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Neuromedin C microinjected into the amygdala inhibits feeding

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Abstract

Bombesin-like peptides including gastrin releasing peptide and neuromedin C are known to inhibit feeding. Bombesin receptors have been found in moderate to high densities in the amygdaloid body, which is essentially involved in the regulation of feeding and body weight. In the present experiments neuromedin C (15, 30, and 60 ng), a carboxyterminal decapeptid fragment of gastrin releasing peptide, was bilaterally microinjected into the central part of the amygdala in *ad libitum* fed male CFY rats. Food intake was measured every 5 min for 30 min and also 60 min following neuromedin C or vehicle microinjections. Fifteen nanograms neuromedin C significantly suppressed liquid food consumption for 5 min and 30 ng for 25 min. However, 60 ng was not effective. Neuromedin C effects were blocked by prior application of the bombesin receptor antagonist [Leu^{13}-ψ(CH_2NH)-Leu^{14}]-bombesin. Neuromedin C treatment increased latency to feeding, decreased food intake, decreased the time spent feeding and their ratio, the number and the duration of feeding episodes during the first 5 min, without modifying body temperature or stereotype activity. Results indicate that neuromedin C may decrease the efficiency of feeding and that activation of bombesin receptors in the central amygdala may reduce appetite.

Keywords: Amygdaloid body; Gastrin releasing peptide; Feeding; Satiety; Behaviour; Body temperature

1. Introduction

Bombesin (BN)-like peptides including gastrin-releasing peptide (GRP), neuromedin C (NMC) and neuromedin B (NMB) are thought to be involved in the regulation of appetite. The BN-like peptide family consists of several amphibian or mammalian peptides, all sharing a common heptapeptide carboxyterminal sequence. NMC has been isolated from porcine spinal cord and is the carboxyterminal decapeptide fragment of GRP [25] (see review for [26]).

After food ingestion, GRP/NMC immunoreactivity increases in the antrum of the stomach [15]. It has been hypothesised that through its neuronal and humoral effects GRP/NMC act as negative feedback satiety signals. Indeed, when BN, GRP or NMC were applied intraperitoneally (i.p.) or intracerebroventricularly (i.c.v.), inhibition of food intake was observed [5,7,19]. Injection of BN-like peptides into the lateral, paraventricular or ventromedial regions of the hypothalamus [17,37], the rostral pole of the nucleus of the solitary tract [3], the trigeminal nucleus or reticular formation decreases food intake [3]. Messenger ribonucleic acids encoding the precursor of GRP in the rat brain [41] and NMC immunoreactivity have been also detected in rat and human brain [34], and radioimmunoassay study found that both GRP and NMC immunoreactivity increases in the hypothalamus during feeding [15].

When applied, i.c.v. BN was an effective modulator inducing a wide range of behavioural alterations. BN increased locomotor activity and rearing and suppressed resting behaviour [17]. BN and NMC increased grooming [11,17] and evoked violent scratching [11]. BN-like peptides (BN, GRP, NMC) have also been shown to produce dose-dependent hypothermia after i.c.v. administration [12].

The amygdala plays an important role in feeding and body weight regulation [6,33]. Autoradiographic [40] and immunohistochemical [27] investigations have revealed moderate to high densities of BN receptors in the central part of amygdala (the central and intercalated nuclei, ACE). In addition, BN-like immunoreactive terminals are present in the ACE [31]. Biological responses mediated by BN-like peptides result from high affinity binding to two pharmacologically distinct BN receptors,

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i.e. GRP-preferring receptor (GRP-R) [35] and NMB-preferring receptor (NMB-R) [18], respectively, and both binding sites were found in the amygdala [39]. It has been suggested that NMC, like GRP, binds preferentially to the GRP-R subtype while BN binds with equal affinity to the GRP-R and NMB-R subtypes [18,39] (see review for [26]).

While the behavioural effects of amphibian BN have been widely characterized in mammals, less is known about the central anorexigenic effects of NMC, which is present in mammals. In our previous experiments, intra-ACE infusion of either BN or GRP [4,38] decreased food intake. The present experiments were designed to examine the ability of intra-ACE infusion of NMC to suppress food intake in rats. Potential effect of NMC on other behaviours or body temperature, changes which could interfere with feeding behaviour, also were examined.

2. Materials and methods

2.1. Subjects

Subjects were 82 male CFY rats (LATI, Gödöllő, Hungary) weighing 300–345 g at the beginning of the experiments. Animals were housed individually and cared for in accordance with institutional (Pécs University Medical School) and international standards {European Community Council Directive of 24 November 1986 (86/609/EEC)}. Rats were kept in a light- and temperature-controlled vivarium (12:12 h light–dark cycle with lights on at 06:00 a.m.; 22 ± 2 °C). Tap water and standard laboratory food pellets (CRLT/N standard rodent food pellet, Charles River Laboratories, Budapest) were available *ad libitum* before experiments. Daily food and water consumption and body weight were measured to the nearest g or ml.

From the 14th preoperative day, rats were allowed to consume liquid diet on a limited access schedule (milk, 136.45 kJ/100 ml, Milk Quick, Nutricia, Hungary) for 2 weeks. Calibrated drinking tubes filled with milk were attached to the front of each home cage. Milk was available between 10:00 a.m. and 03:00 p.m. during the first week. The pre-test exposure period was designed to overcome neophobia and to accustom the rats to the palatable complex food. During the second week, daily access to liquid diet was reduced to 1 h beginning at 10:00 a.m. In the remaining time standard laboratory food pellets were available *ad libitum*. The 1 h liquid food access schedule was maintained daily until the end of the experiments. Rats were excluded from experiments if their liquid food intake did not show stable baseline. Our method made exact consumption measurement possible in 5 min intervals with high accuracy without disturbing animals in their home-cages.

2.2. Surgery

Rats were anaesthetised i.p. with ketamine supplemented with diazepam (Calypsol, 80 mg/kg bw and Seduxen, 2 mg/kg bw, respectively; Richter Gedeon Ltd., Hungary). Stainless steel bilateral guide tubes (22 Gauge) were stereotaxically implanted into the dorsal border of the ACE (coordinates referring to the bregma: AP: 0.0 mm, ML: 4.6 mm and DV: 6.5 mm ventral from the surface of the dura) according to stereotaxic atlas [32]. The tips of cannulae were positioned 0.5 mm above the intended injection site. Cannulae were fixed to the skull with acrylic cement and stainless steel screws. When not being used for injection, the guide tubes were occluded with stainless steel obturators made of 27 Gauge stainless steel wire. Animals were allowed a minimum of 5 days for postoperative recovery before the experiments, during which time they were frequently handled.

2.3. Experiments

2.3.1. Food intake measurements

NMC (4153, Peptide Institute, Louisville, KY) or BN receptor antagonist [Leu^{13}-ψ(CH$_2$NH)-Leu^{14}]-BN (ANT; B-127, Sigma–Aldrich Chemical Co.) were dissolved in 0.15 M sterile saline for bilateral intraamygdaloid microinjections in a volume of 0.4 μl. Drugs or vehicle were microinjected through a 30 Gauge stainless-steel injection needle extending 0.5 mm below the tips of the implanted guide cannulae. Solutions were infused for 1 min into the ACE by automated syringe pumps (Cole Parmer, USA) by means of injection needles attached via polyethylene tubing (PE-10) to 5 μl Hamilton (Bonaduz, Switzerland) microsyringes. Needles were left in place for an additional 1 min after infusion to allow diffusion into the ACE. Awake animals were injected in their home cage and the bilateral injection procedure took 5 min. Milk intake was measured with ml resolution in every 5 min for 30 min and after 60 min. Following the 1 h test, rats were returned to *ad libitum* pellet feeding.

In the feeding experiments, *ad libitum* fed animals were microinjected with 15, 30 or 60 ng NMC into the left and right ACE (13.4, 26.8, 53.5 pmol/side, respectively), resulting in total doses of 30, 60 or 120 ng NMC, respectively. We reported earlier that applications of either 25 pmol BN or 9–53 pmol GRP into the amygdala were effective inducing anorexia [4,38]. The combined effects of antagonist and agonist were also studied. ANT (100 ng; 62.9 pmol/side) was bilaterally administered alone or 15 min prior to microinfusion of NMC (30 ng/side) into the ACE of *ad libitum* fed animals. Thus the total dose used for the animals in the appropriate group was 200 ng ANT or 200 ng ANT + 60 ng NMC, respectively. In this report, all the doses mentioned are meant to be the dose per side value. For testing, non-deprived rats were treated prior to their daily 1 h sessions using a within-subject design with 3 intervening treatment-free days.

2.3.2. Behavioural studies

Using the same feeding procedure, behavioural activities of different group of rats were recorded by a video camera (Sony Handycam camcorder). Individual rats were video-monitored in their home cages, beginning immediately after bilateral microinjections with 30 ng/side NMC or vehicle into the ACE. Experiments were separated by at least 3 days. Results were analysed off-line by two independent observers blind to the treatment. Five behavioural categories were identified and their durations were determined. In the course of an off-line analysis the time spent feeding, locomotion, resting, grooming and scratching was measured in 5 min bins for 30 min. Grooming was defined to include face and body grooming, head washing, sexual (ano-genital) grooming and tail licking. In addition, food intake was measured in the first 5 min and food intake/its duration ratio was calculated during the first 5 min observation period. The number and duration of feeding episodes were also analysed. A feeding episode was defined to end when the rat turned away from the calibrated tube for more than 1 s. The latency to feeding was defined as the latency to initiate eating after being returned to the home cage. The first inter-feeding episode interval was defined as the time between the termination of the first feeding episode (when the rat stopped drinking and turned away from the drinking tube) and the initiation of the subsequent feeding episode.

2.3.3. Body temperature measurements

In a separate experiment the core temperature of animals was measured using a thermometer (Checktemp 2, Singapur) 10 and 30 min after bilateral injection of NMC (30 ng/side) or vehicle. Temperature probe was inserted 50 mm into the colon, and measurements were taken at ambient air temperature at 22 °C.

2.4. Statistical analysis

All results expressed as a means ± S.E.M. One hour food intake measures were subjected to a 2 (vehicle versus one of the NMC dose) × 7 (time points), 30 min behavioural data were subjected to a 2 (vehicle versus 30 ng NMC) × 6 (time points) two-way repeated-measures analysis of variance (ANOVA), for post hoc interpretation within-subject Newman–Keuls test were used (SPSS for Windows 12.0, Chicago, IL). Feeding related data from the video analysis and data from the body temperature measurements were analysed by one-way ANOVA (SPSS for Windows 10.0). The statistical rejection criterion (α) was set at $p < 0.05$ level in the statistical analysis. In all of the experiments, each animal served as its own control.

2.5. Histology

To verify cannula placements, animals were anaesthetised with the same procedure as used for surgery and perfused transcardially with saline (0.15 M) followed by 10% formalin solution. Brains were sliced with a freezing microtome in 40 μm sections and stained with Cresyl violet. Injection sites were reconstructed according to a stereotaxic atlas [32].

3. Results

3.1. Histological determination of cannula placements

Careful histological examination showed that in 68 animals the cannula tips were symmetrically located in the upper part of the ACE. Schematic illustration of cannula placements is shown in Fig. 1. The tracks of cannulae and the tips were determined on the basis of existence of debris and moderate glial proliferation. In 11 rats, position of reconstructed cannulae was located outside the target area and these animals were excluded from statistical analysis. Among these rats, in five cases, cannula tips were symmetrically located 1–2 mm below the target area, so bilateral injections were made in basolateral and lateral amygdaloid nuclei. In six cases, cannula tips were asymmetrical and dorsal, so the tips were localised on one side within the piriform cortex. At the site of injection, in the remaining three cases, brain tissue destruction extending 2–2.5 mm in diameter was observed. Data of these rats were also excluded. In these animals, NMC or ANT injections were ineffective to modify food intake or behavioural patterns.

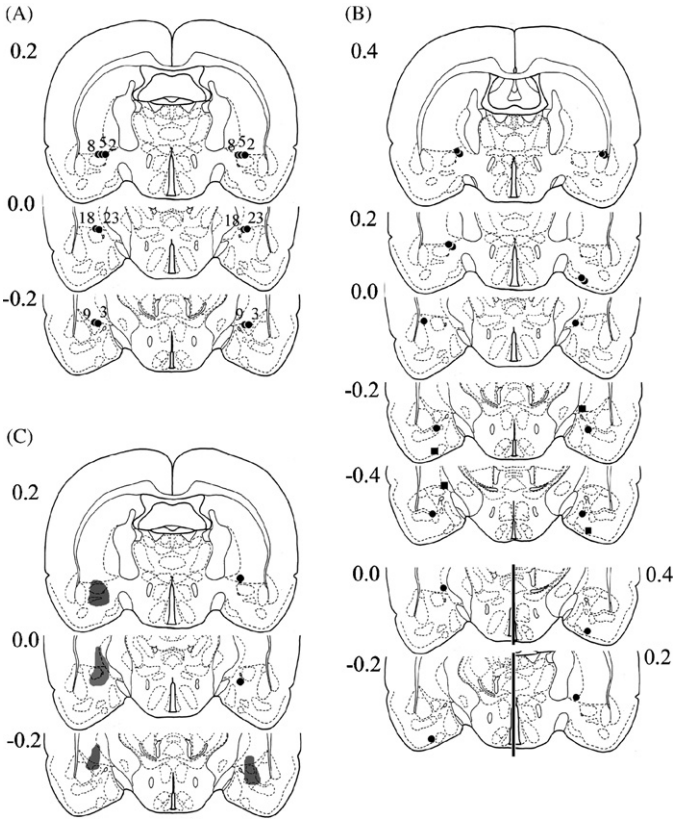

Fig. 1. Illustration of reconstructed injection sites from all of the experiments. Correct bilateral injection placements are indicated as closed circles in the central amygdala ($n = 68$, panel A). Incorrect injection placements are indicated in panel B ($n = 11$) and destruction of the brain parenchyma indicated as grey colored spot are shown in panel C ($n = 3$). Brain structure diagrams of coronal sections are adapted from Pellegrino et al. [32], the numbers refer to anterior–posterior distance from bregma in mm. Identical symbols in panel B (closed circles or squares) indicate coherent injection sites of bilateral injections. Numbers above the closed circles in panel A indicate the animal numbers of the representative injection site.

3.2. Food intake measurements

Within 3 days after surgery, the body weight of all animals reached the preoperative level. Neither hypophagia, nor hypodipsia were observed after the third postoperative day and animals showed continuous increase in body weight. Food intake tests began from the fifth postoperative day. In the present experiments liquid food intake was measured. Figures represent cumulative evaluation of data from measurements during the 60 min tests after NMC or vehicle treatments.

After 15 ng NMC injected bilaterally into the ACE repeated measures ANOVA yielded significant effect of time ($F[6, 42] = 75.320$, $p < 0.0001$) and no significant effect of treatment ($F[1, 7] = 2.664$, $p > 0.05$) on food consumption. *Post hoc* test, however, indicated that NMC treated rats ate significantly less than the vehicle-treated rats in the first 5 min ($p < 0.05$; $n = 8$, Fig. 2A). As illustrated in Fig. 2B ($n = 15$), after bilateral application of 30 ng NMC ANOVA revealed a significant overall interaction between treatments and time points (time: $F[6, 84] = 92.353$, $p < 0.0001$; treatment: $F[1, 14] = 8.776$, $p < 0.01$; time × treatment: $F[6, 84] = 2.271$, $p < 0.05$) on food intake. Follow-up *post hoc* test comparisons indicated that application of 30 ng NMC caused significant cumulative food intake reductions for 25 min (p's < 0.01–0.05, see Fig. 2B). Bilateral application of 60 ng NMC was ineffective to modify food intake (time: $F[6, 42] = 20.745$, $p < 0.0001$; treatment: $F[1, 7] = 0.494$, $p > 0.05$, N.S.; *post hoc* test comparisons: N.S.; $n = 8$, Fig. 2C).

After ANT + NMC treatment statistical analysis yielded significant effect on time ($F[6, 42] = 39.699$, $p < 0.0001$) and no significant difference on treatment ($F[1, 7] = 0.009$, $p > 0.05$). *Post hoc* test comparisons did not indicate significant differences showing that the consumption reducing effect of 30 ng NMC was eliminated by prior intraamygdaloid application of ANT ($p > 0.05$, N.S., $n = 8$, Fig. 3A). When ANT microinjection was applied alone (without NMC treatment) ANOVA revealed significant effect on time ($F[6, 48] = 19.709$, $p < 0.0001$), however, treatment was ineffective to modify food intake ($F[1, 8] = 2.371$, $p > 0.05$, N.S.; *post hoc* test: N.S.; $n = 9$, Fig. 3B).

3.3. Behavioural analysis

In the behavioural experiment, application of 30 ng NMC ($n = 7$) resulted in similar significant decrease of food intake as it was observed in the first experiment during the first 5 min ($F[1, 6] = 8.366$, $p < 0.05$; Table 1). Results of behavioural analysis showed decreasing tendency in feeding time during the first 5 min ($F[1, 6] = 5.097$, $p < 0.10$; Table 1). The food intake/feeding time ratio was significantly decreased (after

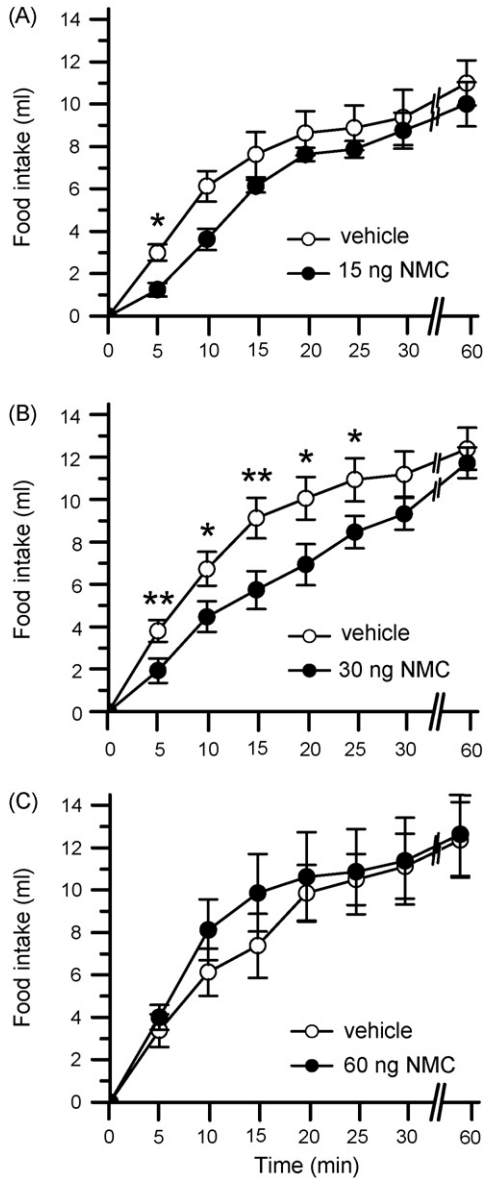

Fig. 2. Cumulative liquid food intake after application of 15 ($n = 8$, A), 30 ($n = 15$, B) and 60 ng NMC/side ($n = 8$, C) or vehicle microinjected into the ACE. Lines with symbols represent mean of food intake in ml (±S.E.M.). Symbol above lines indicates significant difference ($^{*}p < 0.05$, $^{**}p < 0.01$). For more explanation, see the text.

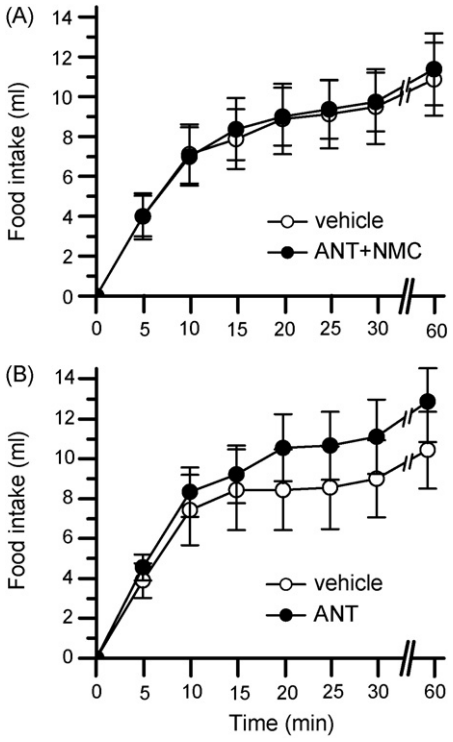

Fig. 3. Cumulative liquid food intake after application of [Leu^{13}-ψ(CH$_2$NH)-Leu^{14}]-BN (ANT) + NMC ($n = 8$, A) and ANT alone ($n = 9$, B), or vehicle. One hundred nanogram ANT was bilaterally applied 15 min before NMC (30 ng/side) microinjection. Lines indicate mean food intake in ml (±S.E.M.) after vehicle or combined drug (ANT + NMC) or ANT treatments. For more explanation, see the text.

vehicle: 3.91 ± 0.82 ml/min; after NMC: 0.98 ± 0.43 ml/min, respectively; $F[1, 6] = 9.021$, $p < 0.05$; in Table 1 see values in s). It was also shown that the number of feeding episodes somewhat decreased ($F[1, 6] = 4.290$, $p < 0.10$; Table 1), however, duration of feeding episodes significantly decreased after NMC treatment ($F[1, 6] = 10.740$, $p < 0.05$; $n = 7$, Table 1). The latency to feeding following NMC treatment was significantly longer than after vehicle injection ($F[1, 6] = 11.587$, $p < 0.05$; $n = 7$, Table 1). The first inter-feeding episode interval after intraamygdaloid NMC treatment was somewhat longer than after vehicle application, however, the difference was not significant ($F[1, 6] = 3.566$, $p < 0.10$; Table 1).

Statistical analysis revealed no significant effects of NMC treatment, as far as observations on feeding ($F[1, 6] = 1.612$, $p > 0.05$), locomotor activity ($F[1, 6] = 0.263$, $p > 0.05$), resting ($F[1, 6] = 0.271$, $p > 0.05$), grooming ($F[1, 6] = 0.618$, $p > 0.05$), or scratching ($F[1, 6] = 1.448$, $p > 0.05$) were concerned during the 30 min behavioural test (Table 2). As the time progressed feeding activity (time: $F[5, 30] = 8.207$, $p < 0.0001$) and locomotion decreased (time: $F[5, 30] = 3.790$, $p < 0.01$) after both treatments and time spent resting slowly increased, however it was not significant (time: $F[5, 30] = 1.964$, $p > 0.05$). Time spent grooming (time: $F[5, 30] = 3.016$, $p < 0.05$) and scratching behaviour (time: $F[5, 30] = 2.931$, $p < 0.05$) slowly increased after both vehicle and NMC application, however, *post hoc*

Table 1
Evaluation of feeding related parameters during the first 5 min of behavioural test after bilateral intraamygdaloid NMC microinjection (30 ng/side) or vehicle treatment

	Vehicle	NMC
Food intake (ml)	4.42 ± 1.25*	0.92 ± 0.57*
Time spent feeding (s)	78.00 ± 21.01	26.28 ± 15.59
Food intake/feeding time ratio (ml/s)	0.065 ± 0.013**	0.016 ± 0.007**
Number of feeding episodes	5.00 ± 1.15	2.04 ± 0.88
Duration of feeding episodes (s)	15.95 ± 3.96$^{\#}$	5.83 ± 2.72$^{\#}$
Latency to feeding (s)	64.00 ± 23.18$^{\#\#}$	231.85 ± 56.87$^{\#\#}$
First inter-feeding episode interval (s)	17.14 ± 8.15	26.14 ± 9.94

Values are means ($n = 7$) ± S.E.M. calculated on the basis of intake in millilitre (ml) and time in seconds (s) after either vehicle or NMC injection. For more explanation, see the text. Symbols indicate significant differences: *, **, $^{\#}$, $^{\#\#}$: $p < 0.05$, respectively.

Table 2
Activity of animals in the home cage after bilateral intraamygdaloid NMC microinjection (30 ng/side) or vehicle (veh) treatment

Observation periods (5 min)	Feeding		Locomotion		Resting		Grooming		Scratching	
	Veh	NMC	Veh	NMC	Veh	NMC	Veh	NMC	Veh	NMC
First	78 ± 21	26 ± 15	184 ± 22	251 ± 14	25 ± 14	15 ± 10	10 ± 5	6 ± 3	1 ± 1	0 ± 0
Second	103 ± 18	145 ± 23	153 ± 12	114 ± 24	30 ± 30	17 ± 17	9 ± 4	20 ± 5	3 ± 2	3 ± 2
Third	84 ± 24	94 ± 19	181 ± 18	154 ± 29	0 ± 0	1 ± 1	16 ± 5	31 ± 12	17 ± 5	18 ± 7
Fourth	28 ± 14	52 ± 17	197 ± 19	143 ± 31	10 ± 10	39 ± 39	28 ± 17	21 ± 9	22 ± 11	39 ± 14
Fifth	28 ± 18	55 ± 23	122 ± 36	123 ± 30	80 ± 51	0 ± 0	43 ± 20	74 ± 21	25 ± 17	47 ± 20
Sixth	16 ± 7	23 ± 13	119 ± 39	123 ± 31	95 ± 47	96 ± 49	42 ± 27	31 ± 12	26 ± 14	25 ± 11

Values are means ($n = 7$) ± S.E.M. calculated on the basis of the time in seconds spent feeding, locomotion, resting, grooming and scratching. For more explanation, see the text.

test have not revealed significant interactions in any observed behaviour in any time points between the treatments. Thus, all animals showed characteristic behavioural "satiety" sequence of progression.

3.4. Body temperature measurements

Analysis of variance yielded no significant effects on core temperature 10 min (after vehicle: 37.4 ± 0.1; after NMC: 37.2 ± 0.2 °C; $F[1, 12] = 2.497$, $p > 0.05$) or 30 min (after vehicle: 38.0 ± 0.15; after NMC: 37.8 ± 0.15 °C; $F[1, 12] = 0.615$, $p > 0.05$; $n = 13$) after administration of 30 ng NMC into the ACE.

4. Discussion

Experimental data suggest that the AMY is essential in the control of hunger-motivated behaviour. While an early study showed that bilateral amygdalectomy in rats caused no changes in food intake and body weight [1], later it was described that either hypophagia [6,8], or hyperphagia [6] developed after lesions of different parts of the AMY. According to Fonberg [6] the dorsomedial–central part of the AMY represents a hunger mechanism while the basolateral region is involved in the regulation of satiety. As far as the ACE is concerned, it has also been shown that specific catecholaminergic microlesions reducing the norepinephrine content in this structure produced hyperphagia and weight increase, while dopamine depletion caused hypophagia and weight decrease [20]. Cell-specific microiontophoretic lesions of the ACE with kainic acid, which destroys neurons in the target area but leaves the passing fibers intact, also induce hypophagia and weight decrease [8]. In the ACE specific glucose-sensitive neurons which have been described [14,21,28] these distinct chemosensitive neurons exhibit characteristic firing patterns during alimentary conditioning and give definite responses to different taste stimulations. The glucose-sensitive gustatory cells are suppressed by microelectrophoretic application of norepinephrine, whereas glucose insensitive neurons are facilitated by dopamine [14]. These data suggest that the ACE may be involved in the integration of feeding-associated humoral, motivational and exogenous chemical information [20]. The AMY is reciprocally connected with the lateral hypothalamus [29] and brain stem having projection to autonomic-related centers such as the dorsal motor nuclei of the vagal nerve, nucleus of the solitary tract and the parabrachial nucleus [10]. The lateral hypothalamus, the nucleus of the solitary tract and the nucleus tegmenti dorsolateralis contain BN/GRP immunostained neurons [31] and these brain structures send projections to the ACE. Although the exact projection and synaptic organization of these BN/GRP containing neurons are still unknown, BN-like immunoreactive terminals and BN receptors have been found in the ACE [27,31,40] (see review for [26]).

In the present experiments direct bilateral application of 15 or 30 ng NMC into the ACE transiently reduced food intake. Although, the highest dose of NMC (60 ng) was ineffective, duration of food intake reduction was longer following the 30 ng dose (26.8 pmol) (25 min) than the 15 ng dose (13.4 pmol) (5 min) of NMC. In our previous experiments with GRP microinjections into the ACE similar observations were made [4]. The lowest and the highest doses of GRP (9.0 and 107.0 pmol/side GRP, respectively) were ineffective and the range of effective doses (i.e. between 20 and 50 pmol/side) [4] was similar to that of NMC in the present experiments (13.4 and 26.8 pmol/side, respectively). Since 60 ng of NMC did not cause anorexia, the effective dose range of NMC appears to be smaller than that of GRP. There can be several explanation for this observation. First, GRP contains 27 aminoacids, while NMC is a 10 aminoacid carboxy terminal fragment of GRP. Thus, NMC seems to be degraded by endogenous peptidases in the brain, supporting the idea that eight aminoacids carboxy terminal fragment contains the active part of the peptide [9,30]. Second, we cannot exclude the possibility of the phosphorylation of BB2 receptors after NMC treatment. Phosphorylation of BB2 receptor appeared to be correlated to the occupation of receptor by agonist. It has been supposed that phosphorylation a prerequisite for either internalization or acute, homologous desensitization [16].

Our present results with NMC are in good agreement with data of others [4,36] that suppression of food intake by GRP was not dose-dependent. Effects of BN (25 pmol/side) application into the ACE was found more potent than GRP because it suppressed feeding even in 24 h food deprived rats [38] while GRP had no effect on food intake after food deprivation [4]. Previous studies also showed that NMC was less potent than BN [11,19,36]. The brief and less efficacious anorectic effect of NMC may be explained by the fact that pyroglutamat at the N-terminal of BN can protect it from enzymatic degradation.

Our experimental data suggest that food intake reducing effects of NMC are pharmacologically specific because it was eliminated by prior application of ANT [Leu^{13}-ψ(CH$_2$NH)-Leu^{14}]-BN. The [Leu^{13}-ψ(CH$_2$NH)-Leu^{14}]-BN antagonist [2] has been shown to have high affinity for GRP-R and extremely low affinity for NMB-R [13], and in other experiments it was more potent on GRP-R than on NMB-R [22]. The finding that [Leu^{13}-ψ(CH$_2$NH)-Leu^{14}]-BN blocked the effect of NMC on food intake suggests that indeed, GRP-R subtypes bound NMC in the ACE resulting suppression of feeding. Our result, that application of the antagonist alone did not modify feeding, is somewhat controversial because i.c.v. injection of [Leu^{13}-ψ(CH$_2$NH)-Leu^{14}]-BN facilitated food intake [24]. However, in these experiments, feeding conditions were different and animals were food deprived in pre-feed paradigm [24].

As far as our experiments on body temperature are concerned, NMC injection into the ACE did not modify core temperature. Our results are consistent with those of others [4,37,38], who reported that BN or GRP injection into the hypothalamus or the amygdala had no effect on body temperature.

In our behavioural experiments, when the activity of animals was video-monitored, similar food intake reduction was detected as in the feeding experiment. Animals started to eat liquid food later following NMC treatment, and in the first 5 min spent less time with feeding, produced fewer and briefer feeding episodes. In our experiment, the food intake/feeding time ratio also decreased after NMC showing that within the same time rats consumed less liquid food after NMC than after vehicle. The results may suggest that NMC suppresses intake by increasing postingestive negative feedback [36] and/or by decreasing the efficiency of feeding. Decrease in the efficiency of feeding may indicate that central effects of NMC interfere with sensorimotor mechanisms involved with ingesting liquid food. Further experiments with computerised lickometer system are necessary to cast light on exact microstructural changes of licking pattern of behaviour after NMC treatment in the ACE, however.

After high dose BN or GRP or NMC injections behavioural alterations including increased grooming were observed [11,17] (see review for [26]). In our experiments, we used the highest dose of NMC that was effective on food intake (i.e. 30 ng), still we did not find apparent changes in grooming recorded in the home cage. Furthermore, our observation is consistent with previous reports showing that after 40 ng BN site-specific injection into the nucleus of the solitary tract or the ACE there was no positive correlation between suppression of food intake and grooming [3,38]. Our behavioural data showed that rats exhibited similar behavioural patterns and expressed behavioural "satiety" sequence of progression after either vehicle or NMC treatments.

Our present results give further supports for the involvement of the AMY in the mechanisms of hunger and satiety and provide evidences that central GRP/NMC system, especially the NMC in the ACE, represents one of the neurochemical signals operating on the mechanisms of satiety. Indeed, there is quite strong evidence that a single meal increases not only the peripheral level of BN-like peptides, but it increases their level in different brain regions [15]. After food ingestion, significant NMC and distinct GRP increases were found in a roughly equivalent degree in the hypothalamus [15]. Our supposition, that NMC in the ACE may be involved in the control mechanisms of satiety, is supported by recent results of in vivo microdialysis studies showing that during ingestion and during the postprandial period the extracellular level of NMC markedly increased in the ACE [23].

5. Conclusion

In conclusion, the present results show that local microinjection of small doses of NMC into the ACE effectively inhibits feeding. This inhibitory effect of NMC is rapid, transient and specific, because it can be eliminated by prior application of ANT and it can not be explained by an increase in grooming or scratching, or by evoking alterations in body temperature. Present data show that NMC may decrease the efficiency of feeding and may act as a specific satiety signal in the ACE, however, the exact mechanisms of NMC action remain to be elucidated.

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