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Aversive Properties of Bombesin in Rats

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MEISENBERG, G., W. H. SIMMONS AND S. A. LORENS. *Aversive properties of bombesin in rats*. PHARMACOL BIOCHEM BEHAV **37**(4) 689–692, 1990.—Aversive properties of bombesin were determined in the conditioned place-preference paradigm in rats and compared with the effects on spontaneous behavior. Bombesin induced excessive grooming and/or scratching behavior at doses of 80 ng, 400 ng, and 2.0 μg ICV. In the conditioned place-preference paradigm, doses of 400 ng and 2.0 μg ICV induced a profound aversion to the environment in which the animals had received peptide treatment. Eighty ng were partially effective, and 16 ng did not induce a significant change in preference. The gooming/scratching behavior was attenuated by pretreatment with 4 mg/kg morphine-sulfate. These results show that bombesin is strongly aversive at doses that induce grooming/scratching behavior. Although the relationship between these different effects is not known, the similarity in their dose-response relationship suggests that they may be mediated by a common mechanism.

Bombesin Place-preference Scratching Grooming Aversion Morphine Pain Rats

BOMBESIN is a tetradecapeptide isolated from the skin of the amphibian *Bombina bombina* (1,7). Although authentic bombesin has not been found in mammalian species, bombesin-like immunoreactivity is widely distributed in the mammalian central nervous system (4, 19, 20). This bombesin-like material is concentrated in synaptosomal fractions (18) and is released by K^+ via a Ca^{2+}-dependent mechanism (18,19). High-affinity binding for bombesin has been demonstrated in the rat brain (18, 21, 24). More recently, the bombesin-like peptides gastrin-releasing peptide (GRP) and neuromedin B and C have been isolated from neural tissue (7, 15, 16, 25). These results suggest that bombesin-related peptides may act as neurotransmitters in the mammalian central nervous system. Various physiological and behavioral alterations are observed after administration of bombesin to the brain or the spinal cord. These include hypothermia (2, 17, 22, 23), hyperglycemia which is mediated by enhanced sympathetic outflow (3,5), analgesia (21), and reductions in food intake (8). In addition, excessive grooming and scratching behavior are observed after intracerebroventricular (ICV) (9, 13, 14) and intrathecal administration (11). Bombesin-like immunoreactivity is concentrated in areas of the brain and spinal cord thought to be involved in nociceptive processes, including the midbrain, hypothalamus, and the dorsal horn of the spinal cord (4, 12, 19, 20). In particular bombesin/GRP-like immunoreactivity has been described in primary sensory neurons (6), and bombesin/GRP receptors are present in the terminal fields of these neurons in the posterior horn of the spinal cord (12). In the present study, we investigated the possibility that the excessive grooming and scratching behavior observed after intracerebroventricular (ICV) administration of bombesin in rats may be related to an activation of nociceptive or related processes. Specifically, we investigated aversive stimulus properties of centrally administered bombesin in the conditioned place-preference paradigm.

METHOD

Animals

Male Sprague-Dawley rats, weighing 275–350 g at the time of surgery, were used. The animals were pair housed at 23 ± 1°C and 50–60% relative humidity in a 12 × 12 hour light-dark cycle, with food and water available ad lib.

Surgery

An intracerebroventricular guide cannula (26 gauge, Plastic Products, Roanoke, VA) was implanted under pentobarbital anesthesia (45 mg/kg IP), using a Kopf stereotaxic instrument. Coordinates were β +0.5, lat 1.0, dv 5.5 mm, with the incisor bar 3.4 mm above the interaural line. The rats were allowed to recover for 8–12 days before the initiation of behavioral testing.

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TABLE 1

EFFECTS OF BOMBESIN ON OPEN-FIELD BEHAVIOR*

N	Treatment	Immobility	Grooming	Scratching	Squ_W	Squ_C	Rearing
10	CSF	34.3 ± 5.0	9.1 ± 2.5	0.2 ± 0.2	100.9 ± 12.7	2.6 ± 0.8	12.6 ± 2.6
7	Bom 16 ng	57.9 ± 22.8	10.6 ± 7.7	0.0	73.4 ± 13.6	5.8 ± 2.8	12.8 ± 5.7
8	Bom 80 ng	36.6 ± 13.2	41.3 ± 8.4†	4.6 ± 2.0	62.6 ± 7.8†	3.1 ± 1.4	7.5 ± 1.5
8	Bom 400 ng	37.1 ± 12.7	71.0 ± 14.3‡	30.9 ± 14.8†	45.5 ± 13.4†	1.5 ± 0.3	4.1 ± 1.6‡
8	Bom 2.0 μg	35.4 ± 14.9	60.6 ± 11.2‡	35.1 ± 9.5‡	64.0 ± 9.0†	5.1 ± 1.8	3.4 ± 1.0‡

*N = number of animals per group; Squ_W = wall squares, Squ_C = center squares entered. Immobility, grooming and scratching are in seconds per 5-minute test session. †$p<0.05$ and ‡$p<0.01$, compared to CSF. ANOVA and Duncan's multiple-range test.

After completion of the experiment, the rats were injected IP with 70 mg/kg pentobarbital, 10 μl Evans blue solution were injected ICV, and the dye distribution was assessed macroscopically. Only results from animals in which the dye solution reached the lower portions of the ventricular system were evaluated. In part of the animals, the brain was cut in 50-μm sections, the sections were stained by the cresylecht violet procedure, and the cannula tracks were visualized. All cannula tracks ended in the lateral ventricle.

Injection

Peptide and vehicle solutions were injected through the implanted guide cannula, using a 33 gauge internal cannula (Plastic Products). Vehicle consisted of 10 μl artificial cerebrospinal fluid containing 128 mM NaCl, 10 mM $NaHCO_3$, 2.8 mM KCl, 1.25 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1.0 mM D-glucose, and HCl to pH 7.3. Morphine-sulfate (4 mg/kg) or vehicle (150 mM NaCl) were injected intraperitoneally (IP) 15–20 minutes before the peptide injection.

Open-Field Test

Ten minutes after the ICV injection of peptide or vehicle solution, the animal was placed in the center of a rectangular open field (100 × 100 cm) whose floor was divided in 25 squares. Its behavior was observed during a 5-minute period. Behavioral parameters which were determined in seconds per test session included grooming, scratching, and periods spent without gross motor activity ("immobility"). In addition, the number of interior squares and perimeter squares entered, as well as rearing, were recorded. The test was performed in a dimly lit room at an environmental temperature of 23 ± 1°C.

Each animal had been adapted to the open field by four 5-minute exposures during the two weeks before the test. In the determination of bombesin effects (Table 1), each animal was injected with only one dose. Statistical evaluation was by analysis of variance, followed by Duncan's multiple-range test. Each bombesin treatment group was compared with the vehicle-injected control. In the experiment on bombesin-morphine interactions (Table 2), a repeated-measures design was employed in which each animal was exposed once to each of the four treatments in random sequence, treatment days being 7 days apart. Evaluation was by analysis of variance including all four treatments, and Duncan's multiple-range test comparing the morphine-CSF group with the NaCl-CSF group, as well as the morphine-bombesin group with the NaCl-bombesin group.

Place-Preference Test

The conditioning chamber consisted of two compartments (39.5 × 25 cm each) which were separated by a guillotine door. One compartment had white walls and a metal grid floor, the other compartment had black walls and floor. This combination had been found to avoid too strongly biased preconditioning preferences for one or the other compartment in most animals. On the first 3 days of behavioral testing, the animals were given free access to the entire apparatus once daily for 15 minutes each, and the time spent in either compartment was recorded. Values from days 2 and 3 were averaged and used as the animals' "preconditioning preference." Most animals preferred the white side, and only those which spent 5–50% of their time in the black compartment were subjected to place-conditioning once daily. During the subsequent conditioning phase (4 days), an internal cannula, connected via a length of silastic tubing to a Hamilton microsyringe, was inserted in the guide cannula, the animal was placed in the conditioning chamber. After entering the previously preferred white compartment, the guillotine door was closed and 10 μl of peptide or vehicle solution were injected over a period of 30 seconds. Subsequently, the animal was confined to the compartment for 25–30 minutes. The animals were subjected to conditioning sessions twice daily, once with peptide or vehicle-injection in the white compartment and once with a sham-injection (internal cannula inserted, but no solution injected) in the black compartment. On day 8 of behavioral testing, the animals were given free access to the whole apparatus for 15 minutes, the time spent in either compartment was recorded. For each individual animal, the relative preference for the two compartments was compared with the preconditioning preference.

The difference scores were used for statistical evaluation by analysis of variance. The preference changes for each of the bombesin treatment groups were evaluated against the preference changes in the CSF group by means of Duncan's multiple-range test.

Like the open-field test, the place-preference test was performed in a dimly lit room at an environmental temperature of 23 ± 1°C.

RESULTS

After ICV injection, bombesin induced dose-dependent grooming and scratching behavior. While doses of 400 ng and 2 μg appeared maximally effective, 80 ng were less active, causing a significant ($p<0.05$) increase in grooming behavior only. The 16 ng dose was essentially inactive (Table 1). Reductions of forward locomotion (peripheral squares entered) and rearing were observed in the same dose range.

In the conditioned place-preference paradigm, a dose-dependent reduction of the time spent in the previously preferred white compartment could be demonstrated (Fig. 1). While vehicle-injected rats showed little change from their baseline preference,

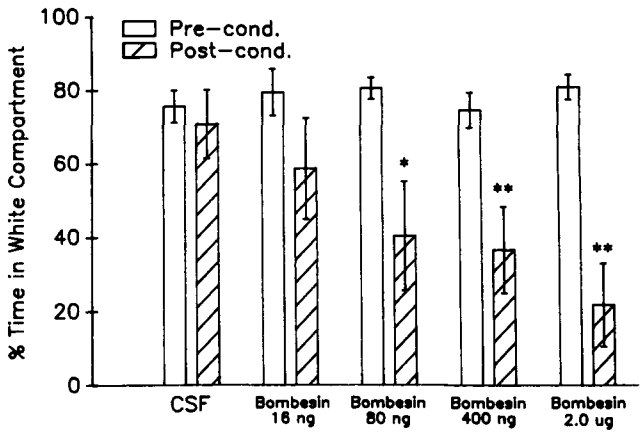

FIG. 1. Effects of bombesin and vehicle in the place-preference test. The figure indicates the percent time which rats spent in the white compartment of the place-preference box both before (preconditioning) and after (postconditioning) intracerebroventricular administration of bombesin (at the indicated doses) or vehicle (artificial CSF) while confined to the white compartment. (Rats per group = 7–10.) *$p<0.05$; **$p<0.01$; ANOVA and Duncan's multiple-range test.

significant decreases were found for the 80 ng, 400 ng, and 2 μg group.

Pretreatment of the rats with 4 mg/kg morphine sulfate IP attenuated the excessive grooming and scratching behavior induced by ICV injection of 400 ng bombesin without suppressing it completely. This dose of morphine did not seem to incapacitate the animals since locomotor activity was generally normal or enhanced in the morphine-vehicle control group (Table 2).

DISCUSSION

The present experiments were designed to test the hypothesis that some of the behavioral changes observed after central administration of bombesin may be due to a stimulation of nociceptive or related mechanisms. These behavioral changes, as described in the present study in rats (Table 1), include a sound stimulation of both grooming and scratching behavior, with slight to moderate reductions of locomotion and rearing in the same dose range. These effects are very similar to those described previously in mice (14,24), effective doses being about 5–10 times higher in rats than in mice. This suggests that the behavioral effects of bombesin are not strongly species-specific and may be mediated by an action on mechanisms of general importance for mammalian behavior.

One possible mechanism for these effects is a stimulatory action on afferent nociceptive or somatosensory pathways resulting in reflex activation of grooming and scratching behaviors. This possibility was originally suggested by the nature of the behavioral response, its persistence in a stressful environmental situation in which similar grooming/scratching behaviors induced by vasopressin and mescaline were suppressed (14), and its lack of tolerance formation after repeated bombesin injections (24). This possibility is further reinforced by the presence of significant quantities of bombesin immunoreactive material in areas of the brain and spinal cord thought to be involved in the transmission and processing of somatosensory and nociceptive stimuli (4, 6, 12, 19, 20). The previously described analgesic properties of centrally administered bombesin (21) do not contradict this possibility because a centrally induced stimulation of somatosensory or nociceptive pathways is not unlikely to inhibit responses to afferent nociceptive stimuli.

The working hypothesis implies the presence of aversive stimulus properties, eventually resulting in grooming/scratching as a defensive set of behaviors. The observation that centrally administered bombesin has apparent aversive stimulus properties (Fig. 1) is therefore in agreement with this hypothesis. Especially the observation that these aversive stimulus properties are present in the same dose range as the grooming/scratching behavior suggests a possible relationship between these effects. The observation that morphine partially antagonizes bombesin-induced grooming/scratching behavior at doses that do not cause gross motor impairment further supports this view, although morphine also caused a reduction of spontaneous grooming behavior in the absence of exogenous bombesin (Table 2). The question of whether morphine attenuates either spontaneous or bombesin-induced grooming behaviors by an action on afferent stimulus processing remains to be evaluated.

There are, however, other possibilities that may account for bombesin's aversive properties. Thus, central administration of bombesin is known to cause substantial hypothermia in rats, especially in a cold environment (2, 17, 22, 23). We do not know whether this hypothermia, which may well be significant at the doses and the environmental temperature in the present experiments, is related to aversive stimulus properties. Another centrally mediated bombesin-effect which may be related to its aversive property is the reduction of food intake observed after bombesin administration in rats (8). This suggests that this peptide may act centrally to induce gastrointestinal discomfort. Bombesin also

TABLE 2

ANTAGONISM OF BOMBESIN EFFECTS BY MORPHINE*

Treatment						
IP	ICV	Immobility	Gr + Scr	Squ_W	Squ_C	Rearing
NaCl	CSF	26.7 ± 17.2	15.4 ± 3.9	111.6 ± 19.4	8.9 ± 3.7	12.0 ± 2.9
Mor	CSF	39.1 ± 25.0	5.4 ± 3.7†	162.1 ± 34.3	9.6 ± 4.1	5.3 ± 2.5
NaCl	Bom	79.1 ± 17.5	80.1 ± 15.5	40.1 ± 7.1	1.4 ± 0.4	0.9 ± 0.4
Mor	Bom	87.9 ± 23.5	31.3 ± 10.9‡	42.0 ± 15.0	1.3 ± 0.3	0.9 ± 0.4

*Mean ± SEM.

Immobility and (grooming + scratching) in seconds/5 minutes; Squ_W = wall squares, Squ_C = center squares entered; repeated-measures design, N = 7; each animal received each treatment once, treatment days were 7 days apart. Doses: bombesin (ICV), 400 ng; morphine (IP), 4 mg/kg.

†$p<0.05$; ‡$p<0.01$; ANOVA and Duncan's multiple-range test.

suppresses gastrointestinal motility after central administration (10,11). Also, the possibility that the bombesin-response in the conditioned place-preference paradigm is related to more subtle behavioral effects of bombesin, such as an action on the adaptation to a novel environment, or on memory processes, cannot be entirely excluded. Thus, either an altered sensitivity to the novelty of the environment, or an increased memory formation to a supposedly aversive testing situation could possibly result in aversion to the bombesin-associated environment.

The sites of action both for bombesin-induced grooming and scratching behavior and aversion are unknown. A possible site of action for the grooming/scratching is in the spinal cord, where similar behaviors have been described after intrathecal doses as low as 4 ng (11). Further studies on the site of action for bombesin-induced aversion are indicated. Studies about an association between the CNS sites mediating the aversive effect and sites at which grooming/scratching behavior, hypothermia, decreased food intake or other behavioral or physiological actions are induced will be useful in understanding these bombesin effects.

In addition, it is not known whether apparent aversive properties of bombesin are dependent on particular aspects of the testing situation. Thus, an influence of environmental temperature on bombesin-induced aversion can be expected if the aversive effect is related to bombesin-induced hypothermia. Familiarity of the animals with the testing procedure, on the other hand, may be an important factor if the peptide acts by changing the motivational impact of novelty or by an action on memory processes.

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