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ScienceDirect
Neuropharmacology 52 (2007) 684–692
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

Region specific galanin receptor/neuropeptide Y Y1 receptor interactions in the tel- and diencephalon of the rat. Relevance for food consumption

C. Parrado [a], Z. Díaz-Cabiale [b], M. García-Coronel [b], L.F. Agnati [c], R. Coveñas [b], K. Fuxe [d], J.A. Narváez [b,*]

[a] Department of Histology, University of Málaga, Faculty of Medicine, Campus de Teatinos sn, Malaga 29080, Spain
[b] Department of Physiology, University of Málaga, Faculty of Medicine, Campus de Teatinos sn, Malaga 29080, Spain
[c] Department of Physiology, University of Modena, Modena, Italy
[d] Department of Neuroscience, Karolinska Institute, Stockholm, Sweden

Received 13 March 2006; received in revised form 15 September 2006; accepted 22 September 2006

Abstract

The aim of this work was to determine the interactions between NPY and GAL receptor (GALR) subtypes in the hypothalamus and the amygdala using quantitative receptor autoradiography to analyze the binding characteristics of NPY-Y1 and Y2 receptor subtypes in the presence and absence of GAL. Food intake in satiated animals was evaluated after intraventricular coinjections of GAL and NPY-Y1 or Y2 agonists. The expression of c-Fos IR in both regions was also investigated. GAL decreases NPY-Y1 agonist binding in the arcuate nucleus by about 15% ($p < 0.01$), but increases NPY-Y1 agonist binding in amygdala (18%) ($p < 0.01$). These effects were blocked with the GAL antagonist M35. Y2-agonist binding was not modified by GAL. GAL blocked the food intake induced by the Y1 agonist ($p < 0.01$). Coinjections of Y1 agonist and GAL also reduced the c-Fos expression induced by the Y1 agonist in the arcuate nucleus and the dorsomedial hypothalamic nucleus but increased c-Fos expression in amygdala. These results indicate the existence of antagonistic interactions between GALR and NPY-Y1 receptors in the hypothalamus and their functional relevance for food intake. In contrast, a facilitatory interaction between GALR and Y1 receptors exists in the amygdala which may be of relevance for fear related behaviour.

Keywords: Autoradiography; NPY Y1 receptor; Galanin; Hypothalamus; Amygdala; Feeding

1. Introduction

Galanin (GAL) is a 29–30 amino acid neuropeptide (Tatemoto et al., 1983) widely distributed in the peripheral and central nervous system (CNS) often co-localized with classical neurotransmitters and other neuromodulators (Jacobowitz et al., 2004). Three galanin receptor (GALR) subtypes (GalR1, GalR2 and GalR3), have been cloned and belong to the G-protein-coupled receptor superfamily (Branchek et al., 2000). In CNS GAL and GALR distributions have been analyzed by in situ hybridization analysis, inmunohistochemistry as well as by radioligand binding (Jacobowitz et al., 2004) in hypothalamus and amygdala. Within the hypothalamus galanin is expressed in a number of neuronal cell populations in the arcuate nucleus (ARC) and the paraventricular hypothalamic nucleus (PVN). These neurons send projections to a large number of hypothalamic nuclei, where GALR1 and GALR2 subtypes exist (O'Donnell et al., 1999). Galanin has been implicated in several higher order physiological functions including learning and memory, pain control, neuroendocrine functions, energy homeostasis and food intake (Crawley et al., 2002). In fact, a role in food intake has been proposed

* Corresponding author.
E-mail address: zaida@uma.es (J.A. Narváez).

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doi:10.1016/j.neuropharm.2006.09.010

since hypothalamic GAL exerts a stimulatory effect on feeding behaviour related specifically to GALR1 and GALR2 receptors subtypes (Gundlach, 2002).

Neuropeptide Y (NPY), a highly conserved 36-amino acid peptide, is involved in several physiological functions and is widely distributed in the nervous system (Lin et al., 2004). NPY is a major regulator of food consumption and energy homeostasis (Kalra and Kalra, 2004) and is expressed mainly in neurons of the ARC and the dorsomedial hypothalamic nuclei (DMN) (Parker and Herzog, 1999) that send their projections to multiple areas. NPY exerts its effects via NPY receptor subtypes Y1–Y6. All receptor subtypes belong to the G-protein coupled receptor superfamily (Lin et al., 2004). To affect feeding and energy homeostasis NPY acts through Y1, Y5 and possibly Y2 receptor subtypes. NPY Y1 agonists stimulate food intake in the ARC and PVN while the role of Y2 receptors seem to be different since they inhibit food intake at low doses (Kalra and Kalra, 2004).

The presence of both GAL and NPY and/or their receptors in many relevant brain regions related to learning and memory, mood, neuroendocrine functions, energy homeostasis and food intake implies that GAL and NPY may complement the physiological actions of one another. Neurons expressing GAL and NPY make synaptic contact with each other in the medial preoptic area and in the magnocellular region of the paraventricular nucleus and the arcuate nucleus (Horvath et al., 1996) and GAL and NPY both dampen sympathetic nervous system function. In the Nucleus tractus solitarius (NTS), GAL and NPY receptors interact specifically with the same Angiotensin II receptor subtype, the AT1 receptor (Díaz-Cabiale et al., 2003, 2005). Moreover, we have shown recently that GAL was able to modify the cardiovascular response produced by a Y1 agonist on mean arterial pressure and heart rate (Díaz-Cabiale et al., 2006). The quantitative autoradiographical results suggested that this functional interaction could take place at the receptor level in the NTS through a GAL receptor/Y1receptor interaction (Díaz-Cabiale et al., 2006).

There is also some evidence of functional GAL and NPY interactions in feeding and energy metabolism (Hohmann et al., 2004; Leibowitz et al., 2005). It has also been recently shown that GAL mRNA is significantly upregulated in the hypothalamus of NPY−/− mice, suggesting that galanin could functionally compensate for the loss of NPY (Hohmann et al., 2004).

Based on these results the aim of this study was to evaluate by quantitative receptor autoradiography the interactions between NPY and GALR in the hypothalamus and the amygdala where both GALR and NPY Y1 and NPY Y2 receptors exist (Caberlotto et al., 1998; O'Donnell et al., 1999; Parker and Herzog, 1999; Sajdyk et al., 2002). The ability of the putative GAL receptor antagonist M35 to block the galanin action was also evaluated. In the present study we have also used combined intraventricular injections of GAL, NPY Y1 and/or Y2 agonists to evaluate their effect on food intake and to obtain a functional correlate to the receptor binding analysis. The expression of c-Fos immunoreactivity (c-Fos IR) in both regions after the intraventricular coinjections of GAL and the NPY Y1 agonist was also investigated to achieve a functional correlate at the cellular level.

2. Materials and methods

2.1. Animals

Male specific free pathogen Sprague–Dawley rats, weighing 225–250 g were obtained from Criffa (Barcelona, Spain) and they were maintained in a humidity and temperature controlled (20–22 °C) room under 12:12-h light/dark cycle (lights on 08:00 and off 20:00 h). They had free access to water and standard laboratory chow unless noted otherwise.

2.2. Quantitative receptor autoradiography

The procedure used here has been described elsewhere (Dumont et al., 1993). Briefly, the rats were killed by decapitation and the brains were rapidly removed and frozen under a CO_2 stream, and then kept at −80 °C until needed. Coronal sections (14 μm thick) were obtained by a cryostat at the bregma level −3.6 mm according to the atlas of Paxinos and Watson (1986) and thaw-mounted on gelatin-coated slides and immediately processed for binding studies.

The sections were preincubated for 60 min at room temperature in a Krebs-Ringer phosphate buffer (KRP) at pH 7.4 and then incubated for 120 min in a fresh preparation of KRP buffer supplemented with 0.1% BSA, 0.05% bacitracin, 25 pM NPY Y1 agonist [^{125}I]Leu^{31}, Pro^{34}PYY or NPY Y2 agonist [^{125}I]PYY 3–36 in the presence or absence of GAL at different concentrations. Non-specific binding was defined as the binding in the presence of NPY 1 μM. At the end of the incubation period, sections were washed four times (2 min each) in ice-cold KRP buffer, dipped in deionised water to remove salts, and rapidly dried under a stream of cold air. Sections were then placed in X-ray cassettes and apposed against Hyperfilms for 6 days.

The effects of GAL on NPY Y1 and NPY Y2 receptor binding were studied in two sets of experiments. In the first set of experiments four groups of animals ($n = 6$ in each group) were used to study the NPY-Y1 agonist binding in the presence of GAL at different concentrations (0.3, 1, 3 and 10 nM). Each concentration tested had its own paired control (same rat) of the same group of animals. A total of twelve brain sections of each rat were used for each concentration tested.

In the second set of experiments the effects of GAL (1 nM) on NPY Y1 agonist binding was evaluated in the presence of M35 (1 nM), a GAL receptor antagonist (Langel and Bartfai, 1998). Six animals were used in this experiment and each animal received four different treatments (control, GAL, M35 and GAL + M35). A total of 40 sections of each rat were obtained and used in this experiment.

Finally, using the same methodology the effects of GAL 1nM on NPY Y2 agonist binding was also evaluated.

2.3. Computer-assisted image analysis

The autoradiograms were analyzed as described previously (Díaz-Cabiale et al., 2000a), using a computer-assisted image analysis system. The computer software NIH was developed by Imaging Research (Brock University, St. Catharines, Ont., Canada). Measurements were made bilaterally using a sampling field of 2.4 ± 0.2 mm^2 (means ± S.E.M.) in the ARC and adjacent basomedial regions and of 6.3 ± 0.1 mm^2 in the entire amygdala. One observation per region and rat was obtained since the average of the measurements was calculated. Prefabricated ^{125}I-labeled polymer strips (Amersham Microscale, Amersham, Little Chalfont, UK) were used to convert the grey values into femtomole per milligram protein values (Díaz-Cabiale et al., 2000a).

2.4. Studies on food intake

This protocol has been used previously (Díaz-Cabiale et al., 2000b). Briefly, the rats were anesthetized with 1.5% halothane/98.5% air mixture (delivered at 1.41 liters/min) and stereotaxically implanted with a unilateral