Brain Research 1648 (2016) 46–53

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/brainres

Research report

Peripheral injection of bombesin induces c-Fos in NUCB2/nesfatin-1 neurons

Kim-Marie Engster, Arthur L. Kroczek, Matthias Rose, Andreas Stengel, Peter Kobelt *

Charité Center for Internal Medicine and Dermatology, Department of Psychosomatic Medicine, Charité – Universitätsmedizin Berlin, Germany

ARTICLE INFO

Article history:
Received 21 March 2016
Received in revised form
5 July 2016
Accepted 7 July 2016
Available online 7 July 2016

Keywords:
Brain-gut-axis, food intake, hypothalamus, nucleus of the solitary tract
Paraventricular nucleus
Rat

ABSTRACT

As anorexigenic hormones bombesin and nucleobindin2 (NUCB2)/nesfatin-1 decrease food intake in rodents. Both hormones have been described in brain nuclei that play a role in the modulation of hunger and satiety, like the paraventricular nucleus of the hypothalamus (PVN) and the nucleus of the solitary tract (NTS). However, the direct interaction of the two hormones is unknown so far. The aim of study was to elucidate whether bombesin directly interacts with NUCB2/nesfatin-1 neurons in the PVN and NTS. Therefore, we injected bombesin intraperitoneally (ip) at two doses (26 and 32 nmol/kg body weight) and assessed c-Fos activation in the PVN, arcuate nucleus (ARC) and NTS compared to vehicle treated rats (0.15 M NaCl). We also performed co-localization studies with oxytocin or tyrosine hydroxylase. Bombesin at both doses increased the number of c-Fos positive neurons in the PVN ($p < 0.05$) and NTS ($p < 0.05$) compared to vehicle, while in the ARC no modulation was observed ($p > 0.05$). In the PVN and NTS the number of c-Fos positive neurons colocalized with NUCB2/nesfatin-1 increased after bombesin injection compared to vehicle treatment ($p < 0.05$). Moreover, an increase of activated NUCB2/nesfatin-1 immunoreactive neurons that co-expressed oxytocin in the PVN ($p < 0.05$) or tyrosine hydroxylase in the NTS ($p < 0.05$) was observed compared to vehicle. Our results show that peripherally injected bombesin activates NUCB2/nesfatin-1 neurons in the PVN and NTS giving rise to a possible interaction between bombesin and NUCB2/nesfatin-1 in the modulation of food intake.

1. Introduction

Bombesin is an anorexigenic tetradecapeptide that was isolated in 1971 from the skin of the European toad *Bombina bombina* (Anastasi et al., 1971). There are several homologs of bombesin in mammals, including gastrin-releasing peptide (GRP) and neuromedin B (Moody and Merali, 2004). Bombesin-like peptides are released in the gastrointestinal tract after the uptake of nutrients (Schusdziarra et al., 1986b). Bombesin binds with high affinity to the GRP receptor (BB_2), and to the neuromedin receptor (BB_1) distributed in the gastrointestinal tract (GI) and in the central nervous system (CNS) (Moody and Merali, 2004; Moran et al., 1988; Sayegh, 2013; Schusdziarra et al., 1986b). High densities of bombesin binding sites were observed in the hippocampus, hindbrain, midbrain, and amygdala and in the hypothalamus (Moody and Merali, 2004; Wada et al., 1991; Zarbin et al., 1985). In line with these findings, microinjections of bombesin into specific brain areas, like hypothalamic nuclei or the nucleus of the solitary tract (NTS) of the brainstem were effective in suppressing food intake (Johnston and Merali, 1988; Kyrkouli et al., 1987). Additionally, it has been observed that the satiating effects of bombesin are attenuated in animals with lesions of hindbrain areas (*e.g.* NTS or the dorsal motor nucleus of the vagus), whereas forebrain lesions did not affect bombesin's effects on food intake (Bellinger and Bernardis, 1984; Geary et al., 1986; Sayegh, 2013, West et al., 1982). This underlines the key role of central – predominantly hindbrain – areas in the bombesin-induced suppression of food intake.

The mapping of brain neuronal circuits recruited by hormone signals has been obtained by assessing changes of the proto-oncogene c-Fos, which allows the identification of activated neurons at the cellular level (Sagar et al., 1988). Studies have shown that intraperitoneally (ip) injected bombesin increased the number of c-Fos positive neurons in the paraventricular nucleus of the hypothalamus (PVN), and in the NTS (Bonaz et al., 1993; Li and Rowland, 1996), an activation likely underlying the peripheral anorexigenic effect of the peptide observed in rodents and humans (Denbow, 1989; Taylor and Garcia, 1985; Woods et al., 1983; Muurahainen et al., 1993; Kulkosky et al., 1982). However, relevant differences between peripheral and central administration of bombesin have been observed with regards to food intake. In

* Correspondence to: Charité Center for Internal Medicine and Dermatology, Department of Psychosomatic Medicine, Charité – Universitätsmedizin Berlin, Campus Mitte, Sauerbruchweg 5, 10117 Berlin, Germany.
E-mail address: peter.kobelt@charite.de (P. Kobelt).

http://dx.doi.org/10.1016/j.brainres.2016.07.006
0006-8993/

contrast to animals icv injected with bombesin, rats injected ip with bombesin display a normal frequency of grooming and do not show a decrease in drinking behavior (Kulkosky et al., 1982). Kulkosky et al. concluded that the specific anorexigenic effect of ip administered bombesin cannot be explained solely by the increase of bombesin in the cerebrospinal fluid (Kulkosky et al., 1982). It can be assumed that ip injected bombesin suppresses feeding additionally through spinal afferent fibers and vagal afferent mechanisms (Michaud et al., 1999; Sayegh, 2013; Stuckey et al., 1985).

Besides bombesin, in rodents the neuropeptide nesfatin-1 suppresses food intake when injected centrally or peripherally (Shimizu et al., 2009; Oh-I et al., 2006; Stengel et al., 2009; Atsuchi et al., 2010). Nesfatin-1 reduces food intake *via* a reduction of meal size and meal frequency in mice (Goebel et al., 2011). Nesfatin-1 is proteolytically cleaved from the precursor protein nucleobindin (NUCB2) and was first described in Oh-I et al. (2006) as "*N*ucleobindin 2-*e*ncoded *s*atiety-and *fat i*nfluencing prote*in*" (Oh-I et al., 2006). Also NUCB2 is biologically active and has the same anorexigenic effect as nesfatin-1 (Oh-I et al., 2006). NUCB2 mRNA and protein expression were detected in different brain nuclei, like hypothalamic nuclei and the NTS (Goebel et al., 2009; Foo et al., 2008).

Administration of sulfated cholecystokinin-8 (CCK-8S) was shown to dose-dependently induce c-Fos in NUCB2/nesfatin-1 immunoreactive neurons in the PVN and NTS (Noetzel et al., 2009) which may contribute to CCK's anorexigenic effects. Interestingly, bombesin induces a BB_2 receptor-stimulated activation of gastric vagal afferent discharge mediated partly through a CCK pathway (Yoshida-Yoneda et al., 1996; Schwartz et al., 1997). In line with this finding, also bombesin might interact with NUCB2/nesfatin-1 to induce its anorexigenic effects. As both anorexigenic hormones induce an activation of hypothalamic and brainstem nuclei, we considered the PVN and the NTS as possible sites of interaction.

Thus, the aim of the present study was to determine whether bombesin injected ip modulates the neuronal activity of NUCB2/nesfatin-1 immunoreactive neurons in the PVN or NTS. Neuronal activity was assessed by measuring c-Fos expression in relevant brain nuclei. It has been reported that oxytocin might be involved in the downstream anorexigenic signaling of nesfatin-1 (Foo et al., 2008; Oh-I et al., 2006; Kohno et al., 2008). Therefore, we also determined whether activated NUCB2/nesfatin-1 immunoreactive neurons are co-localized with ocytocin in the PVN after ip injection of bombesin. Since there is also evidence that oxytocin containing neurons in the PVN are innervated by noradrenergic projections of the A2-cell group in the NTS (Sawchenko and Swanson, 1981, 1982), we lastly quantified activated NUCB2/nesfatin-1 immunoreactive neurons co-localized with tyrosine hydroxylase.

2. Results

2.1. Effects of ip injected bombesin on c-Fos expression and the number of NUCB2/nesfatin-1 immunoreactive neurons in hypothalamic and medullary nuclei

2.1.1. Paraventricular nucleus

After ip injection of bombesin (26 and 32 nmol/kg body weight) we observed a gradual increase in the number of c-Fos positive neurons in the PVN (mean ± SEM: 70 ± 9 and 109 ± 8 neurons/section, respectively) compared to vehicle treated rats (16 ± 4 neurons/section, $p < 0.05$; Figs. 1A and 2A). The number of NUCB2/nesfatin-1 immunoreactive neurons in the PVN did not change after bombesin treatment (237 ± 21 and 220 ± 7 neurons/section, respectively) compared to vehicle treatment (214 ± 3 neurons/section, $p > 0.05$; Fig. 2B). Double labeling showed that the number of c-Fos positive and NUCB2/nesfatin-1 immunoreactive double-labeled neurons in the PVN increased more than tenfold after ip injection of bombesin (32 ± 3 and 57 ± 13 neurons/section, respectively) compared to the vehicle group (4 ± 0.5 neurons/section, $p < 0.05$; Fig. 2A). However, the gradual effect of the two doses of bombesin on c-Fos and NUCB2/nesfatin-1 immunoreactive double-labeling did not reach statistical significance ($p > 0.05$; Fig. 2A).

Triple-staining indicated that c-Fos-positive and NUB2/nesfatin-1 immunoreactive neurons partly co-localized with oxytocin (Figs. 1A,B and 2B). While the number of oxytocin immunoreactive neurons did not change after injection of bombesin (67 ± 19 and 63 ± 8, respectively compared to 51 ± 10 neurons/section; $p > 0.05$ Fig. 2B), this treatment increased the number of c-Fos positive and NUCB2/nesfatin-1 immunoreactive neurons co-localized with oxytocin (2.6 ± 1.3 and 9 ± 4 neurons/section, respectively) compared to vehicle treated rats (0.4 ± 0.2 neurons/section, $p < 0.05$; Fig. 2C).

2.1.2. Nucleus of the solitary tract

In the NTS bombesin administration also led to a gradual increase of c-Fos immunoreactive neurons (46 ± 1 and 91 ± 8 neurons/section, respectively) compared to vehicle treatment (5 ± 2 neurons/section, $p < 0.05$; Figs. 3A and 4A). In contrast to the PVN, the number of NUCB2/nesfatin-1 immunoreactive neurons significantly increased after ip injection of bombesin in the NTS (105 ± 12 and 118 ± 1 neurons/section, respectively) compared to vehicle treatment (61 ± 4 neurons/section, $p < 0.05$; Figs. 3A and 4B). Interestingly, we observed some NTS neurons whose projections were stained by nesfatin-1 antibody (Fig. 3C).

As observed for oxytocin in the PVN, in the NTS the number of tyrosine hydroxylase positive neurons did not change after ip injection of bombesin (26 ± 5 and 25 ± 5 neurons/section, respectively) compared to vehicle treated rats (25 ± 4 neurons/section, $p > 0.05$; Fig. 4B). The number of c-Fos-positive and NUCB2/nesfatin-1 immunoreactive neurons in the NTS significantly increased after ip injection of bombesin (17 ± 3 and 25 ± 3 neurons/section, respectively) compared to the injection of vehicle solution (1 ± 0.1 neuron/section, $p < 0.05$; Fig. 4A). No difference was observed between the two doses of bombesin ($p > 0.05$).

Triple labeling against c-Fos, NUCB2/nesfatin-1 and tyrosine hydroxylase showed that c-Fos-positive and NUCB2/nesfatin-1 immunoreactive neurons were scarcely co-localized with tyrosine hydroxylase in the NTS (Figs. 3A, B and 4C). Quantification of these neurons showed an increase of triple-labeled neurons positive for NUCB2/nesfatin-1, c-Fos and tyrosine hydroxylase after ip injection of bombesin (6.7 ± 0.9 and 8.9 ± 0.3 neurons/section, respectively) compared to vehicle treatment (0.6 ± 0.2 neurons/section, $p < 0.05$; Figs. 3A and 4C).

2.1.3. Arcuate nucleus

In contrast to the PVN and NTS, no changes of c-Fos immunoreactivity were detected in the ARC after bombesin treatment (17 ± 2 and 12 ± 1 neurons/section, respectively) compared to vehicle treatment (14 ± 2 neurons/section, $p > 0.05$; data not shown).

3. Discussion

The present study shows that peripherally injected bombesin affects the activity of NUCB2/nesfatin-1 immunoreactive neurons in conscious non fasted rats. As a response to bombesin injected ip, we observed a four- to seven-fold increase in the number of c-Fos neurons in the PVN and a ten- to eighteen-fold increase in the number of c-Fos neurons in the NTS. In contrast to the PVN, we did not detect any changes in number of c-Fos neurons in the ARC

Please cite this article as: Engster, K.-M., et al., Peripheral injection of bombesin induces c-Fos in NUCB2/nesfatin-1 neurons. Brain Research (2016), http://dx.doi.org/10.1016/j.brainres.2016.07.006