The galanin and galanin receptor subtypes, its regulatory role in the biological and pathological functions

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Summary

The multitalented neuropeptide galanin was first discovered 30 years ago but initially no biologic activity was found. Further research studies discovered the presence of galanin in the brain and some peripheral tissues, and galanin was identified as a modulator of neurotransmission in the central and peripheral nervous system. Over the last decade there were performed very intensive studies of the neuronal actions and also of nonneuronal actions of galanin. Other galanin family peptides have been described, namely galanin, galanin-like peptide, galanin-message associated peptide and alarin. The effect of these peptides is mediated through three galanin receptors subtypes, GalR1, GalR2 and GalR3 belonging to G protein coupled receptors, and signaling via multiple transduction pathways, including inhibition of cyclic AMP/protein kinase A (GalR1, GalR3) and stimulation of phospholipase C (GalR2). This also explains why one specific molecule of galanin can be responsible for different roles in different tissues. The present review summarizes the information currently available on the relationship between the galaninergic system and known pathological states. The research of novel galanin receptor specific agonists and antagonists is also very promising for its future role in pharmacological treatment. The galaninergic system is important target for current and future biomedical research.

Short title: Galaninergic System

Key words

Galanin Galanin receptor Neurotransmission Galaninergic system Regulatory function

1. Introduction

The galaninergic system is one of the specific signaling systems involved in neurotransmission and neuromodulation. The principal molecule of this system is the neuropeptide galanin. Galanin molecule was firstly described more than thirty years ago in the porcine intestine (Tatemoto *et al.* 1983) and this was promptly followed by major mapping studies. The distribution of galanin was reported in the widespread areas in the rat central nervous system and peripheral tissues and the presence of galanin was confirmed in many other species (Lang et al. 2015). Galanin is a principal signaling molecule of the galanin family, so called the galaninergic system, and today the galanin family consists of galanin, galanin-like peptide (GALP), alarin and galanin-message associated peptide (GMAP). The peptide GMAP is actually the precursor protein for the galanin molecule itself and the two possible products of different GALP gene: galanin-like peptide and its alternative form alarin (Webling *et al.* 2012).

Three different types of galanin receptors - galanin receptor 1 (GalR1), galanin receptor 2 (GalR2) and galanin receptor 3 (GalR3) have been described so far (Branchek *et al.* 2000). All of them are members of the G protein-coupled family and are involved in the biosignal transduction. The galaninergic receptors are widely expressed in the mammalian central nervous tissue, however, the presence of these receptors was also confirmed in many peripheral tissues, including the heart, gastrointestinal tract, connective tissue and skin (Lang *et al.* 2007). You can find more details in the part of this review concerning the galanin receptors.

The galaninergic system has been implicated in many biologically diverse functions including arousal/sleep regulation, nociception, learning and memory, depression, inflammation, feeding, pituitary hormone release, stress and anxiety, osmotic regulation and water intake, thermoregulation, reproduction and many others like different parts of metabolism (Fang *et al*. 2012). Also several human diseases, including Alzheimer's disease, epilepsy, diabetes mellitus and chronic pain are associated with the disturbance of the galaninergic system (Branchek *et al.* 2000; Lang et al. 2007). The research of galanin and its agonists and antagonists is also very promising for its future role in pharmacological treatment.

The galaninergic system is one of the crucial research topics of our laboratory. We are dealing with the regulatory issues of different neuropeptides (including galanin) in the central nervous system and various peripheral tissues (Klenerova *et al.* 2008, 2009). The behavioural part of the research activities mostly deals with the research of stress and anxiety using selected animal models (Klenerova *et al.* 2011, 2017). The research is performed on many different levels, including molecular genetic analysis, cell biology, immunofluorescence, immunohistochemistry and various animal tests. One of the main goals of this research is the possibility of future therapeutic use of synthetically prepared modulators of neurotransmission.

2. Galanin and galanin receptors

2.1. Galanin, structure and genes

The neuropeptide galanin is usually composed of 29 amino acids (see Fig. 1) (Lang *et al.* 2007). This applies e.g. for the rat, the porcine or the bovine galanin which all contain a

C-terminal amidated glycine. The human galanin molecule is an exception, since it has a C-terminal nonamidated serine and contains 30 amino acids in total (Schmidt *et al.* 1990; Branchek *et al.* 2000). Human galanin molecule is derived from a 123 amino acid long precursor pro-peptide along with a 59 or 60 amino acid peptide. This peptide is known as galanin message-associated peptide (GMAP) (Lang *et al.* 2007) and his biological function is mentioned in the next paragraph. The galanin precursor peptide is coded by a single gene, which includes 6 small exons (Kofler *et al.* 1996). Human galanin molecule is coded by the galanin gene which was localized by Evans and Shine (1991) to human chromosome 11q13.3.

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Fig. 1. Three dimensional structure of the galanin molecule.

The galanin molecule is usually composed of 29/30 amino acids, C-terminally amidated peptide. The isolated peptide was named galanin because of its N-terminal glycine and C-terminal alanine residue. Galanin is proteolytically processed from a 123- (porcine, human) or 124-(murine and others) amino acid precursor pro-peptide, "preprogalanin", encoded by a single-copy gene (Evans HF and Shine J, 1991).

2.2. Other neuropeptides of the galanin family

A few other proteins have been described to play a role in the galaninergic system signalization. The most important of these proteins is the galanin-like peptide (GALP). GALP is a 60 amino acid long neuropeptide, originally discovered as an endogenous ligand for galanin receptors in the tissues of the porcine hypothalamus and gastrointestinal tract (Ohtaki *et al.* 1999, Lawrence and Fraley, 2011). The GALP gene contains 6 exons in total and its primary structure is quite similar to the structure of the galanin gene (Kageyama *et*

al. 2005). The effects of GALP are mediated by galanin receptor subtypes. The fact that GALP shares homology only with 13 amino acids of the galanin sequence suggests that it might also interact with its own specific receptor. GALP appears to be involved in feeding regulation, energy balance control and reproduction (Kageyama *et al.* 2005).

The second member of this group of alternative protein ligands is the newly described peptide called alarin. Alarin is a peptide composed of 25 amino acids and was firstly described in the tissue of human neuroblastic tumours. Alarin is actually a result of the process of alternative splicing of the GALP gene mRNA molecule (Santic *et al.* 2006). The roles of alarin seem to be mediated by unknown specific alarin receptors, as alarin lacks homology to galanin, and is unable to compete with galanin for known galanin receptors. Little is known regarding the physiological role or pharmacological properties of alarin. To date, the only reported in vivo effects of alarin are to promote vasoconstriction and anti-oedema activity, the stimulation of food intake and the hypothalamo–pituitary–gonadal axis in rats (Boughton *et al*. 2010).

The original protein result of the galanin gene transcription is called galanin message-associated peptide (GMAP) (Lang *et al.* 2007). GMAP is not only the precursor molecule for the galanin creation, but the GMAP was also reported to have different biological functions, the potential role in nociception and immunomodulation was mentioned. However, the complex mechanisms of these biological interactions are not yet fully understood (Webling *et al.* 2012).

2.3. Galanin receptors

So far three main types of specific galanin receptors: GalR1, GalR2 and GalR3 have been described in the living organisms (Branchek *et al.* 2000). All three of them are different

members of the G protein-coupled receptor (GPCR) family. However, their molecular structure, and involvement in the biosignal transduction is different and therefore also their roles in various organs/tissue physiology are rich and pleiotropic (see Fig. 2). Potential association of any of previously discovered orphan receptors and the galaninergic system remain to be proved.

2.3.1. Galanin receptor type 1

The GalR1 molecule was described as the first known galanin receptor ever in the human melanoma cells (Habert-Ortoli 1994). Two years later the same receptor was also identified in the cells of the human gastrointestinal tract (Lorimer and Benya, 1996). Human GalR1 protein is coded by the GalR1 gene which is localized on human chromosome 18q23 (Nicholl *et al.* 1995). The human GalR1 protein itself is composed of 349 amino acids in total: for example the rat GalR1 homologue contains only 346 amino acids and has 92% similarity with human GalR1 (Branchek *et al.* 2000).

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Fig. 2.

The galanin receptors act through stimulation of different second messenger systems. The biological activity of GalR1 and GalR3 stimulation is linked to the activity of adenylate cyclase and cyclic AMP (cAMP) production and stimulation of GalR2 receptor to the phospholipase C (PLC) activity. Gal receptors affect various classes of intracellular G-proteins and influence multiple signal transduction pathways. Based on several presentations Klenerová and Hynie (Klenerova 2013, Klenerova and Hynie, 2013, 2015).

The biological activity of GalR1 stimulation is linked to the activity of adenylate cyclase and cAMP production. Another result of this receptor activation on the cellular level was the prolonged activation of extracellular signal–regulated kinases 1 and 2 (ERK1/2) via Gα inhibitory-subunits (Gi), which modified the expression of cyclic-dependent kinase inhibitor proteins p27Kip1 and p57Kip2, with consequent inhibition of cell proliferation. These data support the concept that GalR1 is a likely tumor suppressor in cancer cells (Kanazawa *et al.* 2007). Additionally, internalization of GalR1 receptor was observed in transfected Chinese hamster ovary cells (Wang *et al.* 1997). This could be a possible mechanism (or one of such mechanisms) for regulating the endogenous galanin signaling cascade.

2.3.2. Galanin receptor type 2

The second identified galanin receptor 2 was initially isolated in the form of an expressed cDNA from the tissue of rat (Smith *et al*. 1997). The later discovered human GalR2 receptor is composed of 387 amino acids - which is 15 more than the originally described rat GalR2 homologue (Bloomquist *et al*. 1998). The human GalR2 is coded by the GaLR2 gene which was mapped to human chromosome 17q25.3 (Fathi *et al.* 1997, Fathi *et al.* 1998).

Also the stimulation of GalR2 receptor affects various classes of intracellular G-proteins and influences multiple signal transduction pathways. The most commonly reported – and probably the most important, pathway mediates the activation of the phospholipase C (PLC) which later increases the hydrolysis of inositol phosphate (Fathi *et al.* 1997). Also other interactions of GalR2 with Gi proteins were already reported. The GalR2 stimulation also leads to the inhibition of adenylate cyclase via Gi proteins, which is similar to the

process of GalR1 stimulation (Wang *et al*. 1997). GalR2 receptor signalizing pathways was also reported to interact with Go-type G-protein which activates mitogen activated protein kinase (MAPK) (Hawes *et al.* 2006). It was also found, that both GalR1 and GalR2 activation inhibits cyclic AMP-responsive element-binding (CREB) protein (Badie-Mahdavi *et al*. 2005). Another interesting result of the GalR2 stimulation is the supposed association with neuronal survival process, which is probably regulated by the AKT signaling pathway [AKT (also referred to as PKB or Rac) plays a critical role in controlling survival and apoptosis] (Ding *et al.* 2006).

2.3.3. Galanin receptor type 3

Galanin receptor type 3 was described as the last known of the galanin receptors. It was initially cloned from the rat tissue and described by Smith et al. (1998). The rat GalR3 cDNA codes for a protein composed of 370 amino acids, the similarity between rat GalR3 and GalR1 molecules is 36% and between GalR3 and GalR2 molecules is 55% (Lee *et al.* 1999). The human GalR3 was firstly cloned by Kolakowski *et al.* (1998) using the human genomic DNA library according to the sequence similarity between GalR1 and GalR2 genes. Using the high-resolution fluorescent in situ hybridization (FISH) the human GalR3 gene was mapped to human chromosome 22q12.2–13.1. Human GalR3 protein contains 368 amino acids in total and has 90 % similarity to rat GalR3 protein amino acid sequence (Kolakowski *et al.* 1998). The biological effects of the GalR3 signalization are still not fully understood and are subject to further research. In general, the pharmacology effects of the GalR3 combine the effects of GalR1 and GalR2 signalization (Branchek *et al.* 2000). GalR3 probably interacts with Gi/o-type G-protein in order to stimulate the activation of an inward K+ influx (Kolakowski *et al.* 1998).

2.3.4. Non-peptide agonists of galanin receptors

So far we have mentioned the peptide agonists of the galanin receptors. Most galanin receptor ligands available today are peptides, vulnerable to enzymatic degradation and unable to cross the blood–brain barrier. However, there is also a small group of non-peptide agonists of galanin receptors (Bartfait *et al*. 2004). So far two different non-peptide general agonists– called galmic and galnon (Fig. 3) - were described. Their effect seems to be limited because of their multiple interaction sites and more research is needed to determine whether they have any important biological role or not (Webling *et al.* 2012). In addition to these two above agonists, there have been described two competitive antagonists of GalR3, SNAP 37889 (IUPAC Name 1-phenyl-3-[3-(trifluoromethyl)phenyl]iminoindol-2-one) and its analog SNAP 398299 (IUPAC Name 1-[3-(2-pyrrolidin-1-ylethoxy)phenyl]-3-[3-(trifluoromethyl)phenyl]iminoindol-2-one which is more water-soluble. These selective antagonists may represent an additional class of therapeutic agents for the treatment of anxiety, depression and cognitive dysfunction (Swanson *et al.* 2005) and the potentional treatment of alcohol use and eating disorders (Ash *et al*. 2011).

GalR2 positive allosteric modulator CYM2503 (9H-fluoren-9-yl)methyl((S)-1(((S)-6(tert-butoxycarbonyl) amino-1-((4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)amino)- 1-oxohexan-2-yl)amino])-3-cyclohexyl-1-oxopropan-2- yl) carbamate) have been shown to potentiate the anticonvulsant activity of endogenous galanin in mouse seizure models (Lu *et al*, 2010) and series of 2,4,6- triaminopyrimidines (Lang *et al.* 2015). However, the potential therapeutical use of these ligands is currently limited. More research will be needed.

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Fig. 3. Structure of galnon.

A low molecular weight galanin receptor agonist galnon, 7-((9-fluorenylmethoxycarbonyl)cyclohexylalanyllysyl)amino-4-methylcoumarin (IUPAC name 9H-fluoren-9-ylmethyl N-[(2*S*)-1-([(2*S*)-6-amino-1-[(4-methyl-2-oxochromen-7- yl)amino]-1-oxohexan-2-yl]amino)-3-cyclohexyl-1-oxopropan-2-yl]carbamate) was discovered by application of a combinatorial library approach. Galnon possesses agonist properties *in vitro* and *in vivo* and strong anticonvulsant properties *in vivo* (Saar *et al.* 2012).

3. Galanin receptors distribution and actions under physiological conditions and in the pathology

The distribution of galanin receptors of all three types were described in various mammalian organs and tissues and recently the aspects of galanin actions have been frequently studied in many areas by several laboratories. Galanin is associated with a lot of central and peripheral receptor-mediated actions including feeding, pain and anterior pituitary hormone regulation, the energy and osmotic homeostasis (Landry *et al.* 2000), reproduction (Gundlach 2002), and cognition (Kinney *et al.* 2002).

The diverse actions of galanin make this peptidergic system an attractive target for modulating obesity, cognitive deficits, analgesia and pituitary dysfunction etc. In the next part of this review we describe the data selected tissues and organs.

3.1. Distribution of the GalRs in central nervous system

GalR1 and GalR2 are widely expressed in the mammalian central nervous system (CNS) (Lang *et al.* 2007). GalR1 was found in the thalamus, the hypothalamus, the amygdala, the olfactory structures, the pons, the medulla and the spinal cord (Hohmann *et al.* 2003). The mRNA of GalR2 is broadly transcribed in the CNS as well, mainly in hippocampus, gyrus dentatus and nucleus arcuatus, supraopticus and corpora mammillaria, spinal trigeminal tract and in the dorsal vagal complex (Burazin *et al.* 2000). The overall expression of the GalR3

in the CNS is low, with the only exception of the hypothalamus (Mennicken *et al.* 2002). Another roles of the galaninergic system signalization in the CNS include the hypothalamic regulation of sleep and arousal processes (McGinty and Szymusiak 2003).

3.1.1. Behavioral processes and anxiety

As a neurotransmitter - galanin was described to affect numerous neurophysiological and behavioural processes. The involvement of galanin in regulation of anxiety and stress is probably one of its most important roles. Numerous studies have reported that galanin administration in the CNS produced an anxiolytic effect (e.g. Bailey *et al.* 2007; Klenerova *et al.* 2011). For example, the general anxiolytic effect of galanin administered bilaterally into the area of the central amygdala was experimentally successfully blocked by the galanin antagonist M40 in laboratory rats (Khoshbouei *et al*. 2002). This anxiolytic effect of galanin was also confirmed in the transgenic (and galanin over-expressing) mice model experiment, where the targeted induction of anxiety with yohimbine substance was impaired. One possible explanation are the reduced levels of noradrenalin and glutamate in the amygdala and the hippocampus (Holmes *et al.* 2002). Another study has shown that the selective GalR3 antagonists have produced anxiolytic effects in rats, mice and guinea pigs, possibly by attenuating the inhibitory effect of galanin (Swanson *et al.* 2005).

Substantial evidence has been obtained that implicates galanin signaling in reward, addictive processes and eating disorders. The selective galanin antagonists are the potentional drugs of treatment of alcohol use and eating disorders (Ash *et al*. 2011).

Neural circuits that affect both stress-related and feeding behavior have been shown to be involved in drug reward behavior and substance abuse and addictionm and are known to be

modulated by galanin (Picciotto *et al.* 2010). GalRs may be attractive targets for the development of novel therapeutics for drug addiction.

3.1.2. Regulatory role of galaninergic system in learning and memory

The role of galaninergic system in learning and memory is also well documented in the literature (Lang *et al.* 2007). It has been shown, that galanin can produce deficits in behavioural performance. Central injection of galanin impaired the process of memory formation and impaired the ability of laboratory rats to perform learning tasks such as the classical Morris water maze (Sundstrom *et al.* 1988). This effect was further studied in different transgenic galanin-overexpressing mice strains. These mice had significant deficits in learning and memory tests (including spatial navigation and olfactory tests) and also in emotional memories which depend on glutamate release from the hippocampus (Mazarati *et al.* 2000). Nevertheless, the sensory and motor abilities, as well as the levels of extracellular noradrenaline and serotonin in the hippocampus were normal (Crawley *et al.* 2002).

3.2. Distribution of the GalRs in peripheral nervous system

The galaninergic neurotransmission system plays an important role also in the peripheral nervous system (Lang *et al*. 2007) where galanin was reported to be involved in the regenerative process following the nerve injury (Ahren *et al.* 2006). The expression of galanin and its receptors is also extensively elevated in the areas of nerve injury (Lang *et al.* 2007). This phenomenon was described by several authors in different animal models – including models of motor and sensory nerve axotomy or crush (Burazin and Gundlach 1998), central nerve transection (Palkovits and Horvath, 1994) or a focal cerebral ischemia (Raghavendra Rao *et al.* 2002).

3.2.1. Regulatory role of galaninergic system in nociception and pain

Galanin mediated neurotransmission also affects several processes in the peripheral nervous system, mainly in the process of nociception and pain in general. Galanin can act both as an inhibitory and excitatory mediator in the nociception process, where the final type of the outcome depends on several variables, like the chronicity of the pain stimulus, the nature (type) of the stimulus or the concentration of galanin in the peripheral nerve area (Liu and Hökfelt, 2000; Flatters *et al.* 2003). It has been shown, that the adults of galanin knock-out mice experimental model have greater sensitivity to acute pain, while galanin overexpressing transgenic mice have reduced responses to acute thermal pain (Blakeman *et al.* 2001; Holmes *et al.* 2003).

3.3. Galanin receptors in heart and central cardiovascular control

Some evidence suggests that Gal may play role in central cardiovascular control, probably acting through different receptor subtypes, but exact mechanism of the action in the heart is not fully elucidated. In our study we have shown the presence of Gal and GALP in left and right atria and ventriculi (Klenerova *et al.* 2014), Gal mostly on plasma membranes of cardiomyocytes and GALP at intercalated discs. To detect intercalated disc we used immunoflurescent detection of Connexin 40. We determined of GalR subtypes in all heart compartments with the largest expression for GalR2 in plasma membranes and nuclei and the lowest for GalR3 in plasma membranes. GalR1 is expressed most intensively in nuclei and also in intercalated discs, in membranes was expression small. The results show different distribution and density of Gal receptors in the heart areas. These findings would suggest that the efficacy of Gal and GALP to induce an effective coupling of its receptors to G proteins could be different depending on the heart localization. The galanin infusion in

humans induced an increase in heart rate (dose dependent) and blood pressure. The galanin antagonist M40 blocks the hypertension induced by Gal (1–15), but not the cardiovascular effects induced by Gal. Although both GalR1 and GalR3 are present in cardiac ganglia, it was suggested that GalR1-activation reduces acetylcholine release from atrial cholinergic neurons (see Lang *et al.* 2015).

4.3. Gastrointestinal tract and pancreas

In the gastrointestinal tract galanin modulates numerous activities, including regulation of transmitter release, secretion and motility. The expression of GalR1 and GalR2 mRNAs were detected in all segments with the highest levels in the large intestine and stomach, respectively. GalR3 mRNA levels were quite low and mostly confined to the colon. The differential distribution of galanin receptors supports the concept that the different effects of galanin suggests the activation of multiple receptor subtypes (Anselmi *et* al. 2005).

Galanin itself was isolated from pancreas islet cells and pancreatic nerves (McDonald *et al.* 1992). β-cells contain mRNA to encode all three galanin receptor subtypes. The role of galanin signalization in pancreas is very complex. Both galanin knockout and GalR1 gene-knockout mice models have shown impaired glucose metabolism (Ahren *et al.* 2004). In patients with diabetes mellitus type II there was a strong positive correlation of the galanin and glucose plasma levels in the fasting state (Legakis *et al.* 2005). Additionally, the study on special mice galanin gene knockout model proved that the galanin neurotransmission also takes part in the pathogenesis of acute pancreatitis (Bhandari *et al.* 2010).

3.5. Bone and and joint tissue cells

Galanin and the expression of GalRs were detected in the bone (McDonald *et al.* 2003; Lang *et al.* 2007). Galanin can facilitate bone formation and enhance repair efficacy after osseous injury, the injection of galanin into the calvaria increased the number and the activity of osteoblasts in mice (McDonald *et al.,* 2007). After bone fracture galanin receptor expression galanin concentration were increased in osteoblast-like cells (McDonald *et al*. 2003). Systemic administration of galnon, a galanin receptor agonist, may stimulate osteoclastic activity, increase resorptive speed of the osteoclastic bone (McGowan *et al.* 2014).

2.3. Regulatory role of galaninergic system in other systems

The galanin was found even in the most peripheral of the tissues and surprisingly, the galaninergic receptors themselves are very scarce in the skin (Kofler *et* al. 1996). The role of galanin in the skin is therefore still not very well understood. Galanin probably influences the skin immunity, a significant increase of galanin was found in the inflamed skin. Furthermore, the GMAP peptide has some antimicrobial activity against *Candida albicans* and alarin has similar activity against *Escherichia coli* (Lang *et al*. 2015).

Galanin has also probably a significant role in the ontogenetic development (Lang *et al.* 2015). Various studies reported a dynamic expression of galanin and its receptors in the stem cells. This phenomenon was first described in the mice stem cells (Anisimov *et al*. 2002) and later also in human stem cells and even in cancer cells (Assou *et al.* 2007). The overall expression of galanin during embryonic development decreases steadily, which means, that the galanin signalization itself may be linked to the potential of the stem cells (Bhattacharya *et al.* 2005).

3. The role of galanin in human diseases

The role of galanin and the galaninergic system itself was already discovered in pathophysiology of several human diseases, namely Alzheimer´s disease, diabetes and epilepsy. The brain in individuals with Alzheimer's disease is characterized by various neurofibrillary tangles and neural plaques. It was described that this degeneration of the neuronal collateral network leads to extensive upregulation of galanin expression in the remaining neurons mainly in the basal forebrain (Chan-Palay 1988). However, it is still not clear whether this upregulation is actually a contributing factor to the disease, mere result of the nerve injury, or maybe even a compensatory action to maintain cholinergic transmission (Lang *et al.* 2007). Epileptic seizures have been shown to rapidly deplete galanin. Galanin role in epilepsy and seizures is again very complex and not yet fully understood. It was shown that the activation of GalR1 is anticonvulsant, while the GalR2 activation is proconvulsant. Spontaneous seizures have been already described in the GalR1 knockout mice experimental model, whereas the seizures are absent at all in the galanin knockout mice model (Mazarati *et al*. 2000; Fetissov *et al.* 2003).

Another newly described role of galanin signalization concerns the oncology issue. Expression of galanin has been identified in several types of human tumours, namely pheochromocytoma, pituitary adenoma, neuroblastic tumours, gastrointestinal cancer, squamous cell carcinoma, brain tumours, melanoma, breast cancer and embryonal carcinoma (Rauch and Kofler, 2010). The more detailed role of the galaninergic signalization in colorectal cancer invasiveness has been described recently (Nagayoshi et al. 2015). The co-expression of galanin and its receptors with variety neuropeptides supports a role for galanin in tumor cell pathology via autocrine/paracrine mechanisms (Berger *et al.* 2004).

As has been already mentioned, neuropeptide expression (including expression of galanin peptide and alarin) has been proven in various tumours. It has been shown, that the expression levels correlate with the level of differentiation or aggressiveness of the tumour. It is supposed that neuropeptides may show pro- or antiproliferative activity on cancer cells and so a therapeutic use could be possible (Lang *et al.* 2015).

Possible therapeutic effect of triple treatment with octreotide, galanin and serotonin on colorectal cancer has been studied. The triple therapy was shown effective in animal model in the treatment of human colon cancer xenografts. The volume and weight of rat as well as human colon carcinoma in xenografts has been reduced – by necrosis, induced apoptosis and reduced proliferation and expression of epidermal growth factor receptor (EGFR) of cancer cells. However, there will be need of further clinical trials to confirm benefit of this usage (El-Salhy 2005). The galaninergic system may also play role in other human diseases, namely alcoholism, chronical pain, bowel and skin inflammatory diseases (Lang *et al.* 2007).

4. Potential therapeutic usage of galanin family

Galanin has numerous and pleiotropic biological roles including various involvements in human diseases as described above. Galanin receptors agonists and antagonists are therefore promising substances for future pharmacologic treatment, namely for example as potential anticonvulsants (Saar *et al.* 2002, Webling *et al.* 2012). The research on this field is very broad; however there are still many problems in designing potential human drugs that we need to overcome first. It is not only the problem of the therapeutic usage of the galaninergic system modulation; this issue extends to the neurotransmitter system in general (Lang et al. 2015). The issues include relative heterogeneity of neurotransmitter molecules

among species which is considerable complication for drug trials. Moreover, the molecules of different neurotransmitters are too big to pass the hematoencephalic barrier, so the central effect of a such drug shall be considerably limited. Additionally, the general effect of neurotransmitters agonists can be potentially harmful, since such a drug will stimulate all receptors at once. On the other, antagonists can primarily affect the pathologically up-regulated areas of particular neurotransmission first (Lang et al. 2015). In addition, future research on galanin pathophysiology will be best advanced by the application of novel experimental tools and approaches.

5. Conclusion

The galaninergic neurotransmission system is one of the newly described signaling systems. The principal signaling molecule is the neuropeptide galanin and 3 different types of cell surface galanin receptors have been described in different types of organs and tissues so far. Galanin receptor stimulation starts the G-protein mediated cell signalization cascades and has pleiotropic effect both on the level of cell, tissue and organism itself. Several human diseases, including Alzheimer's disease, diabetes mellitus and epilepsy, have been already reported to be associated with disturbances in the galaninergic system signalling. Therefore galanin and its receptors present a promising target for pharmacology research and future treatment possibilities. This review will attempt to summarize the significant body of in vitro and in vivo studies conducted so far, concerning the effects of galaninergic system.

Conflict of Interest

There is no conflict of interest

Acknowledgement

This study was supported by the grants PROGRES Q25/LF1 and SVV–2017– 260 377 from Charles University, Prague, Czech Republic.

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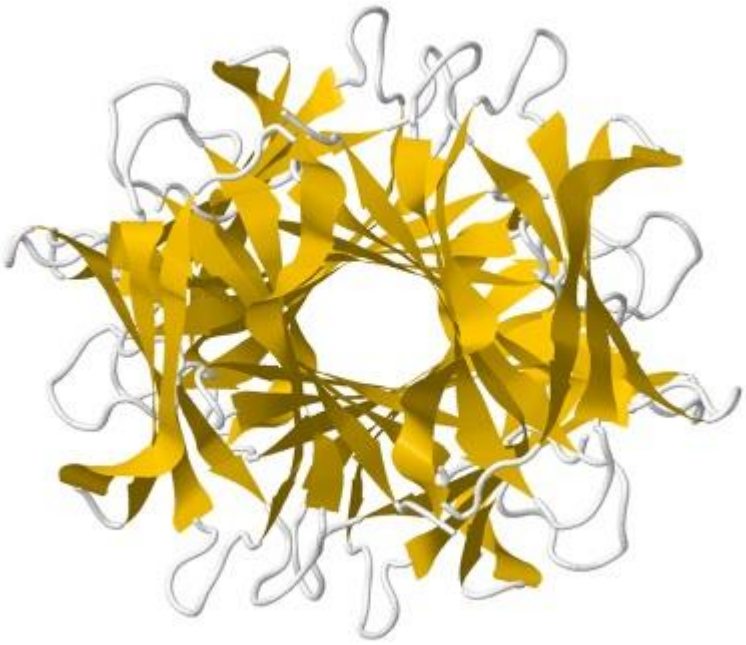

Fig. 1. Three dimensional structure of the galanin molecule.

The galanin molecule is usually composed of 29/30 amino acids, C-terminally amidated peptide. The isolated peptide was named galanin because of its N-terminal glycine and C-terminal alanine residue. Galanin is proteolytically processed from a 123- (porcine, human) or 124-(murine and others) amino acid precursor pro-peptide, "preprogalanin", encoded by a single-copy gene (**Evans HF and Shine J,** 1991).

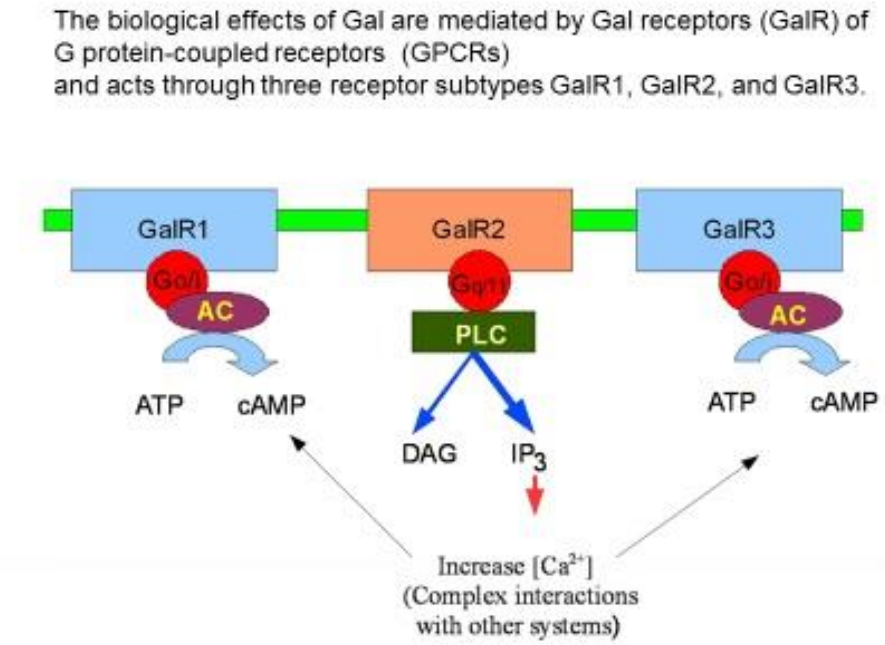

Fig. 2.

The galanin receptors act through stimulation of different second messenger systems. The biological activity of GalR1 and GalR3 stimulation is linked to the activity of adenylate cyclase and cyclic AMP (cAMP) production and stimulation of GalR2 receptor to the phospholipase C (PLC) activity. Gal receptors affect various classes of intracellular G-proteins and influence multiple signal transduction pathways. Based on several presentations Klenerová and Hynie (Klenerova 2013, Klenerova and Hynie, 2013, 2015).

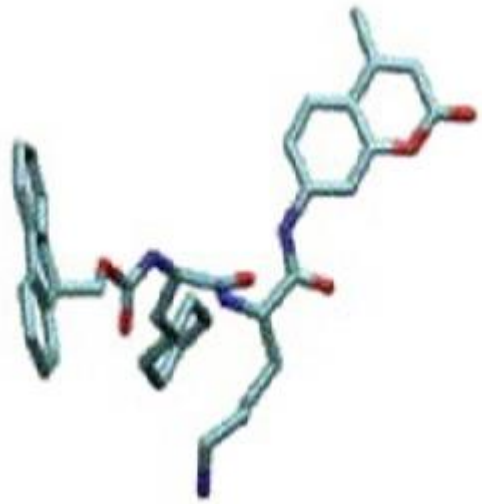

Fig. 3. Structure of galnon.

A low molecular weight galanin receptor agonist galnon, 7-((9-fluorenylmethoxycarbonyl)cyclohexylalanyllysyl)amino-4-methylcoumarin (IUPAC name 9H-fluoren-9-ylmethyl N-[(2*S*)-1-([(2*S*)-6-amino-1-[(4-methyl-2-oxochromen-7- yl)amino]-1-oxohexan-2-yl]amino)-3-cyclohexyl-1-oxopropan-2-yl]carbamate) was discovered by application of a combinatorial library approach. Galnon possesses agonist properties *in vitro* and *in vivo* and strong anticonvulsant properties *in vivo* (Saar *et al.* 2012).