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Short N-terminal galanin fragments are occurring naturally *in vivo*☆

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ABSTRACT

The galanin family currently consists of four peptides, namely galanin, galanin-message associated peptide, galanin-like peptide and alarin. Unlike galanin that signals through three different G protein-coupled receptors; GAL_1, GAL_2, and GAL_3, binding at its N-terminal end, the cognate receptors for other members of the galanin family are currently unknown. Research using short N-terminal galanin fragments generated either by enzymatic cleavage or solid-phase synthesis has revealed differences in their receptor binding properties exerting numerous biological effects distinct from galanin(1-29) itself. Our studies on tissue extracts derived from rat small intestine and bovine gut using chromatographic techniques and sensitive galanin(1-16)-specific radioimmunoassay revealed the presence of immunoreactive compounds reacting with antiserum against galanin(1-16) distributed in distinct elution volumes. These results suggested a possible presence of short N-terminal galanin fragments also *in vivo*. Moreover, employing immunoaffinity chromatography and reverse-phase high performance liquid chromatography (HPLC) followed by mass spectrometry allowed specific enrichment of these immunoreactive compounds from rat tissues and identification of their molecular structure. Indeed, our study revealed presence of several distinct short N-terminal galanin sequences in rat tissue. To prove their receptor binding, four of the identified sequences were synthetized, namely, galanin(1-13), galanin(1-16), galanin(1-20), galanin(6-20), and tested on coronal rat brain sections competing with ^{125}I-labeled galanin(1-29). Our autoradiographs confirmed that galanin(1-13), galanin(1-16), and galanin(1-20) comprehensively displaced ^{125}I-galanin(1-29) but galanin (6-20) did not. Here we show, for the first time, that short N-terminal galanin fragments occur naturally in rat tissues and that similar or identical galanin sequences can be present also in tissues of other species.

Biological significance: This study is first to provide an evidence of the presence of short N-terminal galanin fragments *in vivo* in a biological system and provides further foundations for the previous studies using synthetized short N-terminal galanin fragments.

1. Introduction

Galanin is a neuroendocrine peptide initially isolated from porcine intestine (Tatemoto et al., 1983). It has a widespread distribution throughout the central and peripheral nervous as well as endocrine systems exerting numerous physiological effects, including the control of food intake, learning, memory, nociception, anxiety, stress, depression, inflammation, and also exerting neurotrophic or neuroprotective action (Barreda-Gomez et al., 2014; Borbely et al., 2013; Kinney et al., 2003; Kormos and Gaszner, 2013; Kyrkouli et al., 1990; Lang and Kofler, 2011). The galanin family of peptides is encoded by two separate genes, galanin/galanin message associated peptide (GMAP) prepropeptide (*GAL*) and galanin-like peptide (*GALP*) (Evans et al., 1993; Ohtaki et al., 1999). The mature hormone is processed from a preproprecursor by cleavage of signal peptide and consequent proteolytic processing providing galanin(1-29) (30 amino acids in humans) and GMAP (Evans et al., 1993). Excepting humans, all other species have galanin C-terminally amidated (Evans and Shine, 1991; Lang et al., 2015; Tatemoto et al., 1983). The N-terminal region of galanin (residues 1-14) is highly conserved between species and is involved in receptor binding mediating its biological effects (Land et al., 1991). The function of the C-terminal sequence of galanin that shows higher degree of inter-species variability is not fully elucidated. It has been proposed to serve as protector against proteolytic attacks and may play a role for the recognition of galanin(1-29) by galanin receptors (Bedecs et al., 1995a; Rossowski et al., 1990). Thus variability of C-terminal sequence of galanin can contribute to receptor binding differences observed between species.

So far three G protein-coupled galanin receptors have been identified; GAL_1, GAL_2, and GAL_3. They trigger multiple signaling pathways, including stimulation of phospholipase C, particularly by GAL_2, or inhibition of cAMP/PKA by GAL_1 and GAL_3 receptors respectively

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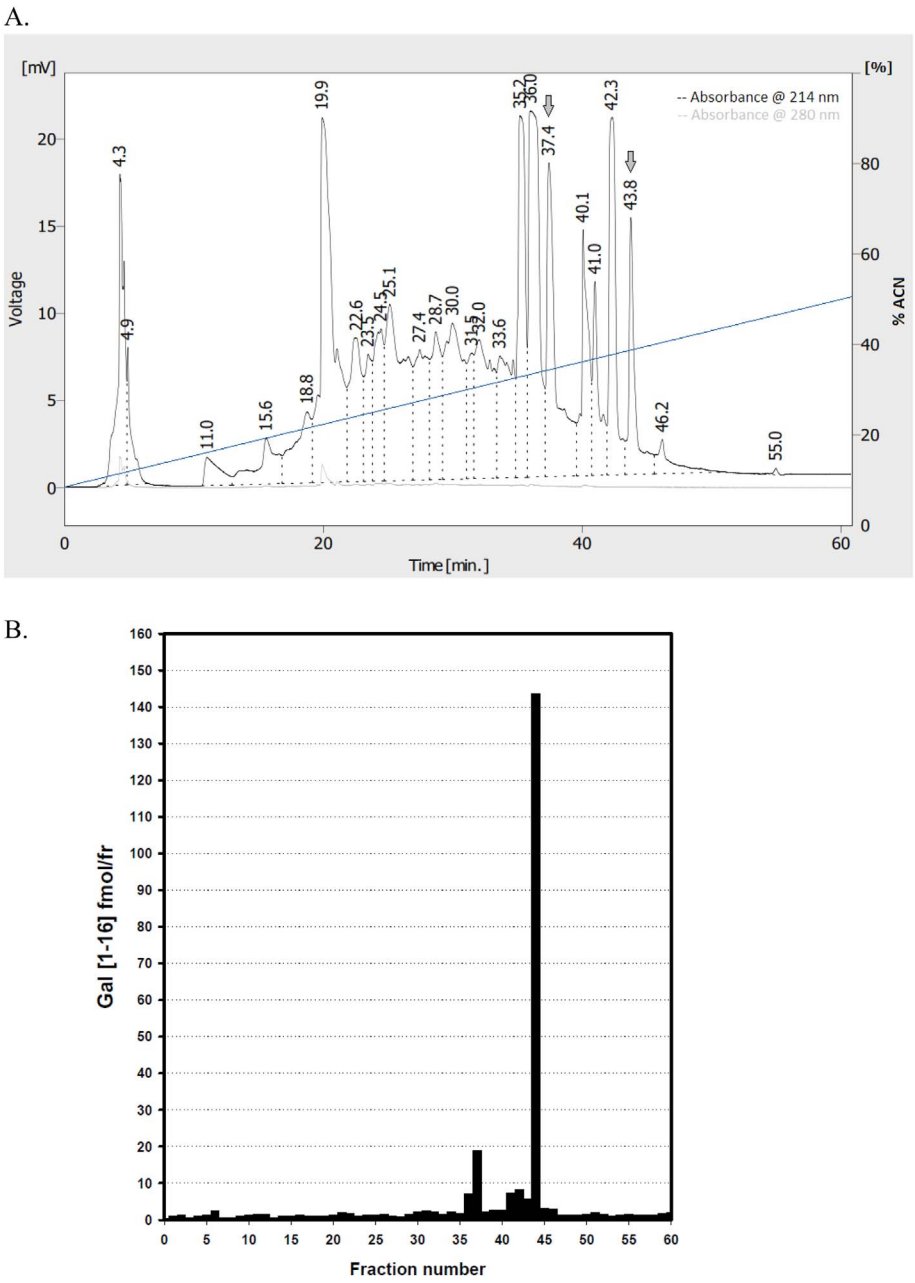

Fig. 1. Detection of immunoreactive compounds in peptide extract isolated from rat small intestine using rabbit antiserum K2 against galanin(1-16). A. The representative chromatogram from five independent experiments from HPLC separation of peptide extract from rat small intestine on C18 HPLC column. Gradient: 0–50% ACN with 0.1% TFA in 60 min (blue line). The arrows show the peaks with eluted immunoreactive material reacting with antiserum K2 in RIA. B. The representative results from five independent RIA analyses based on antiserum K2 of fractions from C18 HPLC separation of peptide extract from rat small intestine.

(Barreda-Gomez et al., 2014; Barreda-Gomez et al., 2005a; Lang et al., 2015; Rezaei et al., 2000; Webling et al., 2012). The distribution of galanin receptors is also tissue-specific. The presence of GAL_1 has been shown in the hypothalamus, ventral hippocampal formation (HiFo), thalamus, amygdala, brainstem and spinal cord (Barreda-Gomez et al., 2005b; Burgevin et al., 1995; Gustafson et al., 1996; Habert-Ortoli et al., 1994; Lu et al., 2005). GAL_2 is highly expressed in several brain regions, particularly in the hypothalamus, hippocampus, the anterior pituitary and lower levels also in the amygdala and regions of cortex (Fathi et al., 1997; Lu et al., 2005). GAL_3 shows the highest expression in the preoptic/hypothalamic area and the pituitary (Lu et al., 2005; Mennicken et al., 2002).

Besides galanin, GALP, a 60 amino acids long neuropeptide binds to the galanin receptors. The structure of GALP is related to galanin since it contains the conserved sequence galanin(1-13) at position 9-21 within its amino acid sequence (Lang et al., 2005; Ohtaki et al., 1999).

Interestingly, several studies have reported different binding properties and distinct functional effects induced by short N-terminal fragments of galanin and GALP that were generated only artificially either by enzymatic cleavage or a solid-phase synthesis until now (Crawley et al., 1990; Floren et al., 2000; Kinney et al., 1998; Land et al., 1991; Lang et al., 2005; Parker et al., 1995; Runesson et al., 2009; Smith et al., 1998; Todd et al., 2000; Wang et al., 1997; Wirz et al., 2005; Wynick et al., 1993). For example, full length galanin(1-29) has shown different distribution of binding sites in the rat brain compared to galanin(1-15) (Hedlund et al., 1992; Melander et al., 1988). Galanin (1-15) has more pronounced electrophysiological effects on pyramidal neurons in the dorsal HiFo than galanin(1-16) and full length galanin(1-29) (Xu et al., 1999). However, the last two showed stronger hyperpolarizing effect on neurons of locus coeruleus (Ma et al., 2001).