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Mechanisms underlying anorexia after microinjection of bombesin into the lateral cerebroventricle

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Abstract

Intracerebroventricular (i.c.v.) injections of bombesin (BN) and gastrin-releasing peptide (GRP) dose-dependently decreased food intake in male Wistar rats fasted for 17 h. Neuromedin B (NMB) did not show any effect on food intake. After BN administration, locomotor activity did not significantly change, compared with a vehicle-injected group. The anorexia induced by BN (0.3 μg) was perfectly inhibited by pretreatment with a GRP-receptor antagonist, [D-Tyr^6]BN(6–13) methyl ester (10 μg), an NO synthase inhibitor, L-nitro-arginine (30 μg), and a PKG inhibitor, H-9 (2 μg). The cGMP concentration in the hypothalamus increased 1 h after administration when compared with the vehicle-injected group. On the other hand, an NMB-receptor antagonist, BIM23127 (10 μg), and the protein kinase (PK) C inhibitors, chelerythrine (2 μg) and Gö6983 (2 μg), inhibited only the late phase of the anorexia. A PKC activator, phorbol 12, 13-dibutyrate (3 μg), injected into the ventricle decreased food intake. These findings suggest that BN suppresses food intake mainly mediated through the GRP receptor and NO-cGMP-PKG pathway, and NMB receptor and PKC is partly involved in the late phase of the anorexia.

Keywords: Food intake; Protein kinase; Nitric oxide; Hypothalamus; Bombesin; GRP receptor; NMB receptor

1. Introduction

Feeding behavior is regulated in the CNS by multiple mechanisms, which interact with each other in a complex way (Fekete et al., 2002; Gillard et al., 1998; Hillebrand et al., 2002; Inui, 1999a,b; Nakazato et al., 2001; Williams et al., 2001). The hypothalamus is a key region for regulation of feeding. In the past, feeding behavior was considered to be determined by the balance between the activities of the feeding and satiety center in the lateral hypothalamus and the ventromedial hypothalamus, respectively. Today, in addition to these sites, the hypothalamic paraventricular nucleus (PVN), the arcuate nucleus, the dorsomedial hypothalamus and the perifornical hypothalamus are recognized as regions playing important roles in the regulation of feeding behavior. These nuclei contact each other via multiple neurotransmitters/neuromodulators.

Bombesin (BN), which is a 14-amino acid peptide originally extracted from frog skin (Anastasi et al., 1971), is one of the most powerful substances showing anorexic effects in the hypothalamus. In mammals, gastrin-releasing peptide (GRP), neuromedin B (NMB) and neuromedin C function as endogenous ligands (McDonald et al., 1979; Minamino et al., 1988), and their receptors are widely distributed in the CNS (Ladenheim et al., 1992; Ohki-Hamazaki et al., 1997a,b; Yamada et al., 1999). BN receptors have been subclassified into four subtypes, NMB, GRP, BRS-3 and BRS-4 receptors, although the fourth subtype has been found only in amphibian tissues at present (Alexander et al., 2001; Nagalla et al., 1995). All of the BN receptor subtypes have been demonstrated to couple Gq protein and activate phospholipase C/protein kinase (PK) C in cultured cells (Alexander et al., 2001; Katsuno et al., 1999). On the other hand, in vivo, signal transductions involving some BN-induced effects are gradually being defined. One of these is a thermoregulatory effect. Our previous paper shows that BN-induced hypothermia is mediated through GRP receptors and

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involves, at least in part, the activation of PK C. However, our findings cannot recognize any contribution of MAP kinase, tyrosine kinase and Rho kinase in the BN-mediated thermoregulation (Tsushima et al., 2003), even though these kinases are contained in the signal transduction of BN using cultured cells (Némoz-Gaillard et al., 1998; Nishino et al., 1998; Rozengurt, 1998). In addition, BN is found to inhibit gastric acid secretion after increased release of nitric oxide (NO) in the dorsal motor nucleus of the vagus followed by activation of the vagus, although none of the BN receptor subtypes have been investigated (Beltrán et al., 1999; Martinez and Taché, 2000).

Ladenheim et al. hypothesized that peripherally injected BN decreased food intake mediated through BN receptors of both GRP and NMB subtypes in the caudal hindbrain around the fourth ventricular wall (Ladenheim et al., 1996a,b; 1997). In addition to the caudal hindbrain, BN is assumed to physiologically regulate feeding behavior in the hypothalamus. There have been some reports on hypophagia after microinjections of BN/BN-related peptides into the hypothalamus (Hillebrand et al., 2002; Kyrkouli et al., 1987; Stuckey and Gibbs, 1982). The release of BN-like peptides in the PVN depends on the ingestion of food (Plamondon and Merali, 1994), and BN receptors and BN-related peptides are distributed in the hypothalamic nuclei (Ladenheim et al., 1992; Wada et al., 1990; Yamada et al., 1999). Moreover, GRP-receptor and BRS-3 knockout mice are obese (Landenheim et al., 2002; Ohki-Hamazaki et al., 1997b). Therefore, BN receptor family in the CNS plays a role in the regulation of feeding behavior. However, signal transduction in anorexia is still unclear. In this study, we investigate the mechanisms for BN-induced anorexia after intracerebroventricular (i.c.v.) injection and compare these mechanisms with those in the BN-induced hypothermia that we have already described using the same methods (Tsushima et al., 2003). At first, the receptor subtypes in the hypothalamus mediating anorexia were examined. In addition, PKs involved in anorexia were investigated.

2. Materials and methods

2.1. Animals and surgical procedure

Male Wistar rats weighing 290–350 g (Japan SLC, Hamamatsu, Japan) were used in this study. They had free access to drinking water and food pellets, and were housed in a temperature-controlled room at 22–24 °C with a 12-h light/dark cycle (light on/off: 08:00/20:00). The experiments were carried out according to the Guidelines for Animal Care and Use of our university.

A guide cannula (AG-8; Eicom, Kyoto, Japan) was implanted into the right lateral ventricle (5.7 mm anterior to the lambda, 1.8 mm lateral to the midline and 3.5 mm below from the surface of the skull) (König and Klippel, 1963) using small screws and dental cement under anesthesia with pentobarbiturate (40 mg/kg, i.p.), as described in our previous studies (Tsushima and Mori, 2000, 2001; Tsushima et al., 2003). During a recovery period of 1 week, they were acclimated to the experimental conditions and handling. The rats were housed individually and all of the experiments were conducted in home cages.

When the experiments were finished, the microinjection sites were histologically verified under a microscope. Data were collected from the animals that showed appropriate placement of the guide cannula into the lateral ventricle.

2.2. Measurement of food intake

Food was removed approximately 17 h prior to the experiments, but the rats were able to freely access drinking water. At 10:30 on the following day, a microinjection cannula (AMI-8; Eicom), connected to a microsyringe with a polyethylene tube containing a pretreatment drug and a BN receptor ligand, was inserted into the guide cannula instead of a dummy cannula. Fifteen min later, the pretreatment drug was injected into the ventricle through the microinjection cannula. Another drug was injected at 11:30. The total volume of drug solution was 10 μl and the drugs had been introduced in advance into the polyethylene tube connecting the microinjection cannula and the microsyringe. The microinjection cannula was kept in the guide cannula for 5 min after drug administration to avoid a countercurrent of drug solution. Then, the dummy cannula was re-inserted, and pre-weighed food pellets and water were given to the rats in the home cage. The weight of the food pellets was measured at 12:00, 12:30, 13:00, 13:30, 14:30 and 15:30.

The doses of the inhibitors used in this study are appropriate using the microinjection method (Sacchetti and Bielavska, 1998; Tsushima and Mori, 2000, 2001; Tsushima et al., 2003).

2.3. Measurement of locomotor activity

The locomotor activity was measured as counts interrupting sensor beams using an activity monitor Automex II (Columbus Instruments, Ohio, USA) with the satiated animals. During the measurement, the rats were allowed free access to food and water. The procedure of drug microinjections was the same for the measurement of food intake. The measurements were carried out 0.5, 1.0, 1.5, 2.0 and 3.0 h after BN administration.

2.4. Measurement of cGMP concentration

One hour after BN (0.3 μg) or vehicle injections, the rats were sacrificed by decapitation and the brain was immediately removed. The dissected right hypothalamus (Glowinski and Iversen, 1966) was stocked at −80 °C until the measurement. The measurement was conducted using a commercial kit (Yamasa, Tokyo, Japan). Extraction and other procedures were carried out according to the manual

supplied with the kit. Briefly, after the hypothalamus was homogenized by sonication (Tomy Seiko, Ultrasonic disruptor, UD-201) in 6% trichloroacetic acid (TCA) on ice, it was centrifuged at 3000 rpm for 15 min at 4 °C. The pellet was reconstituted in 6% TCA and centrifuged under the same conditions. This supernatant was added to the first supernatant, and then the pellet was washed with water-saturated ethanol twice. This material was then lyophilized and reconstituted in distilled water. The content of cGMP in the succinylized sample was determined by radioimmunoassay. Protein content was measured by the Bradford method (Bio-Rad Laboratories, CA, USA).

2.5. Statistic analysis

The results are expressed as the mean±S.E.M. Statistical difference was considered to be significant when P value was under 0.05 by one-way ANOVA followed by Fisher's post hoc test.

2.6. Drugs

The following drugs were used: BN (Sigma Chemical, St. Louis, MO, USA), human GRP, NMB, phorbol-12, 13-dibutyrate (PDBu), chelerythrine chloride (CHE) (Research Biochemicals International, Natrick, MA, USA), H-9 (*N*-[2-amonoethyl]-5-isoquinolinesulfonamide dihydrochloride; Seikagaku, Tokyo, Japan), L-nitro-arginine (LNA) (Peptide Research Institute, Osaka, Japan), Gö6983 (Go) (Calbiochem, La Jolla, CA, USA). [D-Tyr^6]BN(6-13) methyl ester (TBNME), and BIM23127 (BIM; D-Nal-cyclo[Cys-Tyr-D-Trp-Orn-Val-Cys]-Nal-NH_2) are generous gifts from Prof. David H. Coy (Tulane University Medical Center, New Orleans, LA, USA). Go was dissolved in DMSO and diluted with sterile physiological saline (Otsuka Chemicals, Tokyo, Japan). The other drugs were dissolved in sterile physiological saline. The chemicals used were the highest grade available.

3. Results

3.1. BN-induced anorexia

As shown in Fig. 1A, food intakes were investigated after i.c.v. injections of BN and structurally related peptides (GRP and NMB) using the fasted rats. BN elicited anorexia dose-dependently from 0.1 μg to 1.0 μg and it was the most powerful anorexia among the three peptides. The anorexia induced by BN at 0.3 μg (0.17 nmol) showed a similar degree to the effects induced by GRP at 1.6 μg (0.56 nmol). I.c.v. injections of NMB at 20 μg (17 nmol) did not elicit any significant effect on food intake, compared with the vehicle-injected group. When the amounts of food intake every 0.5 or 1 h were compared for the vehicle- and BN (0.3 μg)-injected groups, the food intake at 0–0.5, 0.5–1.0 and 1.0–1.5 h after BN administration significantly decreased, while at 1.5–2.0 h after BN administration, food intake significantly increased (BN-injected group: 0.9±0.3 g, n=7 vs. vehicle-injected group: 0.3±0.2 g, n=10, 1.5–2.0 h after administration; P<0.05). At 4 h after administration, the total food intake of the two groups was not statistically different (Fig. 1B). The duration of anorexia was estimated to be 3 h. On the other hand, in the GRP (1.6 μg)-injected group, food intake was significantly decreased only 0–0.5 h after administration (Fig. 1A), and it was increased 1.5–2.0 h after administration, compared with the vehicle-injected group (GRP-injected group: 1.3±0.3 g, n=6 vs. vehicle-injected group: 0.3±0.2 g, n=10: P<0.05). The total intakes for 2 h were not different between the GRP- and vehicle-injected groups (Fig. 1B).

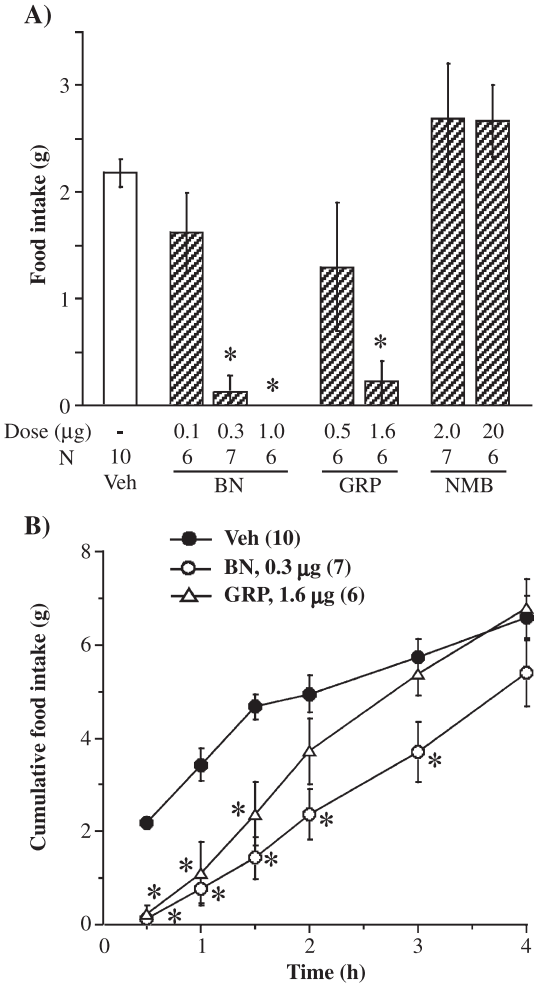

Fig. 1. Anorectic effects induced by i.c.v. injection of the GRP receptor subtype agonists, BN and GRP, and a NMB receptor subtype agonist, NMB. A) Food intake (g) for 0–0.5 h after administration of the three agonists. B) Time course of the BN- and GRP-induced effect. Because the pretreatment with saline or saline containing 5% DMSO did not influence food intakes after injection of BN or saline, the both results were included in the values of the open and closed circles. The values of the vehicle- and BN-injected groups were used as the controls for Figs. 2–4. Numbers in the parentheses express experimental numbers. Values are the means±S.E.M. *P<0.05 vs. the vehicle-injected group.

Table 1
Locomotor activity after i.c.v. injections of bombesin

Drugs	*n*	Time after administration (h)				
		0–0.5	0.5–1.0	1.0–1.5	1.5–2.0	2.0–3.0
Vehicle	7	2108±234	1845±290	1069±352	540±211	850±537
BN (0.3 μg)	7	1471±304	1325±301	1290±216	1178±386	1045±317

Values are counts interrupting sensor beams (the mean±S.E.M.).

Because feeding behavior is influenced by locomotor activities, these activities were examined for 3 h after BN (0.3 μg) administration. The animals injected with BN interrupted the sensor beams in the cage to the same degree as those injected with vehicle (Table 1). I.c.v injections of BN did not influence locomotor activity. After the BN treatment, there were no changes in grooming and scratching behavior, and did not show any sign of malaise/nausea, compared to the vehicle-injected group.

Saline or saline containing 5% DMSO as vehicle for pretreated drugs did not influence food intake after BN or saline administration.

3.2. Effects of receptor antagonists on the BN-induced anorexia

The effects of a GRP-receptor antagonist, [D-Tyr^6]BN(6-13) methyl ester (TBNME), and an NMB-receptor antagonist, BIM23127 (BIM), on the BN-induced anorexia were investigated (Fig. 2). All of the anorexia induced by BN (0.3 μg) was blocked by pretreatment with 10 μg of TBNME into the lateral ventricle. Two micrograms of TBNME only blocked the decrease in food intake 1.0–1.5 h after BN administration (vehicle+vehicle: 1.2±0.3 g, *n*=10; vehicle+BN: 0.7±0.2 g, *n*=7; 2 μg TBNME+BN: 1.6±0.5 g, *n*=6). On the other hand, although pretreatment with the high dose (10 μg) of BIM did not show any significant inhibition of the BN-induced anorexia 0–0.5 h after administration, the BN-injected group with the pretreatment ingested more food pellets 0.5–2.0 h after administration than the BN-injected group without the pretreatment. Therefore, at 2.0 h after administration, the total food intake was almost the same for the two groups. BIM showed an inhibitory effect only on the late phase of the BN-induced anorexia. The lower doses, 2 μg of BIM showed no effect on the BN-induced anorexia.

Two or ten micrograms of either of the two antagonists did not change the basal food intake.

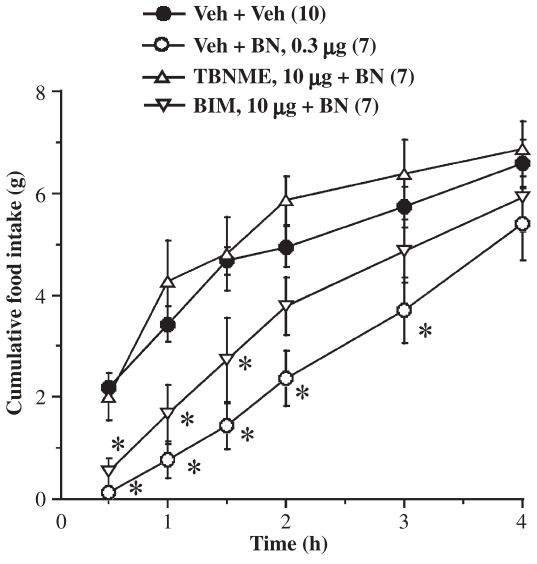

Fig. 2. Inhibitory effects of a GRP receptor subtype antagonist, [D-Tyr^6]bombesin(6–13) methyl ester (TBNME), and a NMB receptor subtype antagonist, BIM23127 (BIM), on the BN-induced anorexia. The antagonists were pretreated into the lateral ventricle 45 min before BN administration. Numbers in the parentheses express experimental numbers. Values are the means±S.E.M. *P<0.05 vs. the vehicle-injected group.

3.3. Involvement of PKC in the BN-induced anorexia

The effects of two PKC inhibitors on the BN-induced anorexia were examined, because the BN receptors have been demonstrated to couple Gq protein and activate PKC. Chelerythrine (CHE) is a non-specific antagonist to the PKC isoforms. Gö6983 (Gö) preferentially inhibits cPKC when compared with its effects on nPKC and aPKC. CHE and Gö did not influence the early phase of decreased food intake induced by BN (0.3 μg), but they inhibited only the late phase of the BN-induced anorexia, just as did the NMB receptor antagonist (Fig. 3A). The inhibitors alone did not influence the vehicle-induced effect.

Phorbol 12, 13-dibutyrate (PDBu) is a popular PKC activator. I.c.v. injection of PDBu also decreased feeding 0–1.5 h after administration, compared with the vehicle (saline containing 5% DMSO)-injected group (Fig. 3B). The 2-h total feeding was not different, however, from the vehicle-injected group. Its duration was shorter than that of the BN-injected group.

3.4. Involvement of NO-cGMP-PKG pathway in the BN-induced anorexia

The effect of an NO synthase inhibitor, L-nitro-arginine (LNA), on the BN-induced anorexia was also investigated, because certain effects of BN are mediated through the release of NO. Thirty micrograms of LNA entirely blocked the anorexia (Fig. 4A). LNA at 10 μg did not influence the BN-induced effect. Individual injections of LNA showed no effects on the basal food intake. The above experiment suggested that increased NO release after BN administration resulted in anorexia.

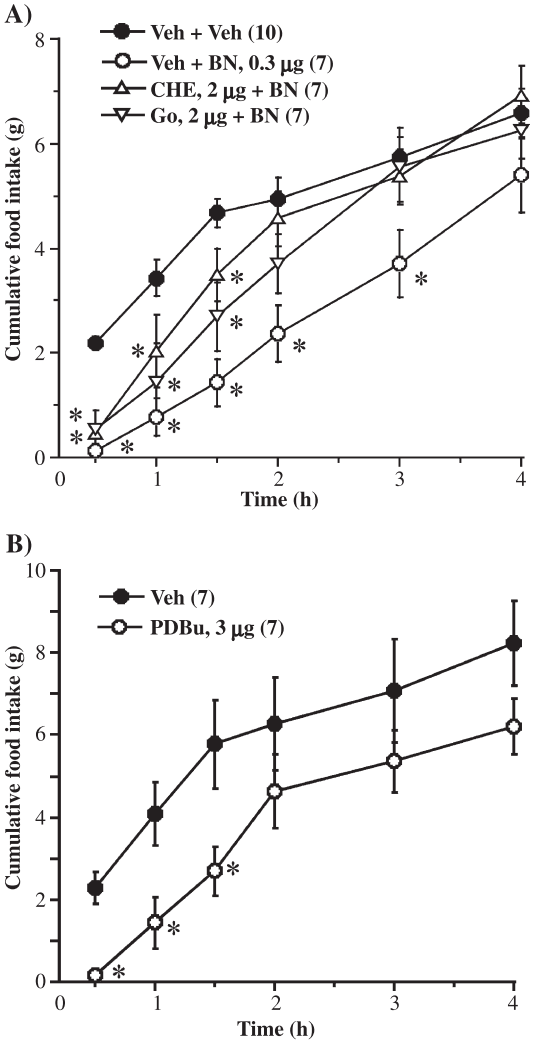

Fig. 3. A) Effects of the protein kinase C inhibitors, chelerythrine (CHE) and Gö6983 (Gö), on the BN-induced anorexia. B) Anorectic effects induced by i.c.v. injection of a PKC activator, PDBu. Numbers in the parentheses express experimental numbers. Values are the mean±S.E.M. *P<0.05 vs. the vehicle saline or saline containing 5% DMSO)-injected group.

NO is well known to activate guanylate cyclase and increase cGMP synthesis resulting in activating protein kinase G. BN (0.3 μg) injections increased cGMP concentration in the hypothalamus 1 h after administration (vehicle: 11.1±1.3; BN: 20.4±3.0 pmol/mg protein; n=8; P<0.05). H-9 (2 μg) was used as a PKG inhibitor in the pretreatment. This inhibitor alone did not influence feeding behavior. The BN (0.3 μg)-induced anorexia was definitely inhibited by H-9 (Fig. 4B).

4. Discussion

In this study, i.c.v. injections of BN elicited the powerful anorexia without any other changes in behavior, although Meisenberg et al. (1990) have demonstrated grooming, scratching and aversion to the environment in rats injected with BN into the ventricle. However, the contribution of malaise/nausea or taste aversion cannot be completely excluded from the mechanisms of the anorexia. I.c.v. injections of BN produced a decreasing tendency of locomotor activity, and hypothermia that was partly inhibited by TBNME and the PKC inhibitors, CHE and Go (Tsushima et al., 2003). Concerning changes in the taste after BN administration, there are some reports demonstrating the anorexia with and without taste aversion, and it remains unclear (Ervin et al., 1995; Gibbs et al., 1979; Vanderweele et al., 1985). In this study, a taste aversion test was not examined after BN administration.

4.1. BN receptor subtype in the BN-induced anorexia

The BN receptor subtype mediating the anorexia appears to be primarily GRP subtype, since BN and GRP showed more powerful effects than those induced by NMB, and the BN-induced effect was entirely inhibited by the GRP

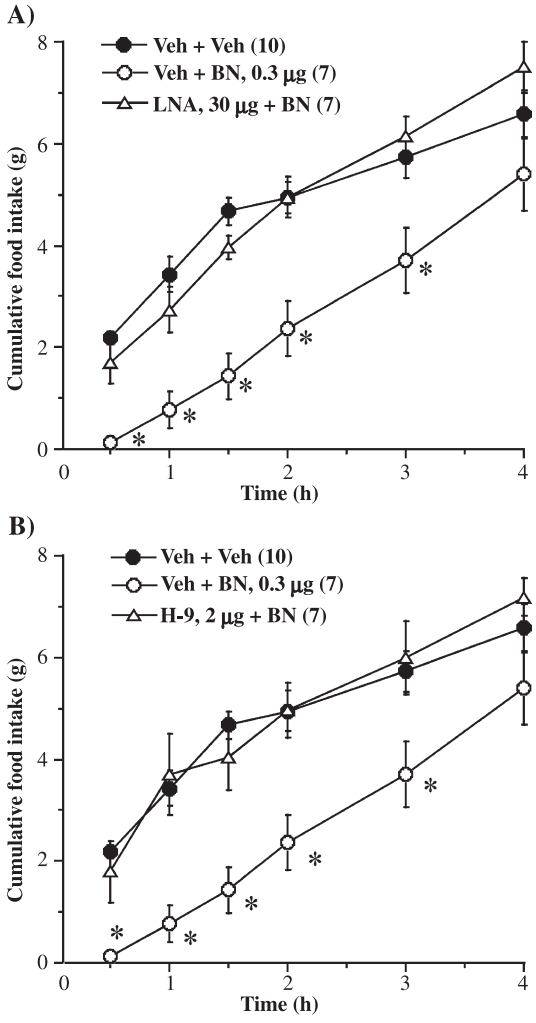

Fig. 4. A) Effects of an NO synthase inhibitor, L-nitro-arginine (LNA), on the BN-induced anorexia; B) Effects of a protein kinase G inhibitor, H-9, on the BN-induced anorexia. Numbers in the parentheses express experimental numbers. Values are the mean±S.E.M. *P<0.05 vs. the vehicle-injected group.

receptor subtype antagonist, TBNME. However, BIM also inhibited only the late phase of the BN-induced effect. The NMB receptor subtype may be involved in the BN-induced anorexia, especially in its late phase, although NMB did not decrease food intake. Drugs injected into the lateral ventricle run into the fourth ventricle after a short time. The late effect will probably be produced there. NMB receptor around the fourth ventricle participates in feeding regulation (Ladenheim et al., 1997). The dose of NMB used in this study may not be enough to produce the effect after its delivery to the fourth ventricle. In addition, there are the following possibilities: 1) The inhibitory effects of BIM are mediated through GRP receptor, because BIM has a low affinity for GRP receptor (Orbuch et al., 1993). However, we have demonstrated that a higher dose of BIM than the dose used in this study does not influence BN-induced hypothermia mediated through GRP receptor (Tsushima et al., 2003), 2) BIM may function as a somatostatin antagonist (Orbuch et al., 1993). The anorexia is possible to mediate through an increased release of somatostatin after BN administration. Somatostatin at high doses injected into the ventricle is demonstrated to decrease food intake (Feifel and Vaccarino, 1990), and 3) BIM also inhibits urotensin II receptors (Herold et al., 2003). However, it is unclear whether urotensin II shows any effects on food intake, or BN promotes its release.

There are some reports on food intake after injection of BN and the related peptides into the fourth ventricle (Flynn, 1993; Ladenheim et al., 1996a, 1997). They suggest that the action site is caudal hindbrain. Probably, BN binds the receptors there and their stimuli are sent to the center regulating food intake in the hypothalamus via neurons. It is supposed that these signals are involved in anorexia after peripheral injections of BN. On the other hand, BN injected into the lateral ventricle, at least mostly, affects the hypothalamus around the lateral/third ventricle, although distinct action site(s) in the hypothalamus for the BN-induced anorexia is(are) not obvious. Histochemical studies show a broad distribution of GRP receptors in the CNS and relatively high distribution of NMB receptors in the hypothalamus (Ladenheim et al., 1992; Ohki-Hamazaki et al., 1997b). Indeed, injections of BN into the multiple sites in the hypothalamus suppress food intake (Kyrkouli et al., 1987; Stuckey and Gibbs, 1982).

Recently, knockout mice of the three BN receptor subtypes were reported (Landenheim et al., 2002; Ohki-Hamazaki et al., 1999; Wada et al., 1997). In the GRP receptor knockout mice, GRP injected into the ventricle does not decrease food intake. Therefore, GRP receptors surely play a role in the regulatory mechanisms of appetite. GRP receptor knockout mice significantly increase body weight after 45 weeks, compared with wild-type mice (Landenheim et al., 2002). The regulation of appetite is very important for living animals and multiple mechanisms participate in its regulation. If one mechanism does not function, the others compensate its role. Mice lacking NMB receptors have normal body weight (Ohki-Hamazaki et al., 1999). On the other hand, BRS-3 deficient mice become obese and BRS-3 probably contributes to the regulation of food intake. This receptor subtype exists in the hypothalamic nuclei regulating food intake (Ohki-Hamazaki et al., 1997b; Yamada et al., 1999). We cannot investigate the role of BRS-3 in appetite using wild-type mice, because there are no selective agonists/antagonists for BRS-3.

4.2. Involvement of PKC

All three BN receptor subtypes in mammals couple Gq protein and are thought to activate PKC (Alexander et al., 2001). In fact, BN is demonstrated to increase the secretion of gastrin via activation of PKC in antral gastrin cells (Moore et al., 1999). Other cultured cell lines promote PKC activity after administration of BN and related peptides (Brough et al., 2000; Némoz-Gaillard et al., 1998). There is much evidence that PKC is involved in the effects induced by BN and related peptides using cultured cells.

In this study, the BN-induced anorexia was shown to be also induced via activation of PKC, because CHE and Gö, PKC inhibitors, diminished the effect, and PDBu, a PKC activator, produced anorexia. However, the inhibitory effect of CHE and Gö was partial and recognized only in the late phase of the BN-induced anorexia, suggesting that other mechanism(s) is(are) present in the early phase. In the BN-induced hypothermia, the involvement of PKC is also partial (Tsushima et al., 2003). The time course of the inhibition by the PKC inhibitors was similar to that by the NMB receptor antagonist. The late phase of the BN-induced anorexia might involve the activation of PKC and NMB receptor.

4.3. Involvement of NO-cGMP-PKG pathway

It has been reported that BN or BN-related peptides produce effects mediated through mechanisms other than the activation of PKC, such as increased intracellular concentration of Ca^{2+}, NO release, and activation of Rho kinase, MAP kinase and tyrosine kinase, and so on (Katsuno et al., 1999; Némoz-Gaillard et al., 1998; Nishino et al., 1998; Rozengurt, 1998). The rapid inhibitory effect of LNA on the BN-induced effect shows the involvement of NO in the early phase. Increased NO concentration is well known to promote guanylate cyclase followed by an increase in PKG activity. In this experimental system, BN increased cGMP in the hypothalamus and the BN-induced effect was inhibited by H-9, a PKG inhibitor. This inhibitory effect was different from that of the PKC inhibitors and remarkably occurred in the early phase, suggesting that the NO-cGMP-PKG pathway and the GRP receptor are involved in the early phase of the BN-induced anorexia. BN has been demonstrated to promote NO synthesis in both the CNS and the peripheral nervous system (Beltrán et al., 1999; Castaneda et al., 2000).

There are some reports showing involvement of NO in regulation of feeding behavior. For example, leptin decreases the activity of NO synthase in the CNS, the NO_x concentration in the CSF, and food intake (Calapai et al., 1998; Morley et al., 1999). Neuropeptide Y and ghrelin show the opposite phenomena, increases in NO and food intake (Morley et al., 1999; Gaskin et al., 2003). These results appear to link the anorexia to a decrease in NO. On the other hand, food-deprived condition decreases NO concentration in the hypothalamus (Ueta et al., 1995). In this study, BN increased NO concentration resulted in the anorexia. These are inconsistent with the above phenomenon. Roles of NO in the regulation are not clear. Because our experiment was carried out under the condition of food deprivation, increased NO concentration by BN may merely recover it to basal level. Where, when and how much is the change in NO concentration after drug administration are probably important. It is difficult to measure NO concentration in several sites with time course after drug administration.

BN has been reported to release NO in the dorsal motor nucleus of the vagus (Beltrán et al., 1999; Martinez and Taché, 2000). The other effect of BN, the hypothermia, was not mediated through PKG (unpublished data).

4.4. Conclusion

BN receptor-mediated regulation of feeding mainly involves the GRP receptor and NO-cGMP-PKG pathway, and additionally its late phase partly involves the NMB receptor and PKC activation.

We previously demonstrated that BN-induced hypothermia is mediated through the GRP receptor subtype, and not the NMB receptor subtype. Moreover, the effect partially involves the activation of PKC (Tsushima and Mori, 2001), but does not involve the activation of PKG (unpublished data). Therefore, the GRP receptor may work in different signal transduction pathways for different sites and/or effects.

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