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Galanin as a modulator of anxiety and depression and a therapeutic target for affective disease

Review Article

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Summary. Galanin is a 29 amino-acid (30 in humans) neuropeptide with a close functional relationship with neurotransmitter systems implicated in the pathophysiology and treatment of depression and anxiety disorders. In rodent models of depression-related behavior, treatment with galanin or compounds with agonist actions at galanin receptors has been shown to affect depression-related behaviors and the behavioral and neurochemical effects of antidepressants. Treatment with clinically efficacious antidepressants alters galanin and galanin receptor gene expression in rodents. Rodent anxiety-like behaviors appear to be modulated by galanin in a complex manner, with studies showing either increases, decreases and no effects of galanin treatments and galanin mutations on anxiety-like behavior in various tasks. One concept to emerge from this literature is that galanin recruitment during extreme behavioral and physiological provocations such as stress and opiate withdrawal may serve to attenuate negative emotional states caused by noradrenergic hyperactivation. The specific galanin receptor subtypes mediating the anxiety- and depression-related effects of galanin remains to be determined, with evidence supporting a possible contribution of GalR1, GalR2 and GalR3. While our understanding of the role of galanin as a modulator of emotion remains at an early stage, recent progress in this rapidly evolving field raise possibility of that galanin may represent a target for the development of novel antidepressant and anxiolytic drug treatments.

Keywords: Galanin – Neuropeptides – Stress – Anxiety – Depression – Rodent

Localization of galanin in neural circuits implicated in emotion

Galanin is a 29 amino-acid (30 in humans) neuropeptide, first isolated from the porcine gut by Tatemoto, Mutt and colleagues more than 20 years ago (Tatemoto et al., 1983). The peptide is highly conserved and across species is abundant in the central nervous system (CNS) (Melander et al., 1985a). In the rodent brain, galanin shows a marked pattern of colocalization with the major ascending monoamine systems. In rats, galanin-like immunoreactivity is detected in brainstem norepinephrine-producing cells of the locus coeruleus (LC), the serotonin-producing neurons of the dorsal raphe nucleus (DRN) and the midbrain dopaminergic ventral-tegmental area (VTA) (Hokfelt et al., 1998b; Holets et al., 1988; Holmes et al., 1995; Lu et al., 2005a; Melander et al., 1986; Merchenthaler et al., 1993; Skofitsch and Jacobowitz, 1986). Functionally, galanin is known to inhibit of the neuronal firing and/or release of norepinephrine, serotonin, dopamine, as well as glutamate and acetylcholine (Kehr et al., 2002; Melander et al., 1985b; Pieribone et al., 1995; Seutin et al., 1989; Zini et al., 1993). These actions of galanin are mediated through three known G-protein coupled receptor subtypes (GalR1, GalR2, GalR3), expressed in midbrain monoamine-producing nuclei as well as their forebrain projection sites of the rodent brain, including amygdala, hippocampus, septum, and hypothalamus (Branchek et al., 2000; Burazin et al., 2000; Hawes and Picciotto, 2004; Hohmann et al., 2003; Iismaa and Shine, 1999; Kolakowski et al., 1998; Larm et al., 2003; Mennicken et al., 2002; O'Donnell et al., 1999; Wang et al., 1997; Waters and Krause, 2000). Importantly, studies in human and primate brain indicates that galanin-like immunoreactivity can also be detected in

midbrain and limbic regions (Beal et al., 1988; Kordower et al., 1992; Kordower and Mufson, 1990).

Taken together, the anatomical and neuromodulatory characteristics of galanin-containing neurons and galanin receptors suggest that the peptide might play a role in mediating various higher-order behavioral functions. In this context, a corpus of data has shown that galanin regulates behaviors related to cognition, nociception, seizure, sexual behavior, feeding, sleep, and reward (for reviews see Hokfelt et al., 1998a, 1999; Mazarati et al., 2001; Saper et al., 2001; Wrenn and Crawley, 2001). Interestingly, many of these behaviors are abnormal in patients with mood disorders. In addition, there is now growing evidence from animal models that galanin modulates emotion-related behaviors (for reviews, see Holmes and Picciotto, 2006; Lu et al., 2005a). As with other neuropeptide systems implicated in emotion, such as corticotropin-releasing factor, vasopressin, neuropeptide Y, and substance P, these findings have led to interest in targeting galanin as a potential novel therapeutic target for depression and anxiety disorders (Contarino and Gold, 2002; Griebel, 1999; Hokfelt et al., 1999; Holmes et al., 2003a; Holsboer, 2003; Xu et al., 2004). The present review provides a brief update on research on galanin's role in modulating stress and emotion using rodent preclinical models, and considers the potential for developing galanin-targeting anxiolytics and antidepressants.

Galaninergic modulation of rodent depression-related behaviors

Early work, since extended, by Weiss and colleagues found that administration of galanin either intracerebroventricular (icv) or into the dopaminergic ventral tegmental area produced an increase in immobility in the rat forced swim test (FST) (Weiss et al., 1998, 2005), a profile consistent with an increase in depression-like behavior in this model (Cryan and Holmes, 2005). More recently, mice genetically engineered to constitutively overexpress galanin in brain via coupling of the galanin gene to the platelet-derived growth factor B promoter (PDGF-B) (Holmberg et al., 2005) have been found to exhibit increased depression-related behavior and abnormal monoaminergic responses in the FST (Kuteeva et al., 2004; Yoshitake et al., 2004). Furthermore, in the Flinders Sensitive Line of rat, a genetic model selected for high FST immobility, there is elevated galanin binding in the serotonergic DRN, and reduced galanin-like immunoreactivity in the DRN and hippocampus (Bellido et al., 2002; Husum et al., 2003). In the context of the aforementioned galaninergic modulation of monoamine neurons, the localization of depression-related effects of galanin to dopaminergic and serotonergic nuclei is intriguing given evidence implicating the monoamines in the pathophysiology and treatment of depression (Ressler and Nemeroff, 2000). Recent data has begun to provide insight into how galanin might interact with monoamines to modulate depression-related behaviors and antidepressant efficacy.

Lu and colleagues found that rats either chronically treated with the serotonin reuptake inhibitor (SSRI), fluoxetine, or subjected to electroconvulsive shock showed a significant increase in levels of galanin mRNA in the DRN, but not forebrain regions such as the hippocampus and amygdala (Lu et al., 2005b; Stenfors et al., 1989). In addition, chronic fluoxetine treatment increased galanin mRNA in the major norepinephrine-producing nucleus, the locus coeruleus (Lu et al., 2005b; Toppila et al., 1995). Sleep deprivation produced a similar effect (Lu et al., 2005b; Toppila et al., 1995); an interesting finding given sleep disturbances found in depression (Wirz-Justice and Van den Hoofdakker, 1999) and evidence that galanin modulates sleep (Saper et al., 2001). Lu and coworkers went on to show that the antidepressant-like effects of chronic fluoxetine treatment in the FST was blocked by icv administration of the peptidergic galanin antagonist M40 prior to FST testing (Bartfai et al., 2004; Lu et al., 2005b). The temporal lag between the start of antidepressant treatment and the onset of clinical benefits is believed to reflect the need for downstream neural adaptations that are as yet unclear (Duman, 1998; Manji et al., 2001). The findings of Lu et al. suggest that upregulation in galanin function could be an important contributor to these permissive adaptations, and as such raise the possibility that promoting galanin activity could itself exert antidepressant effects (Lu et al., 2005a). In this context, a small pilot study reported that intravenous administration of galanin had an antidepressant effect in human subjects diagnosed with depression (Murck et al., 2004).

Given the available literature, data suggesting that increased galanin function may be antidepressant-like are not easy to reconcile with the earlier studies suggesting a pro-depressive effect of galanin, sometimes on the same endpoint measures such as FST behavior (see Table 1). The mechanism of action by which increased galanin function would produce antidepressant-like effects also remains to be clarified. Based on the current dogma that SSRIs exert there therapeutic effects, at least initially, by increasing extracellular serotonin availability, a simple model would predict that increased galaninergic inhibition of serotonergic activity would counter, rather than

Table 1. Effects of galanin treatments and mutations on human depression and rodent depression-related behaviors

Treatment/mutation	Species	Endpoint measure	Behavioral effect	Reference
Galanin iv	Human	Hamilton rating scale	Antidepressant	Murck et al., 2004
Galanin icv	Rat	Forced Swim test	None	Weiss et al., 1998
	Mouse	Tail suspension test	None	Holmes et al., 2005
Galanin intra-VTA	Rat	Forced Swim test	Pro-depression-like	Weiss et al., 1998
Galanin antagonist, M15, intra-VTA	Rat	Forced Swim test	Antidepressant-like	Weiss et al., 1998
Galanin intra-hypothalamus	Rat	Forced Swim test	None	Weiss et al., 1998
Galanin antagonist, M40, icv	Rat	Forced Swim test	Blocked chronic-fluoxetine-induced antidepressant-like effect	Lu et al., 2005
Galanin agonist, galmic	Rat	Forced Swim test	Antidepressant-like	Bartfai et al., 2004
Galanin agonist, galnon	Rat	Forced Swim test	Antidepressant-like	Lu et al., 2005
GalR3 antagonist, SNAP 37889	Rat	Forced Swim test	Antidepressant-like	Swanson et al., 2005
Galanin overexpression (DβH promoter)	Mouse	Tail suspension	None	Holmes et al., 2005
Galanin overexpression (PDGF-B promoter)	Mouse	Forced Swim test	Pro-depression-like	Kuteeva et al., 2005
GalR1 knockout	Mouse	Tail suspension	None	Holmes et al., 2005
GalR2 knockout	Mouse	Tail suspension	None	Gottsch et al., 2005

iv, Intravenous; icv, intracerebroventricular; DβH, dopamine β-hydroxylase; PDGF-B, platelet-derived growth factor B promoter

mimic or augment, antidepressant-like efficacy. For example, in vivo microdialysis studies have demonstrated that icv galanin significantly decreases the ability of antidepressants to increase extracelluar fluid levels of serotonin or norepinephrine in the rat ventral hippocampus (Yoshitake et al., 2003).

One potentially critical factor contributing to the complexity of galanin's effects on monoamine function and depression-related behavior is functional differences in galanin receptor subtypes. It is only recently that this question been addressable however, with the development of subtype-specific pharmacological compounds and the generation of mutant mice with functional inactivation of specific galanin receptor subtypes. The three known galanin receptor subtypes (GalR1, GalR2, GalR3) exhibit partially differential distribution in midbrain and limbic areas mediating emotional behaviors (Branchek et al., 2000; Burazin et al., 2000; Hohmann et al., 2003; Iismaa and Shine, 1999; Kolakowski et al., 1998; Larm et al., 2003; Mennicken et al., 2002; O'Donnell et al., 1999; Wang et al., 1997; Waters and Krause, 2000). Galanin receptor subtypes also activate different signal transduction pathways. While GalR1 and GalR3 couple to inhibitory Gi/Go proteins and attenuate cAMP levels, GalR2 signaling through Gq/G11 causes elevation of intracellular calcium and may be excitatory in certain cell types (Kolakowski et al., 1998; Wang et al., 1999). These differences raise the possibility that activation of specific subtypes could determine the nature of galaninergic effects on monoamine function and depression-related behavior. For example, the actions of galanin on this behavior could depend upon the receptor subtype preferentially activated in a given species, brain region or model system.

In this context, chronic fluoxetine administration has been found to increase GalR2, but not GalR1, binding in the rat DRN (Lu et al., 2005b). Lu and colleagues proposed that a selective upregulation of GalR2 on DRN neurons, resulting in a relative shift from GalR1/GalR3 to GalR2 signaling, might contribute to fluoxetine's antidepressant-like effects via GalR2-mediated excitation of DRN neurons and subsequent augmentation of forebrain 5-HT release (Lu et al., 2005b). This hypothesis will require further testing, for example by using subtype-selective compounds as they become available. Currently, however, there are no brain-penetrant small molecule compounds with a high degree of selectivity for GalR1 versus GalR2. Systemic treatment with either the non-peptide, non-specific galanin agonist galnon, or the more GalR1 selective galmic have recently been shown to produce antidepressant-like effects in the rat FST (Bartfai et al., 2004; Lu et al., 2005b). A caveat to these data is that galmic has rather low affinity for GalR1, while both drugs are known to have off-target actions at sites known to mediate antidepressant responses, including the 5-HT_{1A} receptor and serotonin transporter (Floren et al., 2005; Sollenberg et al., 2005). Further studies will help ascertain these antidepressant-like effects are mediated by galanin, for example by blocking their effects with a specific galanin antagonist.

Galanin receptor 'knockout' mice provide an alternative approach to delineate subtype function. Knockout mice lacking the GalR1 receptor subtype (Jacoby et al., 2002) have been phenotyped and found to display a normal phenotype in the tail suspension test (TST), a model of depression-related behavior thought to be similar but not necessarily synonymous with the FST (Holmes et al., 2005). Antidepressant-like responses to acute treatment with fluoxetine or the norepinephrine reuptake inhibitor, desipramine, were also unaltered in GalR1 knockout mice. These data suggest that GalR1 does not mediate depression-related effects of galanin, and that GalR2 or GalR3 may be more important modulators of such behaviors. However, it should be noted that neither icv galanin in non-mutant mice, nor transgenic overexpression of galanin in noradrenergic neurons, altered TST behavior in this study (Holmes et al., 2005). Moreover, a separate study has found that GalR2 knockout mice also exhibit normal baseline behavior in the TST (Gottsch et al., 2005). As such, it remains possible that either the mouse or the TST are not sensitive to galaninergic modulation of depression-related behavior, and that a depression-related role for GalR1 would be detectable in another model such as the mouse or rat FST. Another possibility is that constitutive (i.e., life-long, tissue-ubiquitous) mutation of galanin, GalR1 or GalR2 results in compensatory changes that underestimates the normal function of these molecules (Crawley, 2000; Holmes, 2001; Holmes et al., 2004). Nonetheless, even without a clear baseline phenotype on these tasks, galanin receptor knockout mice could still prove to be a useful tool for verifying the behavioral specificity of receptor agonists and antagonists.

The comparatively sparse and discrete distribution of GalR3 in brain has meant this receptor subtype has been understudied for its behavioral functions relative to GalR1 and GalR2. However, recent data have implicated GalR3 in depression-related behavior. Swanson and colleagues found that either acute or chronic systemic administration of a novel small molecule GalR3-selective antagonist, SNAP 37889, produced antidepressant-like effects in the rat FST (Swanson et al., 2005). As a possible mechanism subserving these effects, SNAP 37889 and the related compound, SNAP 398299, partially reversed the ability of galanin to inhibit DRN firing and serotonin release in the ventral hippocampus (Swanson et al., 2005). While further work will be needed to extend these encouraging findings to other models and to replicate them in other laboratories (depression-related effects of a GalR3-acting compound have been reported in abstract form by another group; (Barr et al., 2004)) and then the clinic, they suggest another potentially promising target for developing a galanin-targeted antidepressant.

Galaninergic modulation of rodent anxiety-related behaviors

As with research on the putative role of galanin in modulating depression, research on galanin and anxiety is a nascent, but rapidly evolving field. Various manipulations of galanin, including administration of galanin, galanin fragments, small molecule galanin receptor ligands, or genetically-engineered alterations in endogenous galanin have produced diverse effects on rodent anxiety-related behaviors (see Table 2). Early work by Heilig and colleagues showed that icv galanin had anxiolytic-like effects in the rat Vogel conflict test (Bing et al., 1993), while direct injection of galanin into the amygdala produced the opposite effect on the same task and failed to alter behavior on the elevated plus-maze (Moller et al., 1999).

More recently, Morilak and colleagues found that intra-amygdala injection of the peptidergic galanin antagonist, M40, exerted anxiogenic-like effects in the rat elevated plus-maze, but only in animals that had been subjected to restraint stress and treatment with the α2-adrenergic autoreceptor antagonist, yohimbine (Khoshbouei et al., 2002a). Using in vivo microdialysis, the study also showed that galanin was released in the amygdala by the combination of stress and yohimbine. These effects have been replicated (Barrera et al., 2006) and interpreted as evidence that galanin is released in the amygdala in response to the high noradrenergic activity produced by the combination of stress and pharmacological blockade of negative feedback inhibition of norepinephrine release. The precise anatomical and mechanistic basis of this relationship remains to be determined. Barrera et al. (2006) recently demonstrated that lesioning the noradrenergic input from the locus coeruleus to the amygdala did not block these anxiolytic-like effects of galanin in this paradigm, suggesting that the source of galanin was not co-released from noradrenergic neurons.

An interesting parallel with these data from rats has been obtained in mutant mice engineered to conditionally overexpress galanin in epinephrine and norepinephrine-synthesizing neurons via coupling of the mouse galanin gene to a human dopamine β-hydroxylase (DβH) promoter (Crawley et al., 2002; Steiner et al., 2001). Phenotypic analysis demonstrated that both this line, as well as the PDGF-B-coupled galanin overexpressing transgenic line

Table 2. Effects of galanin treatments and mutations on fear and anxiety-related behaviors

Treatment/mutation	Species	Endpoint measure	Behavioral effect	Reference
Galanin icv	Rat	Vogel conflict test	Anxiolytic-like	Bing et al., 1993
	Rat	Shock-induced freezing	None	Holmes et al., 1994
	Mouse	Elevated plus-maze	None	Karlsson et al., 2005
	Mouse	Novel open field test	None	Karlsson et al., 2005
	Mouse	Light/dark exploration test	None	Karlsson et al., 2005
	Mouse	Pavlovian fear conditioning	None	Karlsson et al., 2005
Galanin intra-amygdala	Rat	Vogel conflict test	Anxiogenic-like	Möller et al., 1999
	Rat	Elevated plus-maze	None	Möller et al., 1999
Galanin antagonist, M40, intra-amygdala	Rat	Elevated plus-maze	Blocked anxiolytic-like effect of combined restraint stress + yohimbine treatment	Khoshbouei et al., 2002a
Galanin antagonist, M40, intra- lateral septum	Rat	Defensive burying	Anxiolytic-like	Echevarria et al., 2005
Galanin antagonist, M40, intra-BNST	Rat	Elevated plus-maze	Blocked anxiogenic-like effect of restraint stress	Khoshbouei et al., 2002b
	Rat	Social interaction	Blocked anxiogenic-like effect of restraint stress	Khoshbouei et al., 2002b
GalR3 antagonist, SNAP 37889	Mouse	Stress-induced hypothermia	Anxiolytic-like	Swanson et al., 2005
	Rat	Social interaction test	Anxiolytic-like	Swanson et al., 2005
	Rat	Vogel conflict test	Anxiolytic-like	Swanson et al., 2005
	Guinea pig	Separation-induced vocalization test	Anxiolytic-like	Swanson et al., 2005
Galanin overexpression (DβH promoter)	Mouse	Novel open field test	None	Holmes et al., 2002
	Mouse	Elevated plus-maze	None	Holmes et al., 2002
	Mouse	Light/dark exploration test	Blocked anxiogenic-like effect of yohimbine	Holmes et al., 2002
Galanin overexpression (PDGF-B promoter)	Mouse	Elevated plus-maze	None	Kuteeva et al., 2005
	Mouse	Novel open field test	None	Kuteeva et al., 2005
	Mouse	Light/dark exploration test	None	Kuteeva et al., 2005
GalR1 knockout	Mouse	Elevated plus-maze	Anxiogenic-like	Holmes et al., 2003
	Mouse	Novel open field test	None	Holmes et al., 2003
	Mouse	Light/dark exploration test	None	Holmes et al., 2003
	Mouse	Emergence test	None	Holmes et al., 2003
GalR2 knockout	Mouse	Novel open field test	None	Gottsch et al., 2005
	Mouse	Stress-induced hypothermia	None	Gottsch et al., 2005

iv, Intravenous; icv, intracerebroventricular; BNST, bed nucleus of the stria terminalis; DβH, dopamine β-hydroxylase; PDGF-B, platelet-derived growth factor B promoter

noted above, exhibited normal baseline anxiety-like behavior in several anxiety-related tasks including the elevated plus-maze, open field and the light/dark exploration tests (Holmes et al., 2002; Kuteeva et al., 2005). Similarly, in non-mutant C57BL/6J mice, icv administration of galanin was found to exert no anxiety-related effects across a range of tasks that were sensitive to the anxiolytic-like effects of neuropeptide Y icv treatment in the same experiment (Karlsson et al., 2005). Interestingly, however, further testing of the DβH-coupled transgenic line demonstrated that galanin overexpression rendered mice insensitive to the anxiogenic-like effects of yohimbine treatment as compared to their wild-type littermates (Holmes et al., 2002).

Together, these data provide tentative support for the hypothesis that galanin may be activated under conditions of high noradrenergic activity such as those evoked by stress or pharmacological challenge and thereby preferentially exert anxiolytic actions under these pathological states. In this context, a number of studies have shown that various forms of stress (e.g., social, exercise, cold, pain, immobilization) increase prepro-galanin gene expression in the locus coeruleus, as well as forebrain regions such as the amygdala and hypothalamus (Holmes et al., 1995; Makino et al., 1999; O'Neal et al., 2001; Palkovits, 2000; Sweerts et al., 1999, 2000). Interestingly, other stressors, e.g., footshock and chromic mild stress, have been found to either produce no effect, or decrease galanin mRNA expression in these same brain regions (Sergeyev et al., 2005; Soares et al., 1999). In addition, galanin is expressed in the paraventricular nucleus of the hypothalamus with corticotropin-releasing factor and vasopres-

sin (Mazzocchi et al., 1992) and is known to modulate hypothalamic-adrenal-pituitary (HPA)-axis responses to stress. Administration of galanin can either increase or decrease stress-induced activation of the HPA-axis depending upon the site of administration (Hooi et al., 1990; Khoshbouei et al., 2002b; Malendowicz et al., 1994).

Further, indirect evidence of a role for galanin in modulating anxiety-related behaviors in relatively stressful conditions has been obtained from studies using GalR1 knockout mice (Jacoby et al., 2002). These mutants showed heightened anxiety-like behavior relative to wild-type controls, but only on the elevated plus-maze, and not the light-dark exploration, emergence or novel open field tests (Holmes et al., 2003b). The same study found that, in non-mutant C57BL/6J mice, exposure to the elevated plus-maze produced a greater activation of the HPA-axis than the other tests (Holmes et al., 2003b). In addition, GalR1 KO mice were impaired on trace fear conditioning, a form of emotional learning that is impaired by certain stressors (Wood and Shors, 1998). Interestingly, while one study found that GalR2 knockout mice showed normal anxiety-like behavior on the novel open field and stress-induced hypothermia tests (Gottsch et al., 2005), work on a separate line of GalR2 knockout mice published in abstract form has revealed an anxiogenic-like phenotype that is again specific to the elevated plus-maze (Bailey et al., 2005). One interpretation of these data is that the elevated plus-maze generates a relatively high level of anxiety-like behavior that is necessary to recruit galanin, and in doing so reveals the anxiety-related role of GalR1 and GalR2 in their absence.

The notion that a certain threshold level of emotional provocation or neuropathology may be required to recruit galanin's anxiety-related effects is concordant with the more general model that neuropeptides principally exert their neurophysiological effects during strong stimulation of an interacting principal neurotransmitter system (Consolo et al., 1994; Hokfelt et al., 1987; Lundberg et al., 1983). Interestingly, there is evidence that anti-noradrenergic actions of galanin may extend to other pathological states. Opiate withdrawal is characterized by an array of behavioral and neurophysiological changes including noradrenergic hyperactivation and mood disturbances (Maldonado and Koob, 1993). In mice, opiate withdrawal has been shown to produce increased GalR1 mRNA levels and radioligand binding in the locus coeruleus (Zachariou et al., 2000). Demonstrating the functional relevance of these changes, a recent study found that DβH-coupled galanin-overexpressing transgenic mice and non-mutant C57BL/6J mice systemically treated with the galanin receptor agonist, galnon, show attenuated withdrawal while, in contrast, galanin knockout mice exhibit greater withdrawal severity than wild-type controls (Zachariou et al., 2003).

A model that posits an anxiolytic-like effect of galanin under conditions of stress and hyperarousal has intuitive appeal in terms of developing a clinically efficacious anxiolytic compound with a low side-effect burden (Holmes et al., 2003a; Holmes and Picciotto, 2006). However, as discussed in a recent commentary by Barrera and colleagues, the available evidence indicates that galaninergic modulation of anxiety is likely to be more complex than a stress/anti-noradrenergic models permits (Barrera et al., 2005). For example, administration of the galanin antagonist, M40, directly into the BNST found to reduce anxiety-like behavior in restraint-stressed rats tested on the elevated plus-maze or social interaction test (Khoshbouei et al., 2002a) while, in a separate study, M40 injected into the lateral septum reduce anxiety-like behavior in the shock-probe burying test (Echevarria et al., 2005). These effects are opposite to that produced by intra-amygdala galanin in the same laboratory, and suggest a pro-anxiety, rather than anxiolytic-like, effect of endogenous galanin in the BNST and lateral septum. Clearly, the anxiety-related effects of galanin are complex, and elucidation of the role of the peptide in anxiety might be complicated by differences in its effects across species, brain regions and behavioral paradigms.

As with research on possible antidepressant properties of galanin, a greater understanding of the relative contribution of galanin receptor subtypes may go some way to clarifying galaninergic modulation of anxiety-related behaviors. The development and evaluation of selective GalR1 and GalR2 compounds is still awaited. However, a recent study has shown that a novel GalR3-selective antagonist, SNAP 37889, exhibits anxiolytic-like effects across a range of anxiety tests and species (rat social interaction and Vogel conflict tests, mouse stress-induced hyperthermia, guinea pig separation-induced vocalization test) (Swanson et al., 2005).

These novel findings illustrate the rapid progress in elucidating galanin's role in anxiety and depression. Further developments in this exciting field of preclinical research are anticipated in the near future. The results of these studies will be critical in deciding whether to proceed with costly drug development and subsequent clinical trials to evaluate the anxiolytic and antidepressant efficacy of novel compounds targeting galanin.

References

Bailey KR, Hohmann JG, Pavlova M, Crawley J (2005) Behavioral phenotypes of galanin receptor subtype 2 null mutant mice Society for Neuroscience meeting, Washington DC. Abstract number 999.3

Barr AM, Lu X, Kinney JW, Lucero J, Haberhauer L, Ceide S, Somogyi L, Rebek J, Bartfai T (2004) Positive effects of systemically active galanin receptor agonists in rodent preclinical tests of antidepressant activity. Society for Neuroscience meeting, San Diego. Abstract number 345.15

Barrera G, Echevarria DJ, Poulin JF, Laforest S, Drolet G, Morilak DA (2005) One for all or one for one: does co-transmission unify the concept of a brain galanin "system" or clarify any consistent role in anxiety? Neuropeptides 39: 287–290

Barrera G, Hernandez A, Poulin JF, Laforest S, Drolet G, Morilak DA (2006) Galanin-mediated anxiolytic effect in rat central amygdala is not a result of corelease from noradrenergic terminals. Synapse 59: 27–40

Bartfai T, Lu X, Badie-Mahdavi H, Barr AM, Mazarati A, Hua XY, Yaksh T, Haberhauer G, Ceide SC, Trembleau L, Somogyi L, Krock L, Rebek J Jr (2004) Galmic, a nonpeptide galanin receptor agonist, affects behaviors in seizure, pain, and forced-swim tests. Proc Natl Acad Sci USA 101: 10470–10475

Beal MF, Gabriel SM, Swartz KJ, MacGarvey UM (1988) Distribution of galanin-like immunoreactivity in baboon brain. Peptides 9: 847–851

Bellido I, Diaz-Cabiale Z, Jimenez-Vasquez PA, Andbjer B, Mathe AA, Fuxe K (2002) Increased density of galanin binding sites in the dorsal raphe in a genetic rat model of depression. Neurosci Lett 317: 101–105

Bing O, Moller C, Engel JA, Soderpalm B, Heilig M (1993) Anxiolytic-like action of centrally administered galanin. Neurosci Lett 164: 17–20

Branchek TA, Smith KE, Gerald C, Walker MW (2000) Galanin receptor subtypes. Trends Pharmacol Sci 21: 109–117

Burazin TC, Larm JA, Ryan MC, Gundlach AL (2000) Galanin-R1 and -R2 receptor mRNA expression during the development of rat brain suggests differential subtype involvement in synaptic transmission and plasticity. Eur J Neurosci 12: 2901–2917

Consolo S, Baldi G, Russi G, Civenni G, Bartfai T, Vezzani A (1994) Impulse flow dependency of galanin release in vivo in the rat ventral hippocampus. Proc Natl Acad Sci USA 91: 8047–8051

Contarino A, Gold LH (2002) Targeted mutations of the corticotropin-releasing factor system: effects on physiology and behavior. Neuropeptides 36: 103–116

Crawley JN (2000) What's wrong with my mouse? Behavioral phenotyping of transgenic and knockout mice. Wiley-Liss, New York

Crawley JN, Mufson EJ, Hohmann JG, Teklemichael D, Steiner RA, Holmberg K, Xu ZQ, Blakeman KH, Xu XJ, Wiesenfeld-Hallin Z, Bartfai T, Hokfelt T (2002) Galanin overexpressing transgenic mice. Neuropeptides 36: 145–156

Cryan JF, Holmes A (2005) The ascent of mouse: advances in modelling human depression and anxiety. Nat Rev Drug Discov 4: 775–790

Duman RS (1998) Novel therapeutic approaches beyond the serotonin receptor. Biol Psychiatry 44: 324–335

Echevarria DJ, Hernandez A, Diogenes A, Morilak DA (2005) Administration of the galanin antagonist M40 into lateral septum attenuates shock probe defensive burying behavior in rats. Neuropeptides 39: 445–451

Floren A, Sollenberg U, Lundstrom L, Zorko M, Stojan J, Budihna M, Wheatley M, Martin NP, Kilk K, Mazarati A, Bartfai T, Lindgren M, Langel U (2005) Multiple interaction sites of galnon trigger its biological effects. Neuropeptides 39: 547–558

Gottsch ML, Zeng H, Hohmann JG, Weinshenker D, Clifton DK, Steiner RA (2005) Phenotypic analysis of mice deficient in the type 2 galanin receptor (GALR2). Mol Cell Biol 25: 4804–4811

Griebel G (1999) Is there a future for neuropeptide receptor ligands in the treatment of anxiety disorders? Pharmacol Ther 82: 1–61

Hawes JJ, Picciotto MR (2004) Characterization of GalR1, GalR2, and GalR3 immunoreactivity in catecholaminergic nuclei of the mouse brain. J Comp Neurol 479: 410–423

Hohmann JG, Jureus A, Teklemichael DN, Matsumoto AM, Clifton DK, Steiner RA (2003) Distribution and regulation of galanin receptor 1 messenger RNA in the forebrain of wild type and galanin-transgenic mice. Neuroscience 117: 105–117

Hokfelt T, Bartfai T, Crawley J (1998a) Galanin: basic research discoveries and therapeutic implications. New York Academy of Sciences, New York

Hokfelt T, Broberger C, Diez M, Xu ZQ, Shi T, Kopp J, Zhang X, Holmberg K, Landry M, Koistinaho J (1999) Galanin and NPY, two peptides with multiple putative roles in the nervous system. Horm Metab Res 31: 330–334

Hokfelt T, Millhorn D, Seroogy K, Tsuruo Y, Ceccatelli S, Lindh B, Meister B, Melander T, Schalling M, Bartfai T (1987) Coexistence of peptides with classical neurotransmitters. Experientia 43: 768–780

Hokfelt T, Xu ZQ, Shi TJ, Holmberg K, Zhang X (1998b) Galanin in ascending systems. Focus on coexistence with 5-hydroxytryptamine and noradrenaline. Ann N Y Acad Sci 863: 252–263

Holets VR, Hokfelt T, Rokaeus A, Terenius L, Goldstein M (1988) Locus coeruleus neurons in the rat containing neuropeptide Y, tyrosine hydroxylase or galanin and their efferent projections to the spinal cord, cerebral cortex and hypothalamus. Neuroscience 24: 893–906

Holmberg K, Kuteeva E, Brumovsky P, Kahl U, Karlstrom H, Lucas GA, Rodriguez J, Westerblad H, Hilke S, Theodorsson E, Berge OG, Lendahl U, Bartfai T, Hokfelt T (2005) Generation and phenotypic characterization of a galanin overexpressing mouse. Neuroscience 133: 59–77

Holmes A (2001) Targeted gene mutation approaches to the study of anxiety-like behavior in mice. Neurosci Biobehav Rev 25: 261–273

Holmes A, Heilig M, Rupniak NM, Steckler T, Griebel G (2003a) Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. Trends Pharmacol Sci 24: 580–588

Holmes A, Kinney JW, Wrenn CC, Li Q, Yang RJ, Ma L, Vishwanath J, Saavedra MC, Innerfield CE, Jacoby AS, Shine J, Iismaa TP, Crawley JN (2003b) Galanin GAL-R1 receptor null mutant mice display increased anxiety-like behavior specific to the elevated plus-maze. Neuropsychopharmacology 28: 1031–1044

Holmes A, Lachowicz JE, Sibley DR (2004) Phenotypic analysis of dopamine receptor knockout mice; recent insights into the functional specificity of dopamine receptor subtypes. Neuropharmacology 47: 1117–1134

Holmes A, Li Q, Koenig EA, Gold E, Stephenson D, Yang RJ, Dreiling J, Sullivan T, Crawley JN (2005) Phenotypic assessment of galanin overexpressing and galanin receptor R1 knockout mice in the tail suspension test for depression-related behavior. Psychopharmacology (Berl) 178: 276–285

Holmes A, Picciotto MR (2006) Galanin: a novel therapeutic target for depression, anxiety disorders and drug addiction. Curr Drug Targets CNS Neurol Disord 5: 225–232

Holmes A, Yang RJ, Crawley JN (2002) Evaluation of an anxiety-related phenotype in galanin overexpressing transgenic mice. J Mol Neurosci 18: 151–165

Holmes PV, Blanchard DC, Blanchard RJ, Brady LS, Crawley JN (1995) Chronic social stress increases levels of preprogalanin mRNA in the rat locus coeruleus. Pharmacol Biochem Behav 50: 655–660

Holsboer F (2003) The role of peptides in treatment of psychiatric disorders. J Neural Transm [Suppl] 50: 17–34

Hooi SC, Maiter DM, Martin JB, Koenig JI (1990) Galaninergic mechanisms are involved in the regulation of corticotropin and thyrotropin secretion in the rat. Endocrinology 127: 2281–2289

Husum H, Van Kammen D, Termeer E, Bolwig G, Mathe A (2003) Topiramate normalizes hippocampal NPY-LI in flinders sensitive line 'depressed' rats and upregulates NPY, galanin, and CRH-LI in the hypothalamus: implications for mood-stabilizing and weight loss-inducing effects. Neuropsychopharmacology 28: 1292–1299

Iismaa TP, Shine J (1999) Galanin and galanin receptors. Results Probl Cell Differ 26: 257–291

Jacoby AS, Hort YJ, Constantinescu G, Shine J, Iismaa TP (2002) Critical role for GALR1 galanin receptor in galanin regulation of neuroendocrine function and seizure activity. Brain Res Mol Brain Res 107: 195–200

Karlsson RM, Holmes A, Heilig M, Crawley JN (2005) Anxiolytic-like actions of centrally-administered neuropeptide Y, but not galanin, in C57BL/6J mice. Pharmacol Biochem Behav 80: 427–436

Kehr J, Yoshitake T, Wang FH, Razani H, Gimenez-Llort L, Jansson A, Yamaguchi M, Ogren SO (2002) Galanin is a potent in vivo modulator of mesencephalic serotonergic neurotransmission. Neuropsychopharmacology 27: 341–356

Khoshbouei H, Cecchi M, Dove S, Javors M, Morilak DA (2002a) Behavioral reactivity to stress: amplification of stress-induced noradrenergic activation elicits a galanin-mediated anxiolytic effect in central amygdala. Pharmacol Biochem Behav 71: 407–417

Khoshbouei H, Cecchi M, Morilak DA (2002b) Modulatory effects of galanin in the lateral bed nucleus of the stria terminalis on behavioral and neuroendocrine responses to acute stress. Neuropsychopharmacology 27: 25–34

Kolakowski LF Jr, O'Neill GP, Howard AD, Broussard SR, Sullivan KA, Feighner SD, Sawzdargo M, Nguyen T, Kargman S, Shiao LL, Hreniuk DL, Tan CP, Evans J, Abramovitz M, Chateauneuf A, Coulombe N, Ng G, Johnson MP, Tharian A, Khoshbouei H, George SR, Smith RG, O'Dowd BF (1998) Molecular characterization and expression of cloned human galanin receptors GALR2 and GALR3. J Neurochem 71: 2239–2251

Kordower JH, Le HK, Mufson EJ (1992) Galanin immunoreactivity in the primate central nervous system. J Comp Neurol 319: 479–500

Kordower JH, Mufson EJ (1990) Galanin-like immunoreactivity within the primate basal forebrain: differential staining patterns between humans and monkeys. J Comp Neurol 294: 281–292

Kuteeva E, Calza L, Holmberg K, Theodorsson E, Ogren SO, Hokfelt T (2004) Distribution of galanin and galanin transcript in the brain of a galanin-overexpressing transgenic mouse. J Chem Neuroanat 28: 185–216

Kuteeva E, Hokfelt T, Ogren SO (2005) Behavioural characterisation of young adult transgenic mice overexpressing galanin under the PDGF-B promoter. Regul Pept 125: 67–78

Larm JA, Shen PJ, Gundlach AL (2003) Differential galanin receptor-1 and galanin expression by 5-HT neurons in dorsal raphe nucleus of rat and mouse: evidence for species-dependent modulation of serotonin transmission. Eur J Neurosci 17: 481–493

Lu X, Barr AM, Bartfai T (2005a) Galanin receptors as novel drug targets for the treatment of depression and anxiety. Drug Dev Res: 227–236

Lu X, Barr AM, Kinney JW, Sanna P, Conti B, Behrens MM, Bartfai T (2005b) A role for galanin in antidepressant actions with a focus on the dorsal raphe nucleus. Proc Natl Acad Sci USA 102: 874–879

Lundberg JM, Terenius L, Hökfelt T, Goldstein M (1983) High levels of neuropeptide Y in peripheral noradrenergic neurons in various mammals including man. Neurosci Lett 42: 167–172

Makino S, Asaba K, Nishiyama M, Hashimoto K (1999) Decreased type 2 corticotropin-releasing hormone receptor mRNA expression in the ventromedial hypothalamus during repeated immobilization stress. Neuroendocrinology 70: 160–167

Maldonado R, Koob GF (1993) Destruction of the locus coeruleus decreases physical signs of opiate withdrawal. Brain Res 605: 128–138

Malendowicz LK, Nussdorfer GG, Nowak KW, Mazzocchi G (1994) The possible involvement of galanin in the modulation of the function of rat pituitary-adrenocortical axis under basal and stressful conditions. Endocr Res 20: 307–317

Manji HK, Drevets WC, Charney DS (2001) The cellular neurobiology of depression. Nat Med 7: 541–547

Mazarati A, Langel U, Bartfai T (2001) Galanin: an endogenous anticonvulsant? Neuroscientist 7: 506–517

Mazzocchi G, Malendowicz LK, Rebuffat P, Nussdorfer GG (1992) Effects of galanin on the secretory activity of the rat adrenal cortex: in vivo and in vitro studies. Res Exp Med (Berl) 192: 373–381

Melander T, Hokfelt T, Rokaeus A, Cuello AC, Oertel WH, Verhofstad A, Goldstein M (1986) Coexistence of galanin-like immunoreactivity with catecholamines, 5-hydroxytryptamine, GABA and neuropeptides in the rat CNS. J Neurosci 6: 3640–3654

Melander T, Hokfelt T, Rokaeus A, Fahrenkrug J, Tatemoto K, Mutt V (1985a) Distribution of galanin-like immunoreactivity in the gastrointestinal tract of several mammalian species. Cell Tissue Res 239: 253–270

Melander T, Staines WA, Hokfelt T, Rokaeus A, Eckenstein F, Salvaterra PM, Wainer BH (1985b) Galanin-like immunoreactivity in cholinergic neurons of the septum-basal forebrain complex projecting to the hippocampus of the rat. Brain Res 360: 130–138

Mennicken F, Hoffert C, Pelletier M, Ahmad S, O'Donnell D (2002) Restricted distribution of galanin receptor 3 (GalR3) mRNA in the adult rat central nervous system. J Chem Neuroanat 24: 257–268

Merchenthaler I, Lopez FJ, Negro-Vilar A (1993) Anatomy and physiology of central galanin-containing pathways. Prog Neurobiol 40: 711–769

Moller C, Sommer W, Thorsell A, Heilig M (1999) Anxiogenic-like action of galanin after intra-amygdala administration in the rat. Neuropsychopharmacology 21: 507–512

Murck H, Held K, Ziegenbein M, Kunzel H, Holsboer F, Steiger A (2004) Intravenous administration of the neuropeptide galanin has fast antidepressant efficacy and affects the sleep EEG. Psychoneuroendocrinology 29: 1205–1211

O'Donnell D, Ahmad S, Wahlestedt C, Walker P (1999) Expression of the novel galanin receptor subtype GALR2 in the adult rat CNS: distinct distribution from GALR1. J Comp Neurol 409: 469–481

O'Neal HA, Van Hoomissen JD, Holmes PV, Dishman RK (2001) Prepro-galanin messenger RNA levels are increased in rat locus coeruleus after treadmill exercise training. Neurosci Lett 299: 69–72

Palkovits M (2000) Stress-induced expression of co-localized neuropeptides in hypothalamic and amygdaloid neurons. Eur J Pharmacol 405: 161–166

Pieribone VA, Xu ZQ, Zhang X, Grillner S, Bartfai T, Hokfelt T (1995) Galanin induces a hyperpolarization of norepinephrine-containing locus coeruleus neurons in the brainstem slice. Neuroscience 64: 861–874

Ressler KJ, Nemeroff CB (2000) Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. Depress Anxiety 12 [Suppl 1]: 2–19

Saper CB, Chou TC, Scammell TE (2001) The sleep switch: hypothalamic control of sleep and wakefulness. Trends Neurosci 24: 726–731

Sergeyev V, Fetissov S, Mathe AA, Jimenez PA, Bartfai T, Mortas P, Gaudet L, Moreau JL, Hokfelt T (2005) Neuropeptide expression in rats exposed to chronic mild stresses. Psychopharmacology (Berl) 178: 115–124

Seutin V, Verbanck P, Massotte L, Dresse A (1989) Galanin decreases the activity of locus coeruleus neurons in vitro. Eur J Pharmacol 164: 373–376

Skofitsch G, Jacobowitz DM (1986) Quantitative distribution of galanin-like immunoreactivity in the rat central nervous system. Peptides 7: 609–613

Soares J, Holmes PV, Renner KJ, Edwards GL, Bunnell BN, Dishman RK (1999) Brain noradrenergic responses to footshock after chronic activity-wheel running. Behav Neurosci 113: 558–566

Sollenberg U, Bartfai T, Langel U (2005) Galnon-a low-molecular weight ligand of the galanin receptors. Neuropeptides 39: 161–163

Steiner RA, Hohmann JG, Holmes A, Wrenn CC, Cadd G, Jureus A, Clifton DK, Luo M, Gutshall M, Ma SY, Mufson EJ, Crawley JN (2001)

Galanin transgenic mice display cognitive and neurochemical deficits characteristic of Alzheimer's disease. Proc Natl Acad Sci USA 98: 4184–4189

Stenfors C, Theodorsson E, Mathe AA (1989) Effect of repeated electroconvulsive treatment on regional concentrations of tachykinins, neurotensin, vasoactive intestinal polypeptide, neuropeptide Y, and galanin in rat brain. J Neurosci Res 24: 445–450

Swanson CJ, Blackburn TP, Zhang X, Zheng K, Xu ZQ, Hokfelt T, Wolinsky TD, Konkel MJ, Chen H, Zhong H, Walker MW, Craig DA, Gerald CP, Branchek TA (2005) Anxiolytic- and antidepressant-like profiles of the galanin-3 receptor (Gal3) antagonists SNAP 37889 and SNAP 398299. Proc Natl Acad Sci USA 102: 17489–17494

Sweerts BW, Jarrott B, Lawrence AJ (1999) Expression of preprogalanin mRNA following acute and chronic restraint stress in brains of normotensive and hypertensive rats. Brain Res Mol Brain Res 69: 113–123

Sweerts BW, Jarrott B, Lawrence AJ (2000) Acute and chronic restraint stress: effects on [125I]-galanin binding in normotensive and hypertensive rat brain. Brain Res 873: 318–329

Tatemoto K, Rokaeus A, Jornvall H, McDonald TJ, Mutt V (1983) Galanin – a novel biologically active peptide from porcine intestine. FEBS Lett 164: 124–128

Toppila J, Stenberg D, Alanko L, Asikainen M, Urban JH, Turek FW, Porkka-Heiskanen T (1995) REM sleep deprivation induces galanin gene expression in the rat brain. Neurosci Lett 183: 171–174

Wang HY, Wild KD, Shank RP, Lee DH (1999) Galanin inhibits acetylcholine release from rat cerebral cortex via a pertussis toxin-sensitive G(i)protein. Neuropeptides 33: 197–205

Wang S, He C, Maguire MT, Clemmons AL, Burrier RE, Guzzi MF, Strader CD, Parker EM, Bayne ML (1997) Genomic organization and functional characterization of the mouse GalR1 galanin receptor. FEBS Lett 411: 225–230

Waters SM, Krause JE (2000) Distribution of galanin-1, -2 and -3 receptor messenger RNAs in central and peripheral rat tissues. Neuroscience 95: 265–271

Weiss JM, Bonsall RW, Demetrikopoulos MK, Emery MS, West CH (1998) Galanin: a significant role in depression? Ann N Y Acad Sci 863: 364–382

Weiss JM, Boss-Williams KA, Moore JP, Demetrikopoulos MK, Ritchie JC, West CH (2005) Testing the hypothesis that locus coeruleus hyperactivity produces depression-related changes via galanin. Neuropeptides 39: 281–287

Wirz-Justice A, Van den Hoofdakker RH (1999) Sleep deprivation in depression: what do we know, where do we go? Biol Psychiatry 46: 445–453

Wood GE, Shors TJ (1998) Stress facilitates classical conditioning in males, but impairs classical conditioning in females through activational effects of ovarian hormones. Proc Natl Acad Sci USA 95: 4066–4071

Wrenn CC, Crawley JN (2001) Pharmacological evidence supporting a role for galanin in cognition and affect. Prog Neuropsychopharmacol Biol Psychiatry 25: 283–299

Xu YL, Reinscheid RK, Huitron-Resendiz S, Clark SD, Wang Z, Lin SH, Brucher FA, Zeng J, Ly NK, Henriksen SJ, de Lecea L, Civelli O (2004) Neuropeptide S: a neuropeptide promoting arousal and anxiolytic-like effects. Neuron 43: 487–497

Yoshitake T, Reenila I, Ogren SO, Hokfelt T, Kehr J (2003) Galanin attenuates basal and antidepressant drug-induced increase of extracellular serotonin and noradrenaline levels in the rat hippocampus. Neurosci Lett 339: 239–242

Yoshitake T, Wang FH, Kuteeva E, Holmberg K, Yamaguchi M, Crawley JN, Steiner R, Bartfai T, Ogren SO, Hokfelt T, Kehr J (2004) Enhanced hippocampal noradrenaline and serotonin release in galanin-overexpressing mice after repeated forced swimming test. Proc Natl Acad Sci USA 101: 354–359

Zachariou V, Brunzell DH, Hawes J, Stedman DR, Bartfai T, Steiner RA, Wynick D, Langel U, Picciotto MR (2003) The neuropeptide galanin modulates behavioral and neurochemical signs of opiate withdrawal. Proc Natl Acad Sci USA 100: 9028–9033

Zachariou V, Thome J, Parikh K, Picciotto MR (2000) Upregulation of galanin binding sites and GalR1 mRNA levels in the mouse locus coeruleus following chronic morphine treatments and precipitated morphine withdrawal. Neuropsychopharmacology 23: 127–137

Zini S, Roisin MP, Langel U, Bartfai T, Ben-Ari Y (1993) Galanin reduces release of endogenous excitatory amino acids in the rat hippocampus. Eur J Pharmacol 245: 1–7

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