–21] had a significant effect on grooming behavior, $F(3, 36) = 6.05, p < 0.01$ (Fig. 1), with groups receiving 0.75 or 1.0 μg having means significantly greater than the control (Dunnett's *t*-test). In Fig. 1, it appears that eating behavior increased significantly in the group receiving 1.0 μg of ET[16–21]. However, this effect is due to one high score, because omitting the score resulted in a *F* value that was not significant. ET[16–21] significantly decreased the time spent resting $F(3, 36) = 5.59, p < 0.01$. Dunnett's *t*-test determined that the means of groups receiving 0.75 and 1.0 μg of ET[16–21] were significantly lower than that of the saline control (Fig. 1). An ANOVA failed to detect a significant effect of ET[16–21] on locomotion. The Kruskal–Wallis one-way ANOVA detected a significant effect of ET[16–21] administration on oral behaviors, $F(3, 36) = 6.36, p < 0.01$, with significantly higher means for groups treated with 0.75 or 1.0 μg of ET[16–21] compared to the mean of the control group (Fig. 1). In a separate Kruskal–Wallis ANOVA, food consumption was not affected by ET[16–21] treatment, $F(3, 36) = 1.03, p > 0.10$.

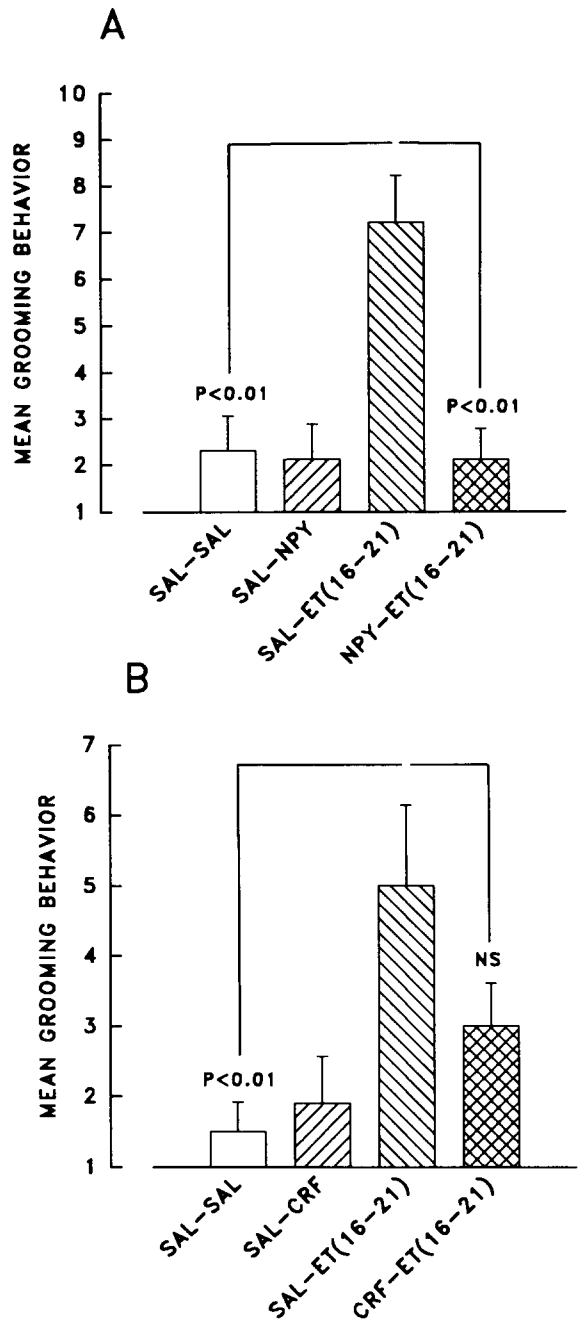

FIG. 2. Effects of NPY (A) and CRF antagonist (B) on ET[16–21]-enhanced grooming. NPY significantly blocks ET[16–21]-induced grooming while CRF antagonist only tends to reduce ET[16–21]-elicited grooming.

In the second experiment, the effect of NPY on ET[16–21]-enhanced grooming behavior was significant, $F(3, 35) = 10.89, p < 0.01$, with the mean of the saline plus ET[16–21] group being significantly greater than the saline control mean (Fig. 2A). Saline-NPY treatment did not affect grooming, as the mean of this group was not significantly different from

ıtrol. The mean of the saline-ET[16–21] group
ly greater than the mean of the group receiving
ɔn treatment of NPY plus ET[16–21] using Tu-
a separate one-way ANOVA, the effect of CRF
ET[16–21]-enhanced grooming was significant,
'1, $p < 0.01$ (Fig. 2B) with the saline-ET[16–
ng a significantly higher mean grooming score
: control. CRF antagonist reduced ET[16–21]-
ıing, but the difference between saline-ET[16–
ɔlus ET[16–21] did not reach significance as it
ʏ study. Based on separate one-way ANOVAs
avior, resting behavior, locomotion, and food
there was no significant treatment effect in ei-
ntagonist or NPY studies.

DISCUSSION

reported here show a dose-dependent increase
response to ET[16–21] central administration
in resting, which was likely due to the increased
oming. ET[16–21] did not significantly affect
ıting behaviors, or the amount of food con-
ɔreased grooming produced by ET[16–21] was
ior treatment with α-helical CRF antagonist

ıdies found that grooming is elicited by CRF
ıis effect was mediated within the paraventricu-
he hypothalamus (11,14). ET3 inhibited drink-
tivated the hypothalamic-pituitary-adrenal axis
might increase grooming by enhancing CRF
ıse the CRF antagonist blocked ET[16–21]-
ıing. However, previous research has demon-
Γ3 modulates the release of dopamine in the
in which it coexists possibly by increasing inter-
n ion concentration and facilitating calcium-
ɔytotic release of dopamine (10). SKF-38393, a
receptor agonist, elicited intense grooming (1,2,19), and at higher doses it produced chewing behavior (1,2). While ET[16–21] may induce grooming behavior by facilitating the release of dopamine, it could also do so by activating CRF which has also been found to enhance dopaminergic activity. ICV administration of 10 μg CRF produced hyperactivity of the mesocortical dopaminergic system which was accompanied by prominent grooming (13).

Although not significant, the trend toward increased eating behavior following 0.75 and 1.0 μg of ET[16–21] may also be due to an activation of dopaminergic mechanisms. Rosengarten et al. (21,22) reported abnormal oral movements, vacuous chewing, and clonic jaw movements following SKF-38393, suggesting these stereotypic behaviors are mediated by a dopaminergic mechanism. Considering that ET[16–21]-treated mice showed increased eating behavior without increased food consumption in the dose–response study, it is possible that ET[16–21] produced stereotypic eating behavior. This activation of eating behavior could be due to ET[16–21] facilitating the release of dopamine on D_1 receptors. Further research is needed to clarify the role of increased D_1 receptor activity in mediating ET[16–21] and CRF induced grooming.

NPY has been demonstrated to have a number of effects on behavior, including enhancement of feeding (12,16,18) and memory enhancement (4). In addition, NPY inhibits grooming behavior (12) but stimulates CRF release (5). Thus, the ability of NPY to inhibit grooming appears to be independent of its effect on CRF.

In rodents it has been suggested that grooming is associated with habituation to stressful stimuli (17). Grooming behavior in rodents is often a displacement phenomena to alleviate stress or to indicate submission (24). In this study, the findings support the idea that stress hormones play an important role in modulating grooming behavior.

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