THE NEUROPEPTIDES GALANIN AND GALANIN (1-15) IN DEPRESSION-LIKE BEHAVIOURS

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Abstract

Galanin is a 29 amino acid neuropeptide widely distributed in neurons within the central nervous system. Galanin exerts its biological activities through three different G protein-receptors and participates in a number of functions, including mood regulation. Not only Galanin but also Galanin N-terminal fragments like Galanin(1-15) are active at the central level. In this work, we review the latest findings in studies on Galanin and Galanin(1-15) in depression-related behaviours. Our focus is on animal models for depression, and we pay some attention to research data obtained in human studies. Since Serotonin (5-HT), especially through 5-HT1A, and Galanin receptors interact at both pre-and postsynaptic level, the development of drugs targeting potential GAL1-GAL2-5-HT1A heteroreceptor complexes linked to the raphe-hippocampal 5-HT neurons may represent new treatment strategies in depression.

Key words: depression, galanin
Introduction

Mood disorders, including depression and anxiety, are among the most prevalent mental illnesses with high socioeconomic impact (Gelenberg, 2010; Wittchen et al., 2011). Although the underlying mechanisms have not yet been clearly defined in the last decade the importance of the role of neuropeptides, including Galanin (GAL), and/or their receptors in the treatment of stress-related mood disorders is becoming increasingly apparent (Kormos et al., 2013).

GAL is a 29 amino acid neuropeptide (Tatemoto et al., 1983) widely distributed in neurons within the central nervous system (CNS) including raphe nuclei, cerebral cortex and hippocampus (Jacobowitz et al., 2004) among other nuclei involved in mood disorders. Three GAL receptor subtypes (GAL$_{1-3}$ receptors) have been described (Branchek et al., 2000; Mitsukawa et al., 2008). GAL$_{1}$ and GAL$_{2}$ receptors, in particular, are found in many regions of the CNS as demonstrated with in situ hybridization, radioligand binding, and immunohistochemical studies (Jacobowitz et al., 2004) and have all high affinity for GAL. GAL$_{1}$ and GAL$_{3}$ receptors mainly activate inhibitory G proteins Gi/Go, while GAL$_{2}$ receptor primarily couples to G$q$/G11 to mediate excitatory signaling (Branchek et al., 2000).

The three GAL receptors participate in a number of functions in the CNS including neuroendocrine levels, mood regulation, pain control, cardiovascular functions, addiction and food intake (Lu et al., 2007; Kuteeva et al., 2008; Mitsukawa et al., 2008; Diaz-Cabiale et al., 2010; Juhasz et al., 2014; Lang et al., 2015)

Not only GAL but also the GAL fragments like GAL N-terminal fragment 1-15 [GAL(1-15)] are active in the CNS (Hedlund et al., 1996; Diaz-Cabiale et al., 2005; Diaz-Cabiale et al., 2010; Millon et al., 2015; Millon et al., 2016). Structure-activity studies described that in the brain, N-terminal fragments of GAL are biologically active, whereas C-terminal fragments are inactive (Diaz-Cabiale et al., 1998) and they act as agonists in several physiological functions (Diaz-Cabiale et al., 1998; Diaz-Cabiale et al., 2005), suggesting a receptor-
mediated action. Although the three GAL receptors subtypes show higher affinity for GAL than for GAL(1-15) (Branchek et al., 1998), the presence of specific binding sites for this GAL fragment in the CNS also in areas lacking $[^{125}\text{I}]$-GAL binding sites indicated a powerful role of GAL fragments, especially in the dorsal hippocampus, neocortex and striatum (Hedlund et al., 1992). Only GAL(1-15), but not GAL, can antagonically modulate the serotonin 5-HT1A receptors in the dorsal hippocampus, and this effect was blocked by the GAL receptor antagonist M35 (Hedlund et al., 1994). In the ventral limbic cortex, N-terminal GAL fragments can more stringy and more potently reduce posjunctionals 5-HT1A receptor recognition than GAL, where high-affinity GAL receptors also exist (Diaz-Cabiale et al., 2000; Diaz-Cabiale et al., 2010). The formation of GAL$_1$/GAL$_2$ heteroreceptors highly specific for GAL fragments will explain the different effects between GAL and GAL(1-15) (Fuxe et al., 2008; Fuxe et al., 2012; Millon et al., 2015).

In this work, we review the latest findings in studies on GAL and GAL(1-15) in depression-related behaviours. Our focus is on animal models for depression, and we pay some attention to research data obtained in human studies.

**GALANIN IN DEPRESSION**

A major impediment in depression research is the lack of validated animals’ models. Animals not only lack consciousness of self, self-reflection and consideration of others but also hallmarks of the disorder such as depressed mood, low self esteem or suicidal intent are hardly accessible in non-human (Deussing, 2006). However, depression, as other mental disorders, constitutes of intermediate or so-called endophenotypes that can be reproduced independently and evaluated in animals, including physiological, endocrinological and neuroanatomical alterations as well as behavioural traits. Numerous behavioural paradigms have been established to elucidate face and construct validity of depression models, including antidepressant-screening test (Deussing, 2006).
The forced swimming test (FST), the most used paradigm to assess depression- and antidepressant-like behaviour, also known as the ‘behavioural despair’ test, was developed by Porsolt et al. (Porsolt et al., 1977) as a rodent model for predicting the clinical efficacy of antidepressant drugs (Bogdanova et al., 2013). The FST takes advantage of the observation that rodents, following initial escape-oriented movements, rapidly adopt a characteristic immobile posture in an inescapable cylinder filled with water. In this paradigm, immobility is interpreted as a passive stress-coping strategy or depression-like behaviour (behavioural despair) (Deussing, 2006).

Fuxe’s Laboratory, around nineties, reported the first evidence of the involvement of GAL in depression. In animal models, GAL administered into the lateral ventricle (i.c.v) reduced serotonin (5-HT) metabolism in ventral limbic cortex, hippocampal formation, and fronto-parietal cortex probably via direct inhibitory actions on dorsal raphe (DR) 5-HT nerve cells reducing their firing rates (Fuxe et al., 1988b). These results suggested based on the 5-HT hypothesis of depression that GAL, may contribute to depression by reducing firing in the ascending 5-HT neurons (Fuxe et al., 1988b).

GAL in the rat ventral tegmental area (VTA) induced in a dose-dependent manner an increased in immobility time in the FST (Weiss et al., 1998), an effect that was blocked by the coinfusion of the GAL receptor antagonist M35. I.c.v. GAL also resulted in an increase of immobility time in FST confirming that GAL may induce depression-like phenotype (Kuteeva et al., 2007). In the rat, intraperitoneal injection of galnon or galmic, GAL receptor agonists, reduced immobility time in the rat FST, indicating an antidepressant-like effect of systemically GAL receptor agonists (Bartfai et al., 2004; Lu et al., 2005) (Table 1).

The role of GAL in depression-like behaviour has been analysed in genetically modified mice. Mice over-expressing GAL under the platelet-derived growth factor-B promoter (GalOE-P) displayed an increased immobility in the FST, suggesting a depression-like behaviour (Kuteeva et al., 2005; Kuteeva et al., 2008). However, Holmes et al. (2005) (Holmes et al., 2005) failed to show such
alterations in GAL over-expressing mice under the dopamine-β-hydroxylase promoter (GalOE-D) and GAL₁ receptor knockout (KO) mice (Table 1). Importantly, GalOE-P but not GalOE-D mice showed an augmentation of hippocampal noradrenaline (NA) and 5-HT release probably indicating that the mechanisms underlying the increase of immobility in GalOE-P can be related to modulation of NA and 5-HT transmission.

In a genetic rat model of depression, the Flinder sensitive line, which displays a high immobility in FST, an up-regulation of the GAL receptor binding sites, is found in the DR, and reduces GAL fiber density in the hippocampus and hypothalamus. The results indicate that enhancement of GAL receptors function in the DR rich in 5-HT neurons could be a mechanism involved in the production of depressive-like activity in this animal model of depression (Bellido et al., 2002).

**Galanin Receptors in depression**

Several studies suggest that GAL₁/GAL₃ receptors can contribute to the pro-depressive effect of GAL. i.c.v infusion of GAL₁ receptor agonist (M617) elevated the immobility time in the FST, similar to GAL itself. Moreover, the depression-like behavior in rats exposed to chronic mild stress was related to an elevated expression of GAL₁ receptor in the ventral periaqueductal gray (Wang et al., 2016). Furthermore, administration of GAL₃ receptor antagonists to rat and mice produced antidepressant-like effects in both FST and the tail suspension test (TST) (Swanson et al., 2005; Barr et al., 2006) (Table 1). The TST is another prominent test, which relies on similar assumptions and interpretations as the FST (Steru et al., 1985). In the TST, animals are suspended by their tails for a defined period of time, and their immobility is assessed (Deussing, 2006).

Stimulation of the GAL₂ receptor, in contrast, produces antidepressant-like effects. The i.c.v. GAL₂ receptor agonist AR-M1896 decreased the immobility time in the FST, while the GAL₂ receptor antagonist (M871) elevated the
immobility time in the FST (Kuteeva et al., 2008). Recent studies shown that systemically active GAL₂ receptor agonist reduced the immobility time in the FST and in the TST, confirming the potential antidepressant effect of GAL₂ receptor (Saar et al., 2013b; Saar et al., 2013a) (Table 1).

GAL₁ receptor (Holmes et al., 2003), GAL₃ receptor (Brunner et al., 2014) and GAL₂ receptor (Bailey et al., 2007) null mutant displayed an anxiogenic-like phenotype in the elevated plus maze; nevertheless, neither GAL₁ receptor knockout (Holmes et al., 2005) nor GAL₂ receptor knock out (Gottsch et al., 2005) mice differed from their wild-type littermate in the TST. On the contrary, transgenic mouse over-expressing GAL₂ receptor-enhanced, green fluorescent protein constructed under the platelet-derived growth factor-B promotor decreased levels of immobility in the FST (Le Maitre et al., 2011). GAL₃ receptor knock out mice displayed a trend toward decreased immobility time in the TST, indicating a reduction in depression-like behaviour (Brunner et al., 2014) (Table 1).

All these results indicate that stimulation of GAL₂ receptor may be responsible for antidepressant-like effects, whereas GAL₁ and GAL₃ receptors seem to contribute to the prodepressive effects of GAL.

In addition, both chronic treatments with the selective serotonin reuptake inhibitor (SSRI) fluoxetine and electroconvulsive treatment increase GAL mRNA levels in the DR of the rat, accompanied by an increase in GAL₂ receptor, but not GAL₁ receptor, binding sites in this monoaminergic nuclei. Moreover, co-administration of GAL receptors antagonist M40 blocked the behavioural effect of fluoxetine in the FST, suggesting that the antidepressant action of fluoxetine can, at least partially, be related to an increase in GAL-mediated transmission (Lu et al., 2005; Kuteeva et al., 2008). Yamada and co-workers found that after four-week treatment with sertraline, a SSRI, GAL but not GAL₂ receptor was upregulated in the ventral dentate gyrus (Yamada et al., 2013). Moreover, the chronic administration of desipramine, a tricyclic antidepressant, paroxetine, a SSRI, and phenelzine, a monoamine oxidase inhibitor, affected mRNA for GAL and GAL receptors (Rovin et al., 2012). In the VTA, the most marked change in
mRNA produced by these drugs was a decrease in mRNA for GAL₂ receptor (Rovin et al., 2012). However, Venlafaxine, a selective serotonin and noradrenalin reuptake inhibitor, does not alter transcript levels of GAL or its three receptors (Petschner et al., 2016). These results suggest that the effects on GAL and GAL receptors are not uniform among antidepressants and may be related to certain pharmacological properties (Petschner et al., 2016).

Some human data support the involvement of GAL and its receptors in mood disorders. Thus, GAL given intravenously induced antidepressant-like effects, indicated by a suppression of REM sleep in healthy male volunteers (Murck et al., 2004). Recently in a genome wide screening, the single nucleotide polymorphism of GAL gene has been associated with major depression (Wray et al., 2012). Moreover, a work reported that variants in genes for GAL, and its receptors confer an increased risk of depression and anxiety in people who experienced childhood adversity or recent negative life events (Juhasz et al., 2014). Furthermore, significant positive correlation between plasma GAL level and depression severity has been demonstrated suggesting that plasma GAL levels may be an important biomarker for depression (Wang et al., 2014). Recent findings showed that GAL, GAL₁ and GAL₃ receptors mRNA levels were increased in DR nucleus and GAL and GAL₃ receptor in Locus Coeruleus (LC) in brains from depressed persons who had committed suicide (Barde et al., 2016).

All these studies suggest the importance of GAL and its receptors in the pathogenesis of depression.

**GALANIN(1-15) IN DEPRESSION**

We have analysed the role of GAL(1-15) on the depression-like behaviour using two tests, the FST and the TST.
In the FST, i.c.v. GAL(1-15) 3 nmol significantly increased the immobility time and decreased the time of climbing by 44% and 46% respectively. In addition, this effect was shown with the administration of GAL(1-15) 6 nmol (Millon et al., 2015). The same pattern of response was observed in the TST, GAL(1-15) at the dose of 3 nmol also significantly increased the immobility behaviour recorded during the 6 min of testing (Millon et al., 2015) (Table 1).

These results in both behavioural tests suggest that GAL(1-15) could evoke a strong depression-like behaviour.

When we compared the effects in FST induced by GAL and GAL(1-15), we observed that the increase in the immobility induced by GAL(1-15) was significantly higher than the one induced by GAL (Millon et al., 2015). Moreover, in climbing behaviour, GAL(1-15) also induced a stronger decrease in climbing response compared with GAL (Millon et al., 2015).

In the open field test and in the light-dark box (Prut et al., 2003), we observed that only GAL(1-15) and not GAL modifies the anxiety parameters (Millon et al., 2015) (Table 1). GAL produced anxiolytic-like effects only in animals tested under heightened stress conditions (Barrera et al., 2005; Millon et al., 2015), on contrary, GAL(1-15) induced an anxiogenic-like effect without a stress situation (Millon et al., 2015).

All these results confirm an important role of GAL(1-15) in mood disorders.

Galanin Receptors involved in GAL(1-15)-mediated effect: siRNA GAL₁ receptor or GAL₂ receptor Knockdown rats.

Gene silencing by RNA interference is as a new method of inhibiting the expression of targeted genes and inducing knockdown of associated proteins both in vitro and in vivo (Nakajima et al., 2012). This RNA interference has been applied in experimental investigations in the treatment of illnesses, including neuropsychiatric disorders (Nakajima et al., 2012). Dharmacom Accell siRNA is
one type of naked siRNA modified chemically resulting in robust silencing of selected genes and knockdown of associated proteins (Nakajima et al., 2012).

We used the Accell siRNA system for generating GAL$_1$ or GAL$_2$ receptors knockdown (KD) rats to analyze the involvement of GAL receptors in GAL(1-15)-mediated effects.

Rats were i.c.v. injected with 5 µg of Accell Smart pool siRNA GAL$_1$ receptor or siRNA GAL$_2$ receptor (Dharmacon), or 5 µl of vehicle (Accell siRNA Delivery Media). We have analysed the reduction produced by siRNAs with a time-course curve performed in siRNA GAL$_2$ or GAL$_1$ receptors animals and we examined the mRNA for GAL$_2$ and GAL$_1$ receptors in the dorsal hippocampus by real-time quantitative polymerase chain reaction (RT-PCR) in these animals.

In figure 1A, it is shown that a single injection of siRNA GAL$_2$ receptor induced a strong reduction of mRNA GAL$_2$ receptor (p<0.05) at day 4 and 6 after siRNA treatment (Millon et al., 2015). However, the icv injection of siRNA GAL$_2$ receptor lacked effect in mRNA GAL$_1$ receptor expression (Fig. 1B) [unpublished results].

The single injection of siRNA GAL$_2$ receptor produced the strongest decreased in the receptor expression 8 days after the injection by 35 % in CA1 of the dorsal hippocampus and by 50% in piriform cortex (Millon et al., 2015).

The same pattern of response in mRNA expression was shown after the administration of siRNA GAL$_1$ receptor. A strong reduction of mRNA GAL$_1$ receptor at 8 days (p<0.001) was produced by siRNA GAL$_1$ receptor (Fig. 1C) (Millon et al., 2015). Again, the Accell siRNA system is specific, since that injection of siRNA GAL$_1$ receptor did not modify the mRNA GAL$_2$ receptor expression (Fig. 1D) [unpublished results].

The Down-regulation of GAL$_2$ or GAL$_1$ receptors did not affect any parameters in the FST or in the open field test (OFT) (Table 1). However, this decrease in GAL$_2$ or GAL$_1$ receptors was enough to block the depression- and anxiogenic-like effect of GAL(1-15) in both tests (Millon et al., 2015). Thus, GAL(1-15) at
the dose of 3 nmol lacked effect on immobility, climbing and swimming time in the FST in siRNA GAL$_2$ receptor or in GAL$_1$ receptor KD rats. In the OFT the same pattern of response was observed, since GAL(1-15) did not affect neither number of entries as the time spent in the central square of open field in GAL$_2$ receptor or in GAL$_1$ receptor KD rats (Millon et al., 2015).

The fact that GAL$_1$ and GAL$_2$ receptors are needed to obtain GAL(1-15)-effect confirms the hypothesis that GAL(1-15) preferring sites may be formed through the formation of GAL$_1}$/GAL$_2$ heteroreceptor complexes which lead to conformational changes in their GAL recognition sites converting GAL$_1$ and/or GAL$_2$ receptors into GAL recognition fragment preferring binding sites with reduced affinity for GAL (Fuxe et al., 2008; Fuxe et al., 2012). In recent work, Borroto-Escuela et al. analyzed the existence of GAL$_1}$/GAL$_2$ heteroreceptor complex in co-transfected HEK cells through Bioluminescence Resonance Energy Transfer (BRET$^2$) giving evidence for the existence of GAL$_1}$/GAL$_2$ heteroreceptor complexes (Borroto-Escuela et al., 2014). A differential role of GAL and GAL(1-15) in signaling cascades Gi/o-Adenylate cyclase (AC)-Protein kinase A (PKA) and Gq-Phospholipase C (PLC)-Protein kinase C (PKC)/Ca$^{2+}$ was observed with cyclic adenosine monophosphate response element (CRE) and nuclear factor of activated T-cells (NFAT) -luciferase reporter gene assays in co-expressing GAL$_1$ and/or GAL$_2$ receptors HEK293T cells (Borroto-Escuela et al., 2014).

The formation of GAL$_1}$/GAL$_2$ heteroreceptor complexes with high affinity for GAL(1-15) can help explain the fact that GAL(1-15) induces a stronger action than GAL at the behavioral level. As described is this review GAL$_2$ receptor may induce antidepressant actions via Gq/G11 mediated GAL$_2$ receptor signaling. It seems possible that in the GAL$_1}$/GAL$_2$ heteroreceptor complex the GAL$_2$ receptor protomer signals in a different way upon activation by its preferred ligand GAL(1-15) leading to its strong depression-like action. Alternatively, the GAL(1-15) activation of the GAL$_1$ receptor protomer may via an allosteric receptor-receptor interaction inhibit the Gq/G11 mediated signaling of the GAL$_2$ receptor protomer and switch it towards Gi/o mediated signaling. In
this way both GAL₁ and GAL₂ receptors protomers become coupled to Gi/o which may lead to the strong depression-like actions observed with GAL(1-15) (Millon et al., 2015). The formation of homodimers and heterodimers among neuropeptide receptors is known (AbdAlla et al., 2005). GAL₁ receptor can form homodimers (Wirz et al., 2005) and heterodimers with 5-HT1A receptors (Borroto-Escuela et al., 2010; Fuxe et al., 2012) and likely with other G-protein coupled receptors.

The existence of GAL₁/GAL₂ heteroreceptors complexes in brain tissue was demonstrated with in situ Proximity Ligation Assay (PLA) to analyze the proximity of GAL₁ and GAL₂ receptors in the dorsal hippocampus and DR nuclei of the KD rats of GAL₂ receptor (Millon et al., 2015). In the siRNA GAL₂ receptor treated animals, PLA- positive red clusters were still observed in the dorsal hippocampus and DR (Millon et al., 2015). However, the number of GAL₁/GAL₂ complex was reduced by 40% in CA1, CA2 and dentate gyrus in dorsal hippocampus and by 60% in the DR nucleus compared with the vehicle group (Millon et al., 2015). In agreement with these results, in the siRNA GAL₂ receptor treated animals the colocalization of GAL₁ and GAL₂ receptors was reduced in the both areas (Millon et al., 2015).

These results strongly indicate that the PLA signals obtained are specific and represent the GAL₁ and GAL₂ heteroreceptor complex. This reduction of the PLA signal was sufficient to block the depression- and anxiogenic-like effects of GAL(1-15), linking them to its actions at the GAL₁/GAL₂ heteroreceptor complex (Millon et al., 2015).

Effects of Galanin on monoamine systems

The physiological/ pathophysiological mechanisms underlying the action(s) of GAL most probably involves modulation of monoaminergic systems, in particular, the LC and DR nuclei. This was further supported by the fact that all three receptors are found in the DR and LC of rats (O'Donnell et al., 1999; Burazin et al., 2000; Mennicken et al., 2002) and that GAL is co-expressed in
almost 40% of the serotonergic neurons in the DR (Xu et al., 1997) and in around 80% of the noradrenergic neurons in the LC (Holets et al., 1988).

I.c.v. GAL produced a reduction in basal NA release in the ventral hippocampus of the awake rat measured by microdialysis, and, significantly attenuated the increase of extracellular hippocampal NA levels evoked by desipramine (Yoshitake et al., 2003). These effects could involve the LC since GAL inhibits LC firing and produces an outward current in rat brain slices through GAL$_{1-3}$ receptors. The application of GAL$_{1-3}$ receptor agonist M961, but not GAL$_2$ receptor agonist AR-M1896, caused hyperpolarisation of these LC neurons (Seutin et al., 1989; Sevcik et al., 1993; Pieribone et al., 1995; Ma et al., 2001).

Moreover, Grenhoff et al. (Grenhoff et al., 1993) observed that “burst” firing of LC neurons (i.e., rapid firing of LC) released GAL from terminals on axons of LC neurons projecting to the VTA, and that the hyperpolarizing influence of GAL on dopamine (DA) cell bodies in the VTA decreased the activity of these DA neurons. This finding suggested that the hyperactivity of LC neurons observed in depression might bring about such depression-related responses by decreasing the neural activity of dopaminergic cell bodies in the VTA as the result of GAL released from LC-derived terminals in the VTA (Weiss et al., 1998).

In relation with the DR and the 5-HT system, i.c.v. GAL reduced 5-HT metabolism in ventral limbic cortex, hippocampal formation, and fronto-parietal cortex probably via direct inhibitory action on DR nerve cells reducing their firing rates (Fuxe et al., 1988b). This result is in agreement with other works where i.c.v. GAL into vicinity of the DR caused a dose-related and long-lasting inhibition of 5-HT release in the ventral hippocampus measured by microdialysis (Kehr et al., 2002). Moreover, immunohistochemistry experiments showed a strong GAL immunoreactivity staining was observed in the DR after GAL administration, suggesting that the DR was the site of action of GAL on
hippocampal 5-HT release (Kehr et al., 2002). In agreement, i.c.v. GAL attenuates the increase in extracellular levels of 5-HT induced by SSRI citalopram, indicating that this inhibitory action persisted under conditions of serotonergic activation following reuptake inhibition by an SSRI (Yoshitake et al., 2003; Kuteeva et al., 2008). Electrophysiological experiments, where GAL inhibits the firing rate of 5-HT neurons, probably via G protein-coupled inwardly-rectifying potassium channel (GIRK) (Xu et al., 1998), could support these findings.

The 5-HT1A receptor seems to be a key receptor in the GALR-5-HT interaction. In the DR, i.c.v. GAL induced a time-dependent reduction in affinity and an increase in the 5-HT1A autoreceptor density (Razani et al., 2000). At postsynaptic level GAL reduced the affinity of the 5-HT1A receptors in the ventral limbic cortex (Fuxe et al., 1988a; Hedlund et al., 1996). Moreover, in hypothermia, locomotor activity and passive avoidance, i.c.v. GAL blocked postsynaptic 5-HT1A receptor function (Misane et al., 1998; Razani et al., 2001; Kehr et al., 2002). This interaction can in part be due to the existence of GAL1-5-HT1AR heteroreceptor complexes in discrete brain regions (Borroto-Escuela et al., 2010).

The GAL(1-15) also modified 5-HT system (Millon et al., 2015). Using a rat medullary raphe-derived cell line RN33B, we observed that GAL(1-15) significantly decreased the 5-HT immunoreactivity in the RN33B cells (p<0.001) (Fig.2). Interestingly this reduction was stronger than the one induced by GAL (p<0.001) (Fig.2) (Millon et al., 2015). This effect of GAL(1-15) on 5-HT immunoreactivity may indicate a possible mechanism contributing to the depression-like actions of GAL(1-15).

In this cell model, we have detected PLA- positive clusters, indicating close proximity of GAL1 and GAL2 receptors and the possible formation of GAL1/GLAR2 heteroreceptor complexes (Millon et al., 2015). Therefore, the formation of GAL1/GAL2 heteroreceptor complexes with high affinities for GAL(1-15) can help explain the fact that GAL(1-15) induces a stronger action than GAL in the reduction of 5-HT in the RN33B. Thus, the strong decrease on
5-HT immunoreactivity induced by GAL(1-15) may indicate a mechanism contributing to the depression-like actions of GAL(1-15) (Fig. 2D) (Millon et al., 2015). Recently, we have described that GAL(1–15) enhances the antidepressant effects induced by 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) acting on 5-HT1A receptors operating as postjunctional or as autoreceptors, confirming the importance of the interaction GALR-5-HT1A receptors (Millon et al., 2016).

**Conclusion**

All these data emphasized the role of GAL and its N-Terminal fragment (1-15) in depression. Moreover, GAL(1-15) induces a stronger depressive effect than GAL through GAL₁-GAL₂ heteroreceptor complexes in the raphe-limbic system. Since 5-HT, especially through 5-HT1A, and GAL receptors interact at both pre- and postsynaptic level, the development of drugs targeting potential GAL₁-GAL₂-5-HT1A heteroreceptor complexes linked to the raphe-hippocampal 5-HT neurons may represent new treatment in depression.

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**Figure 1.** Expression levels of GAL2 receptor mRNA (A) and GAL1 receptor mRNA (C) in dorsal hippocampus. (A,B) Expression levels of mRNA GAL2 or GAL1 receptors (4 rats per group) measured on different days after administration of the Acell smart pool siRNA for GAL2 receptor and (C,D) expression levels of mRNA GAL2 receptor or GAL1 receptor (4 rats per group) measured on different days after administration of the Acell smart pool siRNA for GAL1 receptor. The quantitative PCR results were normalized to the expression levels of GAPDH and expressed as arbitrary units. *p<0.05, **p<0.01 vs Vehicle according to student’s t-test. Modified from (Millon et al., 2015).

**Figure 2.** Close proximity between Galanin receptor 1 (GAL1 receptor) and Galanin receptor 2 (GAL2 receptor) and their effects in 5-HT synthesis and storage in RN33B cells. (A) Detection of close proximity between GAL1-GAL2 receptors in RN33B cells by in situ PLA. Scale bar, 10 μm. (B) Quantification of 5-HT immunoreactivity was analyzed after incubation with GAL and GAL(1-15) ***p<0.001 according to one-way ANOVA followed by Newman Keuls Multiple
Comparison Test. (C) Representative images of 5-HT stained RN33B cells under different conditions are presented. Scale bar, 10 μm. Modified from (Millon et al., 2015). (D) Representative schema illustrating the reduction of 5-HT in the dorsal raphe nuclei induced by the action of GAL(1-15) through the GAL₁/GAL₂ heteroreceptor complexes. Representative projections from DR to cortico-limbic areas are shown in red.
Figure 1

A

GAL$_2$ receptor mRNA expression in GAL$_2$-KD animals

B

GAL$_1$ receptor mRNA expression in GAL$_2$-KD animals

C

GAL$_1$ receptor mRNA expression in GAL$_1$-KD animals

D

GAL$_2$ receptor mRNA expression in GAL$_1$-KD animals

* indicates statistical significance.
Figure 2

Medullary Raphe-derived cell line RN33B

A

RN33B

B

Medullary Raphe-derived cell line RN33B

Control

GAL

GAL(1-15)

5HT Immunoreactivity

0.0

2.5

5.0

7.5

10.0

12.5

15.0

17.5

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***

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0.0

2.5

5.0

7.5

10.0

12.5

15.0

17.5

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0.0

2.5

5.0

7.5

10.0

12.5

15.0

17.5

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***

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C

Control

GAL

GAL(1-15)

D

Frontal Cortex

Hippocampus

Estriatum

Amygdala

Hypothalamus

DORSAL RAPHE

GAL(1-15)
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<td>Galanin</td>
<td>FST</td>
<td>Rat</td>
<td>VTA</td>
<td>↑</td>
<td>Weiss et al., 1998</td>
</tr>
<tr>
<td></td>
<td>FST</td>
<td>Rat</td>
<td>i.c.v.</td>
<td>↓</td>
<td>Barfai et al., 2004</td>
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<tr>
<td>Galnon</td>
<td>FST</td>
<td>Rat</td>
<td>i.p.</td>
<td>↓</td>
<td>Lu et al., 2005</td>
</tr>
<tr>
<td>Galmic</td>
<td>FST</td>
<td>Rat</td>
<td>i.p.</td>
<td>↓</td>
<td>Swanson et al., 2005</td>
</tr>
<tr>
<td>M617 (GAL1 receptor agonist)</td>
<td>FST</td>
<td>Rat</td>
<td>i.c.v.</td>
<td>↑</td>
<td>Kuteeva et al., 2008</td>
</tr>
<tr>
<td>(GAL3 receptor antagonist)</td>
<td>TST</td>
<td>Mouse</td>
<td>i.p.</td>
<td>↓</td>
<td>Barr et al., 2006</td>
</tr>
<tr>
<td>AR-M1896 (GAL2 receptor agonist)</td>
<td>FST</td>
<td>Rat</td>
<td>i.c.v.</td>
<td>↓</td>
<td>Kuteeva et al., 2008</td>
</tr>
<tr>
<td>M871 (GAL2 receptor antagonist)</td>
<td>FST</td>
<td>Rat</td>
<td>i.c.v.</td>
<td>↓</td>
<td>Saar et al., 2013a</td>
</tr>
<tr>
<td>M1160 (GAL2 receptor agonist)</td>
<td>FST</td>
<td>Mouse</td>
<td>i.v.</td>
<td>↓</td>
<td>Saar et al., 2013b</td>
</tr>
<tr>
<td>J18 (GAL2 receptor agonist)</td>
<td>FST</td>
<td>Rat</td>
<td>i.c.v.</td>
<td>↑</td>
<td>Million et al., 2015</td>
</tr>
<tr>
<td>GAL(1-15)</td>
<td>TST</td>
<td>Mouse</td>
<td>0</td>
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<td>Holmes et al., 2005</td>
</tr>
<tr>
<td>GAL1-KO</td>
<td>TST</td>
<td>Mouse</td>
<td>0</td>
<td></td>
<td>Holmes et al., 2003</td>
</tr>
<tr>
<td>GAL2-KO</td>
<td>EPM</td>
<td>Mouse</td>
<td>0</td>
<td>↑*</td>
<td>Bailey et al., 2007</td>
</tr>
<tr>
<td></td>
<td>TST</td>
<td>Mouse</td>
<td>0</td>
<td>↑*</td>
<td>Gottsch et al., 2005</td>
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<td>GAL2 receptor overexpressing</td>
<td>FST</td>
<td>Mouse</td>
<td>0</td>
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<td>Le Maitre et al., 2011</td>
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<tr>
<td>GAL3-KO</td>
<td>TST</td>
<td>Mouse</td>
<td>0</td>
<td>↑*</td>
<td>Brunner et al., 2014</td>
</tr>
<tr>
<td></td>
<td>EPM</td>
<td>Mouse</td>
<td>0</td>
<td>↑*</td>
<td>Brunner et al., 2014</td>
</tr>
<tr>
<td></td>
<td>OFT</td>
<td>Mouse</td>
<td>0</td>
<td>↑*</td>
<td>Brunner et al., 2014</td>
</tr>
<tr>
<td></td>
<td>L/D test</td>
<td>Mouse</td>
<td>0</td>
<td>↑*</td>
<td>Brunner et al., 2014</td>
</tr>
<tr>
<td>GAL1-KD</td>
<td>FST</td>
<td>Rat</td>
<td>0</td>
<td>↓</td>
<td>Million et al., 2015</td>
</tr>
<tr>
<td></td>
<td>OFT</td>
<td>Rat</td>
<td>0</td>
<td>↓</td>
<td>Million et al., 2015</td>
</tr>
<tr>
<td>GAL2-KD</td>
<td>FST</td>
<td>Rat</td>
<td>0</td>
<td>↓</td>
<td>Million et al., 2015</td>
</tr>
<tr>
<td></td>
<td>OFT</td>
<td>Rat</td>
<td>0</td>
<td>↓</td>
<td>Million et al., 2015</td>
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Table 1. Effects of Galanin receptor ligands and genetic animal models in rodent test of depression. FST: Forced Swimming Test; TST: Tail Suspension Test; OFT: Open Field Test; EPM: Elevated Plus Maze; L/D Test: Ligh-Dark test; VTA: Ventral Tegmentar Area; i.c.v.: intracerebroventricular; i.p.: intraperitoneal; i.v.: intravenous; KO: Knock Out; KD: Knock Down; ↑: pro-depressive effect; 0: no effect; ↓: antidepressant effect; ↑*: anxiogenic effect.
GALANIN (1-15) AND THE HETERODIMER GALR1/GALR2 IN DEPRESSION AND ANXIETY-LIKE BEHAVIOURS

Carmelo Millón¹, Antonio Flores-Burgess¹, Manuel Narváez¹, Dasiel O. Borroto-Escuela², Belén Gago¹, Luis Santín³, Estela Castilla-Ortega³, José Ángel Narváez¹, Kjell Fuxe² & Zaida Díaz-Cabiale¹.

Abstract

Galanin is a 29 amino acid neuropeptide widely distributed in neurons within the central nervous system. Galanin exerts its biological activities through three different G protein-receptors and participates in a number of functions, including mood regulation. Not only Galanin but also Galanin N-terminal fragments like Galanin(1-15) are active at the central level. In this work, we review the latest findings in studies on Galanin and Galanin(1-15) in depression-related behaviours. Our focus is on animal models for depression, and we pay some attention to research data obtained in human studies. Since Serotonin (5-HT), especially through 5-HT1A, and Galanin receptors interact at both pre-and postsynaptic level, the development of drugs targeting potential GAL1-GAL2-5-HT1A heteroreceptor complexes linked to the raphe-hippocampal 5-HT neurons may represent new treatment strategies in depression.

Key words: depression, galanin
Highlights

- GAL(1-15) produces depression- and anxiety- like effects in behavioural test.
- In these tests, GAL(1-15) induces stronger effects than GAL.
- GALR1/GALR2 heteroreceptor complexes are involved in GAL(1-15)-mediated action.