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Anorectic action of bombesin requires receptor for corticotropin-releasing factor but not for oxytocin

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Abstract

The marked functional similarities between pharmacological effects of bombesin and of corticotropin-releasing factor (CRF), prompted the formulation and testing of our working hypothesis that BN may elicit its biological effects through the release of CRF. Central pretreatment with CRF receptor antagonists, α-helical CRF-(9–41) (α-CRF-(9–41)) or [D-Phe^{12}, C$^{\alpha}$MeLeu^{37}]CRF-(12–41) (CαMeCRF), blocked the effects of centrally administered bombesin on food intake and related behaviors and partially attenuated the satiety effects of systemically administered bombesin. We also attempted to characterize the specificity of this interaction through the combined use of bombesin with the oxytocin antagonist, [d(CH$_2$)$_5$, Tyr(OMe)^2, Orn^8]vasotocin (vasotocin). Central pretreatment with vasotocin failed to alter bombesin-induced behaviors, suggesting the absence of a pharmacological interaction between these two peptidergic systems. Finally, the CRF antagonist failed to reverse the oxytocin-induced suppression of food intake, indicating that CRF does not have a direct role in the mediation and/or modulation of the effects of oxytocin on food intake. Thus, the present experiments support the contention that bombesin partly mediates its feeding-suppressant effects through interactions with CRF. The specificity of this interaction is supported by the lack of interaction between bombesin and/or CRF with oxytocin. © 1997 Elsevier Science B.V.

Keywords: GRP (Gastrin-releasing peptide); Feeding; Grooming; Central nervous system; Anorexia

1. Introduction

Bombesin, a tetradecapeptide of amphibian origin and its mammalian counterparts, gastrin-releasing peptides (GRP-(1–27) and GRP-(18–27)) and neuromedin Bs (neuromedin B-(1–32) and neuromedin B-(23–32)) are present in discrete brain sites (Moody et al., 1981, 1986; Panula et al., 1982; Chronwall et al., 1985). Central administration of bombesin elicits a number of biological and behavioral actions including the suppression of food intake (induction of a satiety-like state) in a variety of species ranging from rats to humans (Baile and Della-Fera, 1981; Stuckey and Gibbs, 1982; Kyrkouli et al., 1987; Muurahainen et al., 1993). The evolutionary persistence of this pharmacological response suggests a physiological role for these peptides in the control of food intake. Thus, a central blockade of bombesin/gastrin-releasing peptide receptors has been shown to enhance feeding in rats (Flynn, 1993; Merali et al., 1993). Furthermore, increased in endogenous levels of bombesin-like peptides have been observed in postmortem rat hypothalamus, following feeding (Kateb and Merali, 1992; Merali and Kateb, 1993). More recently, meal-related in vivo fluctuations in the release of BN-like peptides at the rat paraventricular nucleus of the hypothalamus have also been demonstrated (Plamondon and Merali, 1994).

Despite these findings, the exact physiological mechanism(s) by which these peptides exert their action on feeding remains largely unknown. It has been demonstrated that satiety induced by systemically administered bombesin is abolished by surgical afferent disconnection of the gastrointestinal tract from the brain (subdiaphragmatic vagotomy combined with thoracic section at T6 level and bilateral rhizotomy from T3 to T6) (Stuckey et al., 1985). Furthermore, effects of systemically administered bombesin are attenuated by central pretreatment with bombesin antiserum or antagonists (Merali et al., 1988; Ladenheim and Ritter, 1993; Motamedi et al., 1993). These

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observations suggest that the effects of systemically administered bombesin are neurally communicated to the brain, where bombesin receptors participate in the suppression of food intake. Attempts to identify the relevant neural circuits, using brain microinjections and lesioning, have revealed that certain hypothalamic and hindbrain structures, such as the paraventricular nucleus (Kyrkouli et al., 1987) and the nucleus tractus solitarii (Johnston and Merali, 1988; Flynn, 1992), are particularly sensitive to bombesin's feeding suppressant effects.

Like that of bombesin, central administration of the 41-amino acid polypeptide, corticotropin-releasing factor (CRF), has been reported to exert potent anorectic effects (Morley and Levine, 1982; Levine et al., 1983; Richard, 1993). In addition, there is some anatomical overlap in the distribution pattern of bombesin and CRF (Panula et al., 1982; Cummings et al., 1983; Chronwall et al., 1985). The possibility of interaction between these two peptidergic systems was supported by our recent observation that many of the autonomic and endocrine effects of bombesin are blocked by CRF antagonists (Merali et al., 1994). Furthermore, we have recently shown site- and peptide-specific fluctuations in bombesin and CRF levels in relation to the animal's feeding status, implying their role in the regulation of food intake (Plamondon and Merali, 1997).

The marked behavioral, anatomic, autonomic and endocrine similarities between CRF and bombesin, prompted us to formulate and test the working hypothesis that bombesin may mediate its effects on feeding and related behaviors via the release of CRF. Thus, the first set of experiments explored the potential role of CRF in the mediation and/or modulation of the behavioral and ingestive effects of bombesin, utilizing two different CRF receptor antagonists, [α-helical CRF-(9–41) (α-CRF-(9–41)) and [D-Phe^{12}, C$^{\alpha}$MeLeu^{37}]CRF-(12–41) (CαMeCRF)], prior to central or systemic administration of bombesin. In subsequent experiments, the specificity of this interaction and the potential mechanism(s) of action involved were investigated using pharmacological blockade of oxytocin receptors. Oxytocin was selected for the study as although it shares some similarities with bombesin in terms of its feeding effects (Arletti et al., 1989; Olson et al., 1991) and immunohistochemical distribution (Swaab et al., 1975; Swanson et al., 1983; Tribollet et al., 1988), it also exhibits some differences regarding biological effects (Lin et al., 1983; Dunn and File, 1987). Thus, the aim of the second set of experiments was to explore the potential interactions between bombesin and oxytocin through the combined use of bombesin and the oxytocin antagonist [d(CH$_2$)$_5$, Tyr(OMe)^2, Orn^8] vasotocin (vasotocin). Finally, since some effects of CRF given centrally are reported to be mediated through oxytocin (Bruhn et al., 1986; Olsen et al., 1991b), the last set of experiments assessed the potential role of CRF in the mediation and/or modulation of oxytocin effects.

2. Materials and methods

All experiments were conducted according to the accepted principles for the care and use of experimental animals stipulated by the Canadian Council on Animal Care and as approved by the University Animal Care Committee.

2.1. Animals

The male Sprague–Dawley rats (300–350 g) used in these experiments were housed individually in sound-attenuated room with controlled temperature (23–24°C), humidity (60%) and light (12 h light/dark cycle; lights on at 7.00 h). All rats were trained to consume their daily food ration during a 4 h food access period between 10.00 and 14.00 h during the light phase. During this time, the rats had access to powdered Purina rat chow (through short tunnels 6.5 × 6.5 × 10 cm, with a grid floor) contained in a bin on top of an electronic balance (accurate to 0.1 g, Omnitech Instruments, OH). Each balance was connected to a microcomputer that measured cumulative food ingestion throughout the 4 h session. Prior to testing, the animals were extensively habituated to the testing apparatus and the feeding protocol until their food consumption stabilized (10–14 days). Rats had unrestricted access to water throughout. All experiments were carried out between 10.00 and 14.00 h, at which time food was available. Rats were tested on alternate days and were left undisturbed on non-test days.

2.2. Surgery

The rats were anesthetized with pentobarbital (65 mg/kg; i.p.) and implanted with stainless steel cannulae (22 gauge) aimed at the 3rd ventricle (4.3 mm posterior to bregma, 0.0 mm lateral to the midline and 4.3 mm ventral to the skull surface) (Paxinos and Watson, 1986). The cannulae were anchored by four stainless steel screws and dental acrylic cement and plugged with a stainless steel wire stylet. During post-surgical recovery and before the start of the experiment, all animals were allowed to adapt to handling for a minimum of 7 days.

2.3. Behavioral measurement

Throughout the first hour of the testing session, the behavior of each rat was visually monitored using a time-sampling procedure (one 5 s observation every 20 s) (Merali and Banks, 1994). The behaviors monitored included: (1) eating: scooping, chewing and/or ingesting rat chow, (2) drinking: licking the water spout, (3) scratching: contact of the hind paw with the side of the face/head or body/flank followed by a scratching action, (4) washing: wiping of the face and crown regions with circular movements of the forelimbs or active licking of the abdomen and/or thorax, (5) resting/sleeping: the animal is inactive with eyes open or is lying in a curled position with eyes closed and (6) exploring: the animal is moving around the cage, sniffing or rearing.

2.4. Peptide and / or antagonist treatments

Saline, bombesin, two CRF antagonists, α-CRF-(9–41) and CαMeCRF, oxytocin and the oxytocin antagonist, vasotocin, were used in these experiments. All drugs were freshly dissolved in 0.9% saline prior to use and administered in the third cerebral ventricle 10–15 min prior to food presentation. Drugs were obtained from Bachem (California) except for the CRF antagonist CαMeCRF, which was generously provided by J. Rivier (the Salk Institute's Clayton Foundation Laboratories for Peptide Biology). The i.c.v. injections were made in awake, unrestrained animals through a stainless steel cannula which extended 0.5 mm below the tip of the guide cannula and into the third cerebral ventricle. Drug solutions were infused in a 3 μl volume delivered over a 30 s period. The cannula was left in place for an additional 1 min (to minimize back-flow) then was replaced by the stylet. For systemic injections, drug solutions were delivered in a volume of 1 ml/kg body weight.

2.5. Histology

Upon completion of each experiment, the animals were killed with an overdose of pentobarbital and intracardially perfused with saline followed by 10% formaldehyde solution. 0.5 μl of India ink was then microinjected through the cannula. Brain slices (30 μm) were then cut with a cryostat and successful cannula placement confirmed by the presence of ink in the 3rd ventricle.

2.6. Experimental design

2.6.1. Experiment 1: Effects of central blockade of the CRF receptors on bombesin response

Using a group of animals ($n = 8$ to 9) trained to a 4 h food access test session as described previously, this experiment assessed the effects of the CRF antagonist, α-CRF-(9–41), on bombesin-induced ingestive and other behavioral effects. The animals were injected with α-CRF-(9–41) (0 (saline), 5 or 10 μg; i.c.v.), which was followed 15–20 min later by bombesin (0.5 μg; i.c.v.) or saline. All drug treatments were repeated across subjects and were separated by at least 48 h.

In the next experiment, the effects of central blockade of CRF receptors on the satiety induced by systemically administered bombesin were determined. Rats ($n = 9$) were trained, treated and monitored as in the first study with the exception that only the most effective dose of α-CRF-(9–41) (10 μg; i.c.v.) was used, followed by peripheral administration of saline or bombesin (6 μg/kg; i.p.).

In order to ascertain that these findings were not unique to the particular CRF antagonist used, a third group of rats ($n = 8$ to 9) was trained, treated and monitored as in the first experiment, except that a different CRF antagonist, CαMeCRF, was used instead of α-CRF$_{9-41}$. Preliminary experiments had revealed that the optimal doses of CαMeCRF were 2 to 5 μg, lower than those of α-CRF-(9–41). This is consistent with a recent report indicating that CαMeCRF was more potent than α-CRF-(9–41) to antagonize CRF-induced locomotor and anxiogenic effects in the rat (Menzagui et al., 1994). Thus, as in earlier experiments, the rats were injected with CαMeCRF (0 (saline), 2 or 5 μg; i.c.v.), followed 15–20 min later by bombesin (0.5 μg; i.c.v.) or saline (control).

All drug treatments were performed 10–15 min before food presentation. The cumulated amount of food consumed was recorded at intervals of 0.5, 1, 2 and 4 h. The incidence of expression of other behaviors was monitored during the first hour of the test session.

2.6.2. Experiment 2: Effects of central oxytocin receptor blockade on bombesin response

A separate group of rats ($n = 8$) was trained to the feeding and testing regimen of experiment 1. In the present study, rats were treated alternately with i.c.v. vasotocin (0 (saline) or 8.9 μg; i.c.v.) 15 min before the central administration of bombesin (0.5 μg; i.c.v.) or saline (3 μl; i.c.v.; control conditions).

2.6.3. Experiment 3: Effects of central CRF receptor blockade on oxytocin response

Another group of rats ($n = 7$ to 8) was used with the experimental regimen described earlier to determine the potential participation of CRF in oxytocin-induced feeding suppression and related behaviors. The rats were injected with CαMeCRF (0 (saline), 5 μg; i.c.v.), followed 15–20 min later by oxytocin (10 μg; i.c.v.) or saline (control).

2.7. Data analysis

Repeated measures analyses of variance (ANOVA) were applied to the feeding and locomotion data with factors treatment (peptide and/or antagonist) and sampling (time). The visually obtained behavioral data were similarly analyzed by individual behavior with factors treatment and sampling. Post hoc comparisons were made using Tukey's test.

3. Results

3.1. Experiment 1: Effects of central CRF receptor blockade on response to central bombesin

Since 2 different doses of the CRF antagonist were used in an attempt to antagonize the effects of BN, the experimental design was not amenable to a 2 × 2 ANOVA. Accordingly, the data for all 5 groups were subjected to a one way ANOVA with repeated measures over time. This analysis revealed a significant treatment (α-CRF-(9–41) and/or bombesin) by sampling (time) interaction on food intake ($F(12, 114) = 8.69$, $P < 0.05$). As illustrated in Fig. 1, post hoc analyses of the simple effects revealed that central administration of bombesin (0.5 μg) suppressed food intake for up to 4 h. Central administration of α-CRF-(9–41) prior to bombesin attenuated the bombesin-induced suppression of food intake at both the 5 and 10 μg doses. This effect was specific as central administration of α-CRF-(9–41) (10 μg) alone failed to alter food intake.

As noted above, the feeding suppressant effects of bombesin as well as the blockade of this effect by a CRF antagonist were most pronounced during the initial hour after treatment. During the initial hour the occurrence of various behaviors (including eating, grooming, exploring and drinking) was also recorded. When the frequency data for individual behaviors were subjected to similar analyses, significant treatment (α-CRF-(9–41) and/or bombesin) by sampling (time) interactions were noted for both eating ($F(12, 96) = 2.47$, $P < 0.007$) and grooming ($F(12, 96) = 2.49$, $P < 0.007$). Post hoc comparisons of the simple effects of the interactions indicated that central bombesin treatment (0.5 μg; i.c.v.) was associated with decreased frequency of ingestive behavior for the full hour; an observation consistent with the amount of chow consumed (data presented in Fig. 1). Although the CRF antagonist (α-CRF-(9–41)) itself did not affect food intake, it dose and time dependently attenuated the bombesin effects; there was a significant attenuation of bombesin anorexia during the initial 30 min, and an almost complete blockade by 45 min (for the higher dose) or 60 min (for the lower dose). With saline, or α-CRF-(9–41) plus bombesin, eating frequency also paralleled the rat's actual food intake where the rats spent most of the hour ingesting food (see Fig. 2). Saline or CRF antagonist treatment had little effect on grooming activity. Bombesin markedly enhanced grooming and this effect was once again antagonized in a dose- and time-dependent manner by the CRF antagonist pretreatment. The exploratory behavior occurred with a relatively low frequency and was affected by the treatment (α-CRF-(9–41) and/or bombesin) ($F(4, 32) = 9.14$, $P < 0.0001$). The effect on exploratory behavior was largely attributable to a slight stimulation of this behavior by bombesin at the 30 and 45 min intervals and this effect was blocked by the high dose of α-CRF-(9–41). The incidence of drinking behavior was relatively low under all treatment conditions (see Fig. 2) and was affected by treatment ($F(4, 32) = 9.05$, $P < 0.0001$). Finally, the frequency of resting behavior was relatively low and was not significantly altered by α-CRF-(9–41) and/or bombesin treatment (data not presented).

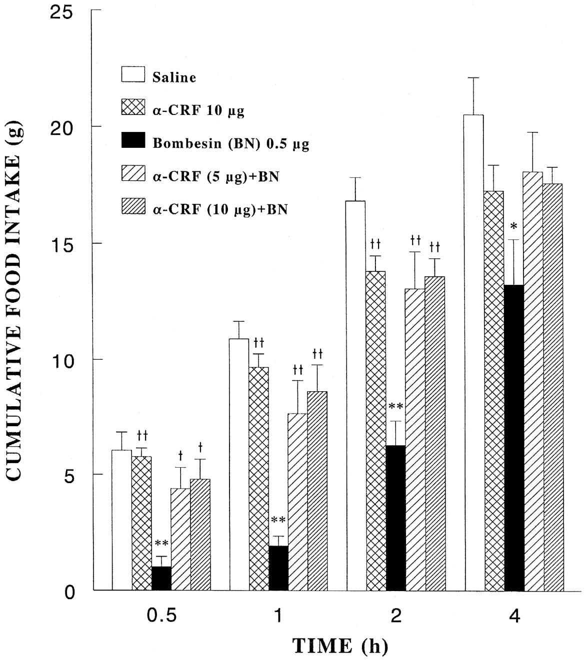

Fig. 1. Bombesin-induced satiety was blocked by central administration of α-CRF. Each column represents the mean ± S.E.M. of the cumulated food consumption (g) at designated time points during the rat's daily 4 h food access period. *, **Significantly different from saline at $P < 0.05$ and $P < 0.01$, respectively. †, ††Significantly different from bombesin at $P < 0.05$ and $P < 0.01$, respectively (Tukey's test).

3.1.1. Effects of central CRF receptor blockade on response to systemic bombesin

To determine whether central CRF receptors also participate in the satiety induced by systemically administered bombesin, we assessed the efficacy of α-CRF-(9–41) (10 μg; i.c.v.) to attenuate the effects of systemic bombesin (6 μg/kg; i.p.). ANOVA of food intake data revealed a significant interaction effect between treatment (α-CRF-(9–41) and/or bombesin) and sampling (time) ($F(12, 120) = 2.58$, $P < 0.004$). Systemic administration of bombesin (6 μg/kg; i.p.) suppressed food intake (by about 50%) for up to 2 h. This suppression was significantly attenuated by central administration of α-CRF-(9–41) (10 μg; i.c.v.) during the first hour of testing, as illustrated in Fig. 3.

Behavioral data for the 2 consecutive 30-min bins were evaluated by ANOVA. The frequency of the monitored behaviors varied as a function of treatment (α-CRF-(9–41) and/or bombesin treatment) and sampling for eating ($F(3, 31) = 3.25$, $P < 0.03$), grooming ($F(3, 31) = 3.08$, $P < 0.04$) and exploring ($F(3, 31) = 4.21$, $P < 0.01$). No significant effects on the drinking and resting frequencies were observed. As mentioned above, central administration of α-CRF-(9–41) partially blocked the satiety induced by peripheral bombesin administration. In agreement with this finding, central pretreatment with α-CRF-(9–41) (10 μg;

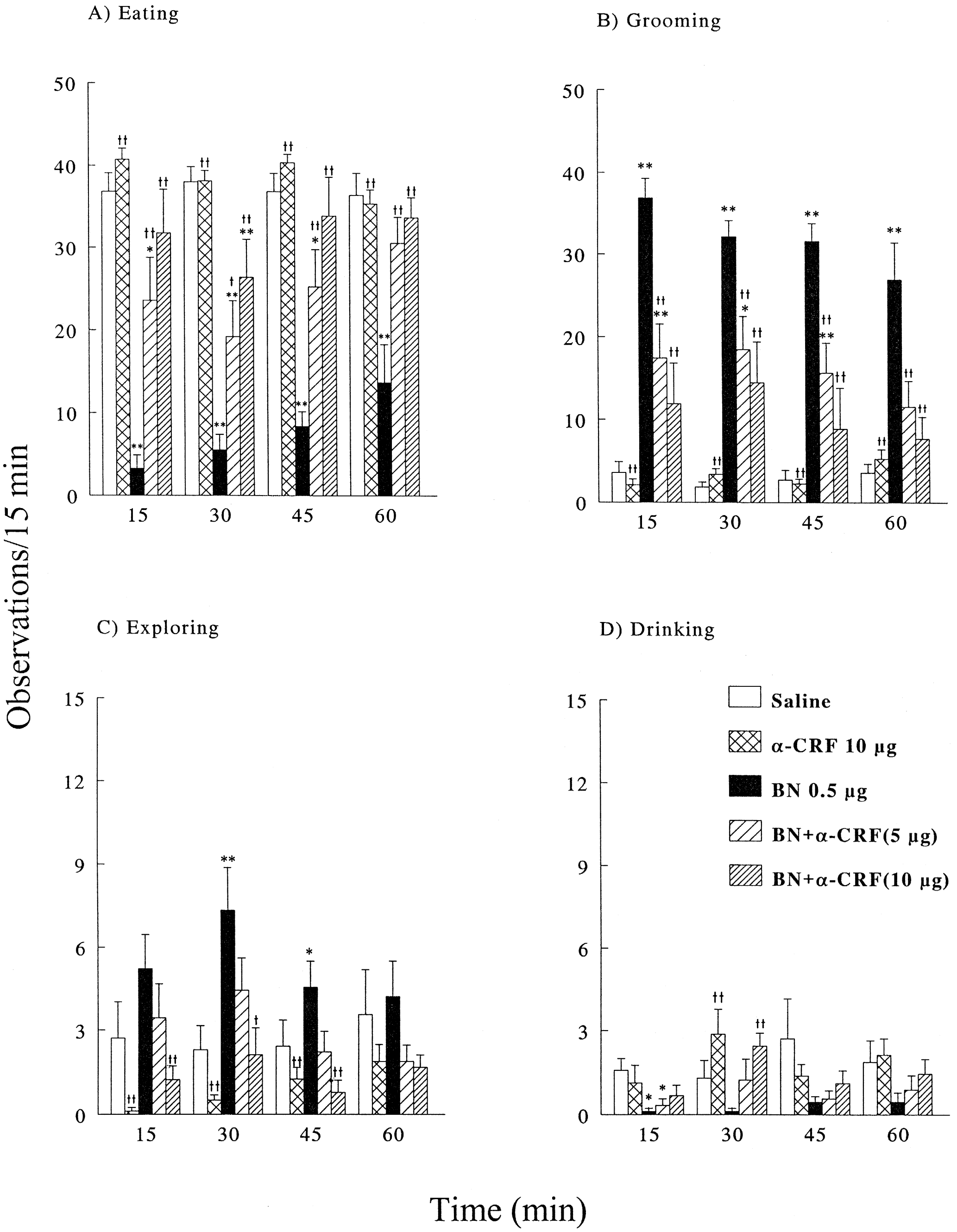

Fig. 2. Effect of blockade of CRF receptors by α-CRF on bombesin-elicited behavioral profile. Each column represents the mean ± S.E.M. of the frequency (incidence/15-min time bins) of eating, grooming, exploring and drinking during the 60-min test session. *, ** Significantly different from saline at $P < 0.05$ and $P < 0.01$, respectively. †, ††Significantly different from bombesin at $P < 0.05$ and $P < 0.01$, respectively (Tukey's test).

i.c.v.) significantly increased eating frequency in BN treated rats. The frequency of grooming was relatively low throughout the test hour but a slight enhancement of grooming was observed in bombesin-treated animals in the first 30 min testing interval and appeared to be reversed by α-CRF-(9–41) pretreatment (see Table 1). The frequency of exploration was also increased by bombesin and this effect was only blocked by α-CRF-(9–41) at the 60-min interval. Finally, drinking and resting incidence were not significantly altered by any of the drug treatments (data not shown).

3.1.2. Effects of a different CRF antagonist on central action of bombesin

This experiment examined whether the above findings were unique to α-CRF-(9–41) or could be reproduced using another CRF receptor antagonist. Thus, a more recently synthesized CRF antagonist, CαMeCRF, was

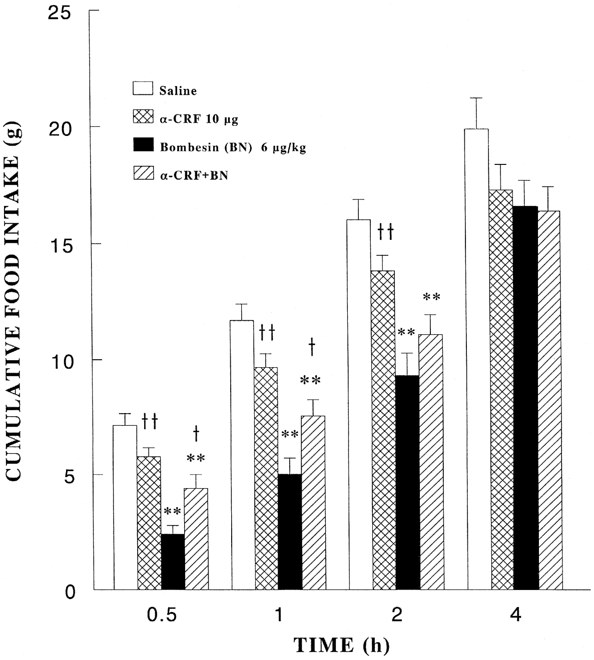

Fig. 3. Central administration of α-CRF attenuated the satiating effects of systemically administered bombesin. Each column represents the mean ± S.E.M. of the cumulated food intake (g) at designated time points during the 4 h access period to food. **Significantly different from saline at $P < 0.01$. †, ††Significantly different from BN at $P < 0.05$ and $P < 0.01$, respectively (Tukey's test).

tested. Statistical analysis of food intake revealed a significant overall interaction between the effects of treatment (CαMeCRF and/or bombesin) and sampling (time) ($F(12, 117) = 2.03$, $P < 0.02$). Administration of CαMeCRF (5 µg; i.c.v.) alone failed to affect food intake. However, like that of α-CRF-(9–41), central administration of CαMeCRF, 15 min prior to bombesin administration (0.5 µg; i.c.v.) dose dependently blocked the bombesin-induced feeding suppression. A complete blockade of bombesin anorexia satiety was obtained with the higher dose of CαMeCRF (5 µg), while only partial attenuation was obtained with the lower, 2-µg dose (see Fig. 4).

Analysis of the behavioral data revealed a significant main effect of treatment (CαMeCRF and/or bombesin) for eating ($F(4, 39) = 21.97$, $P < 0.0001$) and grooming ($F(4, 39) = 23.45$, $P < 0.0001$). ANOVA failed to reveal significant differences between groups for drinking, resting or exploring behaviors.

Under saline or CαMeCRF alone conditions, the rats spent most of the hour ingesting food. Consistent with the food intake data presented in Fig. 4, bombesin alone was associated with a decreased frequency of ingestive behavior during the entire observation period. The frequency of this behavior was also diminished in rats pretreated with the 2 µg dose of CαMeCRF prior to bombesin (see Table 1).

Under saline and CαMeCRF (5 µg) conditions, the frequency of grooming was low and remained unchanged during the entire hour. In contrast, bombesin administration markedly stimulated grooming activity. This bombesin-elicited grooming was significantly attenuated with the 2-µg dose of CαMeCRF and completely blocked by the higher dose (5 µg) of this antagonist (see Table 1).

Table 1
Effect of the various drug treatments on eating and grooming behaviors

Behavior	Eating		Grooming	
Treatment	30 min	60 min	30 min	60 min
Experiment 1				
Saline (i.p)	79.9 ± 1.0	76.0 ± 1.7	5.4 ± 0.9	7.6 ± 1.3
Bombesin (6 µg; i.p.)	29.1 ± 6.3^{b}	45.6 ± 7.7^{b}	15.6 ± 2.7^{b}	11.0 ± 1.6
α-CRF + bombesin	61.7 ± 8.8^{a}	68.4 ± 5.0	9.0 ± 1.7	7.7 ± 1.6
α-CRF(10 µg; i.c.v.)	78.9 ± 1.7	75.8 ± 1.7	5.5 ± 1.2	7.5 ± 1.1
Saline (i.c.v.)	77.8 ± 3.2	75.4 ± 5.7	3.3 ± 1.4	5.2 ± 1.4
Bombesin (0.5 µg; i.c.v.)	6.4 ± 2.0^{b}	18.0 ± 4.9^{b}	71.6 ± 3.8^{b}	61.9 ± 5.7^{b}
CαMeCRF (2 µg) + bombesin	48.7 ± 12.0^{b}	49.6 ± 7.4^{b}	33.0 ± 10.3^{b}	30.7 ± 8.1^{b}
CαMeCRF (5 µg) + bombesin	63.6 ± 8.3	62.6 ± 5.5	19.3 ± 8.1	18.0 ± 4.2
CαMeCRF (5 µg)	72.8 ± 3.7	71.6 ± 4.2	9.8 ± 2.4	7.1 ± 1.7
Experiment 2				
Saline (i.c.v.)	80.0 ± 3.6	65.4 ± 5.2	4.0 ± 1.4	10.4 ± 3.3
Bombesin (0.5 µg; i.c.v.)	9.0 ± 3.6^{b}	16.8 ± 4.9^{b}	75.9 ± 3.8^{b}	65.3 ± 4.9^{b}
Vaso (8.9 µg) + bombesin	2.6 ± 9.9^{b}	19.1 ± 6.4^{b}	59.9 ± 9.6^{b}	62.9 ± 7.9^{b}
Vaso (8.9 µg; i.c.v.)	79.3 ± 2.7	77.9 ± 2.9	5.1 ± 1.4	5.1 ± 1.3
Experiment 3				
Saline (i.c.v.)	80.0 ± 3.6	65.4 ± 5.2	4.0 ± 1.4	10.4 ± 3.3
Oxytocin (10 µg; i.c.v.)	33.0 ± 6.8^{b}	41.1 ± 4.1^{b}	24.3 ± 4.5^{b}	20.4 ± 3.8^{a}
CαMeCRF (5 µg) + oxytocin	28.6 ± 6.5^{b}	37.6 ± 6.5^{b}	31.4 ± 5.8^{b}	24.6 ± 4.2^{b}
CαMeCRF (5 µg)	73.4 ± 3.5	71.6 ± 4.2	9.8 ± 2.4	7.1 ± 1.7

Each cell represent the mean ± S.E.M. of frequency of eating and grooming at the 30 and 60-min test intervals.
[a], [b] significantly different from saline at $P < 0.05$ and $P < 0.01$, respectively (Tukey's test).

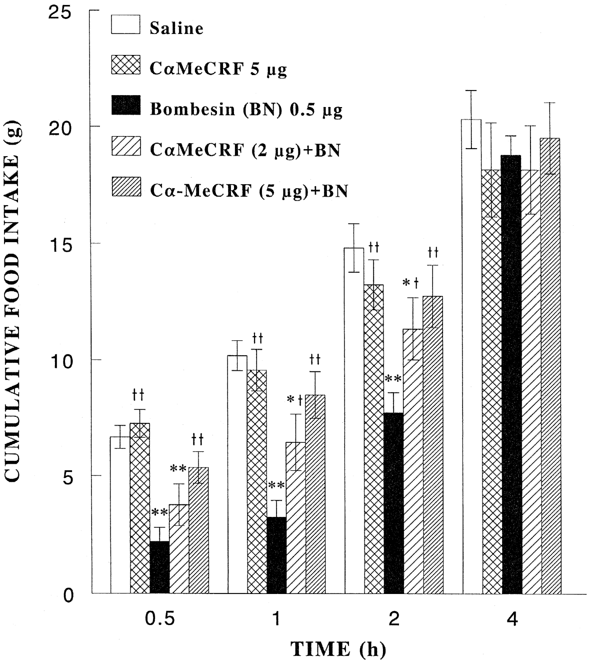

Fig. 4. Blockade central of CRF receptors (using CαMeCRF) dose dependently blocked bombesin-anorexia. Each column represents the mean ± S.E.M. of the cumulated food consumption (g) at designated time points during the rat's daily 4 h food access period. *, **Significant differences from saline at $P < 0.05$ and $P < 0.01$, respectively. †, ††Significantly different from BN at $P < 0.05$ and $P < 0.01$, respectively (Tukey's test).

3.2. Experiment 2: Effects of central oxytocin receptor blockade on bombesin-elicited behaviors

ANOVA of food intake revealed a significant overall interaction between the effects of treatment (vasotocin and/or bombesin) and sampling ($F(9, 63) = 5.75$, $P < 0.0001$). As expected, bombesin treatment (0.5 μg; i.c.v.) significantly suppressed food ingestion for the entire 4 h testing period. Pretreatment with the selective oxytocin antagonist, vasotocin (8.9 μg; i.c.v.), failed to affect bombesin-induced satiety. Administration of vasotocin by itself had no effect on feeding (see Fig. 5).

Analyses of the frequency of various behaviors during the first hour of testing revealed a significant treatment (vasotocin and/or bombesin) by sampling interaction for eating ($F(3, 28) = 4.89$, $P < 0.007$) and grooming ($F(3, 28) = 5.01$, $P < 0.006$). Consistent with the food intake data in Fig. 5, bombesin treatment was associated with a decreased frequency of ingestive behavior during the entire hour of monitoring. There were no significant differences between conditions where rats received bombesin alone or vasotocin plus bombesin. Similarly, both these drug treatments enhanced grooming frequency throughout behavioral testing. In contrast, saline and vasotocin treated animals spent most of the time ingesting food and their grooming frequency remained relatively low throughout the test hour (see Table 1). The frequencies of exploring, drinking and resting behaviors were low for all treatment conditions and no overall statistical differences were found between treatments (data not presented).

3.3. Experiment 3: Effects of oxytocin following central blockade of CRF receptors

This experiment aimed to determine whether CRF receptors participate in the expression of oxytocin effects. Statistical analysis of food intake revealed a significant treatment (CαMeCRF and/or oxytocin) × sampling (time) interaction ($F(15, 144) = 1.73$, $P < 0.05$). As illustrated in Fig. 6, this interaction was attributable to the fact that various treatments affected feeding differentially, in a time-dependent manner. Compared to control conditions (saline) oxytocin (10 μg; i.c.v.) transiently suppressed food intake (for 1 h). Pretreatment with the CRF antagonist, CαMeCRF (5 μg; i.c.v.), prior to oxytocin failed to alter oxytocin-induced feeding suppression. The CRF antagonist was without effect on its own and also failed to alter the feeding-suppressant effects of oxytocin (see Fig. 6).

Analysis of the behavioral data revealed a significant effect of treatment (CαMeCRF and/or oxytocin) for eating ($F(5, 42) = 17.87$, $P < 0.0001$) and grooming ($F(5, 42) = 6.17$, $P < 0.0002$) and a treatment (CαMeCRF and/or oxytocin) × sampling (time) interaction for drinking ($F(5, 42) = 4.62$, $P < 0.001$). The reduction of food intake that followed central administration of oxytocin was

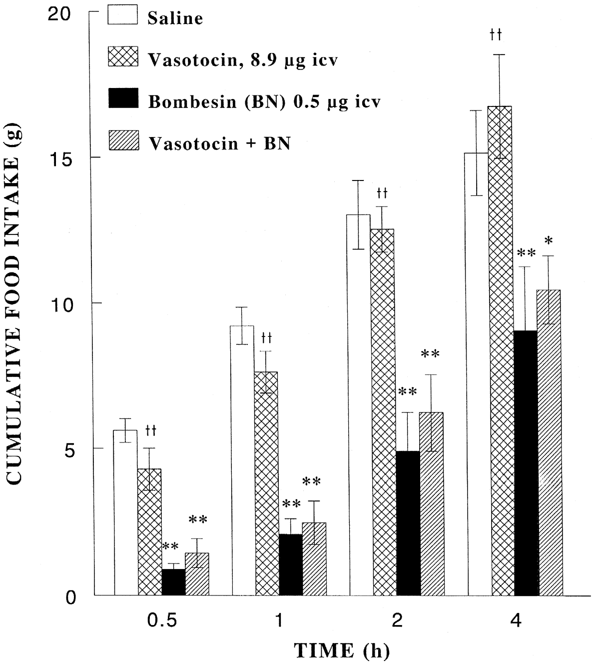

Fig. 5. Blockade of central oxytocin receptors by vasotocin failed to block bombesin-induced satiety. Each column represents the mean ± S.E.M. of the cumulated food consumption (g) at designated time points during the rat's daily access period to food. **Significantly different from saline at $P < 0.01$. ††Significantly different from bombesin at $P < 0.01$ (Tukey's test).

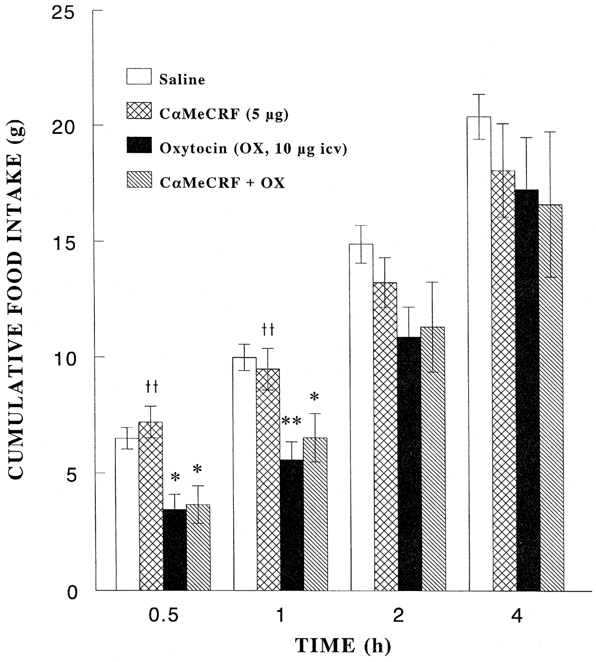

Fig. 6. Central blockade of CRF receptors failed to reverse oxytocin-induced satiety. Each column represents the mean ± S.E.M. of the cumulated food consumption (g) at designated time points during the rat's daily access period to food. *, ** Significantly different from saline at $P < 0.05$ and $P < 0.01$, respectively. ††Significantly different from bombesin at $P < 0.01$ (Tukey's test).

accompanied by a concomitant reduction in eating frequency in oxytocin-treated animals as well as in rats pretreated with CαMeCRF (5 μg) prior to oxytocin administration. The reduced eating frequency was proportional to the treatment effects on food consumption, and lasted for the entire observation hour (see Table 1). The frequency of grooming also appeared enhanced during the entire hour of testing in both the oxytocin-treated rats and the rats pretreated with CαMeCRF (5 μg) prior to oxytocin administration (see Table 1). Finally, the drinking frequency was low for all treatment groups but rats given CαMeCRF (5 μg) alone drank more frequently than saline-treated rats during the initial 30 min. At the 60-min interval, the drinking incidence of both saline- and CαMeCRF-treated rats appeared to have increased more than in the other test groups (data not shown).

4. Discussion

The exact role of CRF in the regulation of food intake is not understood. However, various findings support the contention that CRF plays a central role in the control of various feeding-related events (Richard, 1993). For instance, evidence suggests that CRF may mediate the anorectic effects associated with certain stressful stimuli (Krahn et al., 1986; Shibasaki et al., 1988; Rivest and Richard, 1990).

Based on the functional and anatomical overlap between bombesin and CRF, the present study investigated the potential participation of CRF in bombesin-induced satiety. The present results provide clear evidence implicating CRF in the mediation of anorectic effects of bombesin. Central administration of either of the two different CRF receptor antagonists (α-CRF-(9–41) and CαMeCRF) blocked the behavioral and feeding-suppressant effects of centrally administered bombesin. When administered alone, these CRF antagonists failed to affect food intake, suggesting that the blockade of bombesin-induced anorexia was not attributable to intrinsic properties of these drugs. Moreover, these pharmacological interactions appeared quite specific as bombesin did not interact with oxytocin and the CRF antagonist, CαMeCRF, failed to alter oxytocin-induced satiety. These results are consistent with the notion that bombesin may mediate its behavioral effects partly through activation of central CRF system. We have previously suggested that systemically administered bombesin appears to activate neural circuits within the brain that utilize bombesin-like peptides, to bring about cessation of eating. In this context, it was of interest that a centrally administered CRF antagonist (α-CRF-(9–41)) also attenuated the anorectic effects of systemically administered bombesin, suggesting that at least some of the interactions between bombesin and CRF might occur within the brain. Distinct anatomical brain site(s) where these peptides might interact have not been identified as yet. However, the paraventricular nucleus might be important in this context, as meal-related changes in the release of endogenous bombesin-like peptides have been reported at this site (Plamondon and Merali, 1994). Moreover, CRF has been reported to suppress feeding when it is microinjected into the paraventricular nucleus but not into the lateral or ventromedial hypothalamic nuclei, globus pallidus or striatum (Krahn et al., 1988). Furthermore, exogenous administration of CRF into the paraventricular has been shown to site-specifically increase grooming and locomotion (Krahn et al., 1988; Mönnikes et al., 1992). Thus, it is possible that an increased bombesin release at the paraventricular nucleus before and after spontaneous food intake stimulates the release of CRF from neurons emanating from the paraventricular nucleus or at anatomically related sites, which would induce and/or maintain a satiety-like state in the animal. The contention that some of the effects of bombesin-like peptides may be directly mediated through the release of CRF is also consistent with our earlier observation that α-CRF-(9–41) pretreatment blocked endocrine and autonomic effects of central bombesin (Merali et al., 1994).

The precise receptor-based mechanism(s) by which CRF and bombesin-like peptides may interact remain unknown. The actions of CRF can potentially be mediated via several receptor subtypes. Thus far, at least three different receptor subtypes have been described, namely, CRF_1, $CRF_{2\alpha}$ and $CRF_{2\beta}$ (Behan et al., 1996). Since the CRF antagonists used in the present study do not effectively differentiate between these receptor subtypes, one can not conclusively implicate any particular CRF receptor subtype in the mediation of the bombesin effects. Blockade of CRF receptors using α-CRF-(9–41) failed to alter bombesin membrane binding (unpublished observation) suggesting the inability of bombesin to directly bind to CRF receptors. Although the presence of bombesin receptors on CRF neurons remains to be shown, there is some evidence that these two peptides may be co-localized at some terminal sites.

Regarding the specific physiological mechanisms by which these peptides interact in the regulation of feeding, endocrine, autonomic and behavioral functions, several possibilities for their common actions can be considered. Although central administration of bombesin and CRF has been shown to stimulate adrenocorticothophic hormone release from the pituitary (Rivier et al., 1982; Thomas and Sander, 1985; Sander and Thomas, 1991), it is unlikely that the feeding suppression and grooming induced by these peptides is related to their activation of the pituitary as hypophysectomy failed to alter the behavioral effects of the peptides (Morley and Levine, 1982; Gmerek and Cowan, 1983).

Central administration of CRF or bombesin induces hyperglycemia associated with elevation of plasma glucagon levels and relative lowering of insulin concentrations (Brown et al., 1979; Brown et al., 1982). These glucostatic effects are thought to be secondary to adrenomedullary epinephrine secretion (Brown et al., 1977, 1982). In the light of the transient decline in glucose levels observed immediately preceding initiation of a spontaneous meal (Campfield and Smith, 1986), the satiety effects of these peptides may be partly secondary to their hyperglycemic action. Indeed, glucose infusions have also been shown to inhibit or postpone eating (Novin et al., 1973). Although adrenalectomy failed to alter CRF-induced hyperglycemia (Brown et al., 1982), it significantly attenuated the satiety effects of centrally infused CRF (Gosnell et al., 1983) and this effect was attributable to adrenomedullary epinephrine secretion and not to changes in corticosterone levels (Gosnell et al., 1983). Furthermore, suppression of feeding by CRF appears to be centrally mediated, as intravenous administration of CRF antiserum failed to attenuate the epinephrine release elicited by centrally applied CRF (Brown and Fisher, 1985). In contrast to those of CRF, the central effects of bombesin on circulating levels of glucose, glucagon, and insulin were prevented by adrenalectomy (Brown et al., 1977). Furthermore, adrenalectomy failed to alter the feeding suppressant effects of systemically administered bombesin (Gibbs et al., 1981).

The specificity of the functional interactions between bombesin and CRF were further assessed by exploring potential interactions with another anorectic peptide, oxytocin. Oxytocin was selected as, like bombesin, it not only suppresses food intake (Arletti et al., 1989; Olson et al., 1991) but also induces grooming in rats (Caldwell et al., 1986; Pederson et al., 1988; Olson et al., 1991). Moreover, CRF has been shown to elevate plasma oxytocin secretion (Bruhn et al., 1986) and oxytocin receptor blockade antagonizes CRF-induced anorexia (Olsen et al., 1991b). Central oxytocin has also been implicated in the mediation of another satiety peptide, namely cholecystokinin, as blockade of brain oxytocin receptors attenuated the satiety effects of systemically administered cholecystokinin (Olsen et al., 1991a). Thus it was of interest to determine whether oxytocin might represent a link in the chain of neural events contributing to bombesin-induced feeding suppression. In the present study, the oxytocin antagonist, vasotocin, failed to alter bombesin-induced feeding suppression. Furthermore, oxytocin-induced anorexia was not affected by pretreatment with a CRF antagonist. These findings support the notion of different mechanisms of action for bombesin- and cholecystokinin-induced suppression of food intake. Bombesin effects would require the participation of CRF receptors but be independent of oxytocin receptor participation. These findings are also consistent with the observation that, while increased pituitary secretion of oxytocin is observed following systemic administration of cholecystokinin, which is thought to contribute to cholecystokinin's satiety effects (Verbalis et al., 1986), bombesin fails to alter pituitary oxytocin secretion in rats (Verbalis et al., 1988). It thus seems likely that the effects obtained in the present study are not secondary to blockade of CRF-activated oxytocinergic pathways that elicit feeding suppression. Furthermore, since various stressors stimulate the secretion of pituitary oxytocin (Lang et al., 1983; Gibbs, 1984), it is possible that CRF-induced oxytocin secretion might contribute more specifically to stress-induced anorexia and not to a satiety-like state as noted with spontaneous meals. Finally, the inability of CRF receptor blockade to alter oxytocin-induced anorexia both suggests that the interaction between these two peptides is not bi-directional and further supports the specificity of the functional interaction of bombesin with CRF.

In conclusion, the present findings suggest the participation of CRF in the bombesin-induced suppression of food intake. Our results demonstrate for the first time that blockade of central CRF receptors prevents bombesin's suppressant action on feeding and attenuates bombesin-elicited grooming. This pharmacological interaction appeared to be specific as bombesin did not interact with oxytocin and as blockade of CRF receptors failed to alter the anorectic effects of oxytocin. The observation that bombesin-induced satiety remained unaltered by the blockade of oxytocin receptors suggests that bombesin's anorectic action may not be mediated through CRF activation of oxytocinergic neurons. These finding also provide evidence for the existence of distinct physiological mechanisms underlying the satiety-like effects of bombesin and CCK. Taken together, our findings suggest that bombesin may activate central CRF pathways to produce its effects on feeding and related behaviors. It is possible that bombesin and/or CRF could play a role in the pathophysiology of eating disorders such as anorexia nervosa, cancer anorexia, bulimia and obesity.

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