A role for galanin in antidepressant actions with a focus on the dorsal raphe nucleus

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Selective serotonin reuptake inhibitors, such as fluoxetine (FLX), are the most commonly used drugs in the treatment of major depression. However, there is a limited understanding of their molecular mechanism of action. Although the acute effect of selective serotonin reuptake inhibitors in elevating synaptic serotonin concentrations is well known, the clinical amelioration of depressive symptoms requires 14–21 days of treatment, suggesting that numerous other rearrangements of function in the CNS must take place. In the present study, we demonstrated that 14 days of FLX treatment up-regulated galanin mRNA levels by 100% and GalR2-binding sites by 50%, in the rat dorsal raphe nucleus, where galanin coexists with serotonin. Furthermore, a galanin receptor antagonist, M40, attenuated the antidepressant-like effect of FLX in the forced swim test, a rodent preclinical screen commonly used to evaluate antidepressant-like efficacy. Direct activation of galanin receptors by a galanin receptor agonist, galnon, was found to produce an antidepressant-like effect in the same task. Two other antidepressant treatments also affected the galaninergic system in the monoaminergic nuclei: Electroconvulsive shock elevated galanin mRNA levels in dorsal raphe nucleus, whereas sleep deprivation increased galanin mRNA levels in the locus coeruleus, further underlining the connection between activation of the galaninergic system and antidepressant action of various clinically proven treatments.

Our understanding of the molecular mechanism of action of fluoxetine (FLX), beyond its effect of elevating synaptic serotonin [5-hydroxytryptamine (5-HT)] concentration, is limited. The delay in the onset of clinical antidepressant effect suggests that transcriptional and translational events, leading to functional changes in signaling within the major serotoninergic nucleus dorsal raphe nucleus (DRN) and in its projection areas, may be required for these therapeutic effects (1–3). One potential player in mediating the long-term effects of FLX, besides 5-HT, is the neuropeptide galanin. Galanin, through its three G-protein-coupled receptors, GalR1, GalR2, and GalR3 (4), regulates homeostatic and motivated behaviors including pain perception, sleep, food intake, sexual activity, learning, and memory (5). Galaninergic transmission modulates the activity of monoaminergic neurons in the ventral tegmental area, DRN, and locus coeruleus (LC) (6–10). Galanin receptor subtypes GalR1 (7) and GalR2 are expressed in DRN neurons (11) that can be activated by galanin dendritically released from the dorsal raphe 5-HT neurons (9, 12) or from surrounding galanin immunoreactive terminals (7). In the noradrenergic nucleus LC, an area that is closely connected both structurally and functionally to DRN (13, 14), GalR1 expression is induced by morphine withdrawal (15), and the galanin receptor agonist, galnon, was shown to attenuate several withdrawal signs (16). It is worth noting that drug withdrawal often precipitates symptoms of depression, and depression is a commonly observed withdrawal symptom in humans (17, 18). In addition, decreased galanin expression in DRN, hippocampus, and hypothalamus have been observed in rat models of depression (19–21), and a recent clinical study reported preliminary evidence for an acute antidepressant effect of galanin (i.v.) in depressed patients (22), whereas a few early microdialysis and behavioral studies in rodents suggested depressive actions of galanin (6, 8, 23, 24). We have, however, recently observed that a systemically active galanin receptor agonist, galnic, in a dose that suppresses status epilepticus, has an antidepressant-like effect in the forced swim test (25).

To further explore the relevance of the galanin system for the treatment of depression, we first examined the effects of three clinically validated antidepressant treatments, sleep deprivation (24 h), electroconvulsive shock (four shocks daily for 2 days) and, the most commonly used, chronic FLX treatment (14 days), on the expression levels of galanin and its receptors in the DRN and LC of the rat. The length of each treatment was chosen to correlate with the onset of clinical benefit of each treatment and previous experience in the animal studies (26–29). To further examine the contribution of altered galaninergic signaling to the FLX-mediated antidepressant-like effect, we tested whether a galanin receptor antagonist, M40, can block the antidepressant-like effect of chronic FLX treatment (10 mg/kg i.p., 14 days) and whether a galanin receptor agonist, galnon, can exert an antidepressant-like effect in the rat forced swim test.

Materials and Methods

Animals. Adult male Sprague–Dawley rats (Harlan, Indianapolis), weighing 250–275 g, were given ad libitum access to food and water and were maintained on a 12 h light/dark cycle. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Sleep Deprivation and Electroconvulsive Shock. Sleep deprivation (24 h) was achieved by disturbing the rats whenever sleep behavior was observed. For electroconvulsive shock treatment, rats received four shocks bilaterally each day, delivered by using a constant current Ugo Basile apparatus for small mammals (Varese, Italy; 90 mA, 70 Hz, until a tonic-clonic seizure developed), separated by 1-h intervals, for 2 days.

Immunohistochemistry. Adult male rats were deeply anesthetized and perfused with 4% paraformaldehyde, and 30-μm coronal sections were cut with a cryostat. Sections were permeabilized in 0.1% Triton X-100 for 30 min, blocked with 10% normal goat serum for 1 h, and incubated with the following primary Abs: rabbit polyclonal galanin (Bachem; 1:5000) and mouse monoclonal tryptophan hydroxylase (Sigma; 1:1,000) overnight at room temperature. Goat anti-rabbit Alexa Fluor 594 and goat anti-mouse Alexa Fluor 488 (Molecular Probes) were used at 1: 1000 overnight at room temperature. Goat anti-rabbit Alexa Fluor 594 and goat anti-mouse Alexa Fluor 488 (Molecular Probes) were used at 1: 400 for 2 h at room temperature. The sections were examined with a confocal scanning microscope (Olympus, Melville, NY) equipped with the appropriate filter combinations.

Abbreviations: DRN, dorsal raphe nucleus; 5-HT, 5-hydroxytryptamine; FLX, fluoxetine; LC, locus coeruleus; ACSF, artificial cerebrospinal fluid; i.c.v., intracerebroventricularly; galanin-LI, galanin-like immunoreactivity.

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Tissue Dissections. Tissues used for RNA extraction or binding study were rapidly dissected after killing and immediately frozen on dry ice. All dissections were performed by an experienced neuropathologist with a rat brain slicer. For small structures, like LC and DRN, the punching method was used to assure reproducible dissection.

Quantitative Real-Time PCR. Brain tissue obtained from 12 rats (DRN, LC, and paraventricular nucleus of hypothalamus) or 6 rats (prefrontal cortex, amygdala, and hippocampus) were pooled, and total RNA was prepared with TRIZol reagent (Invitrogen) following manufacturer’s instructions. For galanin mRNA quantification in DRN and LC after FLX treatment, the experiment was repeated with three independent pools of four rats/treatment/brain region. Aliquots of total RNA (2 μg) and oligo(dT) primers were then reverse transcribed with ThermoScript RT (Invitrogen) at 51°C for 60 min. Quantitative PCR was performed by using a Roche Diagnostics LightCycler and LightCycler-Fast Start DNA Master SYBR Green I mix. Specific primers (β-actin: 5′-GGG TAC AGC TTC ACC ACC AC-3′ and 5′-TGCTCAGGAGGACTG-3′; and galanin: 5′-AGGCAAAGGGAATTACC-3′ and 5′-GGTGGCCAAAGGGAATG-3′) were designed to correspond to sequences on different exons to avoid amplification of possible genomic DNA contamination. Real-time PCR assays included an initial 10 min, 94°C step to activate Taq polymerase, followed by 35 cycles of denaturation at 94°C for 10 s, annealing at 58°C for 10 s, and extension at 72°C for 25 s. The results were expressed in arbitrary units normalized by the expression levels of the reference gene, β-actin.

Cell Lines. Stably transfected Chinese hamster ovary cells expressing rat GalR2, and Bowes’ melanoma cells that express human GalR1 were cultivated as described earlier (30, 31).

Membrane Preparation and Binding Assay. DRN and LC were dissected from a total of 16 rats/group and samples from four animals were pooled. Rat brain synaptic membranes (32) and cells were prepared as described (31). Equilibrium binding of [125]Galanin (2,200 Ci/mmol, PerkinElmer) to hippocampal membrane preparations was performed in 150 μl of binding buffer [50 mM TrisCl, pH 7.4/5 mM MgCl2/0.05% (wt/vol) BSA, supplemented with protease inhibitors]. Incubations were at room temperature for 45 min and terminated by rapid filtration through glass fiber filters (Packard, Meriden, CT). After three washes with cold PBS (pH 7.4) containing 0.01% (vol/vol) Triton X-100, the filter was counted with a Cobra II auto-γ-counting systems (Packard Bioscience, Downer Grove, IL). To determine the sum of GalR1 and GalR2 receptor sites in tissues, [125]Galanin was used at a saturating concentration of 1 nM, and nonspecific binding was determined in the presence of 5 μM of galanin (1-29). The number of GalR2 sites was estimated by using 5 μM of the highly selective GalR2 ligand, galanin (2-11) (33), as a competitor. Because GalR3 is not abundant in the regions tested, GalR1 sites were estimated by subtracting GalR2 sites from the sum of GalR1 and GalR2 receptor sites. The selectivity of galanin (2-11) for the GalR2 receptors over GalR1 receptors in the applied concentration has been confirmed by binding assays with GalR1- and GalR2-expressing cells (34).

Surgery. Rats underwent aseptic stereotaxic surgery under ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) anesthesia. A guide cannula 1.4 cm in length, of 26-gauge stainless steel hypodermic tubing (Plastics One, St. Louis) was implanted into the right lateral ventricle at coordinates 0.5 mm posterior, 1.0 mm lateral to bregma, and 3.5 mm ventral to the surface of the skull. A 31-gauge stylet was secured to the guide cannula after the surgery. After surgery, rats were given at least 8 days recovery before the start of behavioral testing.

Drug Treatments. FLX (Sigma) was dissolved in saline and administered i.p. at a dose of 10 mg/kg daily for 14 days for biochemical studies. Separate groups of rats received daily i.p. injections of FLX or saline vehicle for 14 days for testing in the forced swim task. On day 14, rats were administered FLX or saline i.p. at 45 min before the forced swim test and M40 (Bachem) or artificial cerebrospinal fluid (ACSF) intracerebroventricularly (i.c.v) at 15 min before the forced swim test (see methods below). Single i.c.v. microinjections of M40 (8 nmol/2 μl ACSF) or ACSF (2 μl) were performed with a 10-μl Hamilton syringe connected by means of Becton Dickinson polyethylene tubing (PE20) to a 1.5-cm injector (Plastics One) fabricated from 31-gauge hypodermic tubing. The animal was allowed to freely explore a small cage during the infusion of 2 μl over 1.5 min, with each 1 μl separated by 10 s, and an additional 60 s before the injector was withdrawn from the guide cannula. The treatments consisted of equal numbers of rats receiving one of the following combinations of treatments: saline plus ACSF, saline plus M40, FLX plus ACSF, or FLX plus M40. Additional treatment groups (n = 10/group) were examined in a separate forced swim test 45 min after i.p. injection of one of the following: 50% DMSO (wt/vol), galnon (Valpes, Estonia; dissolved in 50% DMSO), or desipramine (Sigma, 15 mg/kg in 50% DMSO). After the forced swim test (1 wk), rats were randomly assigned to treatment groups. Rats were treated with vehicle; galnon, or desipramine. After injection (45 min), rats were subjected to the open field test.

Forced Swim Test. Rats were placed singly in a cylindrical glass container (48 cm height and 21 cm diameter) with tap water (25 ± 1°C) to a depth of 27 cm and tested for 10 min. Water was changed and the cylinder thoroughly rinsed after each test. Tests were recorded on videotape and later scored by an experienced observer blind to the experimental conditions of the rats. Behaviors were scored as either (i) active: characterized as when the rat engages in escape behaviors, typically evident by forepaws splashing above the surface of the water or (ii) inactive: described by minimal locomotor activity. The presence of antidepressant activity in a drug is inferred from its capacity to increase the fraction of time spent in active behavior (35).

Open Field Test. One week after the forced swim test, rats were randomly assigned to treatment groups. Rats were treated with vehicle, galnon, or desipramine: 45 min after injection, rats were placed in an open field. The apparatus consisted of a square arena (100 × 100 cm) with a 40 cm high opaque white wall. The floor was marked into nine equal segments and fluorescent light provided diffuse overhead illumination. Locomotor activity over 5 min was recorded on videotape. Tapes were later scored blind to the experimental condition of the animals. Line-crossing behavior (defined as at least three paws in a square) was tallied and compared between experimental conditions.

Statistical Methods. Data for forced swim test studies by using galnon and desipramine were subject to an ANOVA, with drug treatment as the between-subjects factor. For the M40-FLX study, data were subject to a two-factor between-subjects design, with chronic drug treatment (FLX vs. vehicle) and i.c.v. drug (M40 vs. ACSF) as the main factors. Significant main effects were followed up with Fisher’s least significant difference post hoc test to determine specific group differences. Data for the GalR1- and GalR2-binding sites in DRN and LC after FLX treatment were analyzed by the Student’s t test with the Bonferroni correction applied to control α-level for multiple comparisons with single control group.

Results
Galanin-Like Immunoreactivity (Galanin-LI) Is Abundant in Serotonergic and Noradrenergic Nuclei. Previous immunohistochemical studies on colchicine-treated animals have shown that galanin is widely distributed in the CNS, and galanin-LI usually colocalizes...
with cholinergic, catecholaminergic, and serotonergic markers (9, 10). Using double immunofluorescent labeling techniques, we reexamined the distribution of galanin-LI in the noradrenergic and serotonergic systems in noncolchicine-treated naive rats. In the DRN, as expected from the labeling of serotonergic neurons, tryptophan hydroxylase immunoreactivity was mainly present in cell bodies and primary dendrites. Most tryptophan hydroxylase positive neurons exhibited moderate galanin-LI, whereas nonsero- tonergic galanin-LI fibers and cell bodies were also present (Fig. 1A). In the LC, strong galanin-LI was observed in both cell bodies and fibers, showing almost complete colocalization with the noradrenergic marker, dopamine-β-hydroxylase (Fig. 1B). The merged images demonstrated that almost all of the noradrenergic and serotonergic neurons in the LC were galanin positive.

Quantification of Galanin mRNA in the Rat Brain. Galanin mRNA expression levels, among the six brain regions tested, were highest in paraventricular nucleus of hypothalamus, LC, and DRN (Fig. 2A). High expression levels of galanin mRNA in hypothalamus, LC, and DRN have previously been suggested by Northern blot analysis and in situ hybridization (36, 37). Amygdala and hippocampus have similar levels of galanin mRNA, both representing ∼13% of that of DRN. Galanin mRNA expression was also detected in the prefrontal cortex, with roughly 3% of the DRN level.

Antidepressant Treatments Up-Regulate Galanin mRNA Expression in Several Brain Regions. The effects of three clinically relevant antidepressant treatments, i.e., chronic FLX i.p. injections for 14 days, 24-h sleep deprivation, or electroconvulsive shock for 2 days (four shocks daily with 1 h interval), on galanin mRNA expression were analyzed by using real-time PCR (Fig. 2B). In prefrontal cortex, DRN, and LC, two of three antidepressant treatments produced a marked increase in galanin mRNA levels. In the DRN, the electroconvulsive shock and chronic FLX treatment had similar effects, resulting in a 2-fold increase; in the LC, FLX and sleep deprivation resulted in a 2.2- and 1.8-fold increase, respectively. Additional studies were conducted to better quantify the effects of chronic FLX administration on galanin mRNA levels in DRN and LC on separate pools of tissue samples from animals treated with saline and FLX. The results (Fig. 2C) were similar to those obtained from larger pools of samples (Fig. 2B).

Chronic FLX Treatment Increases GalR2-Binding Sites in DRN. In control animals, both LC and DRN showed high levels of galanin-binding sites (Fig. 3), with GalR1 sites estimated at 58 fmol/mg protein in DRN and 72 fmol/mg protein in LC. Approximately one-third of total galanin-binding sites corresponded to GalR2 receptors (33% in DRN and 32% in LC), a finding that is consistent with previous observations on GalR1 and GalR2 mRNA distribution obtained by using in situ hybridization (11, 38).

In the DRN, FLX treatment significantly increased the number of GalR2 sites by 58%, without changes in the number of GalR1-
binding sites (Fig. 3). Electroconvulsive shock had no effect on either GalR1 or GalR2 sites. In the prefrontal cortex, hippocampus, and amygdala, none of the antidepressant treatments led to significant changes in the levels of either GalR1 or GalR2 receptors (data not shown).

Galanin Receptor Antagonist, M40, Attenuated Antidepressant-Like Effect of FLX in the Forced Swim Test. The Kᵢ of M40 for GalR1 and GalR2 receptors were 1.82 nM and 5.1 nM, respectively (Fig. 4A). The data on the effects of FLX and M40 in the forced swim test (Fig. 4B) was subjected to a two-factor ANOVA. Results indicated a significant main effect of treatment \[F(1,140) = 5.568, P < 0.05\] and a significant interaction between pretreatment (FLX or saline) and treatment (M40 or ACSF) \[F(1,140) = 5.520, P < 0.05\]. Subsequent post hoc analysis showed that FLX, when administered at 10 mg/kg (i.p. 14 days), produced a significant increase in the time that rats spent active in the forced swim test, suggestive of an antidepressant-like effect \((P < 0.05, \text{FLX plus ACSF vs. saline plus ACSF})\). The galanin receptor antagonist M40, when infused 15 min before the forced swim test, at a dose shown to block galanin-induced increase in food intake (8 nmol, i.c.v.), significantly attenuated the increased time spent active in the forced swim test, thus antagonizing the putative antidepressant-like effect of FLX \((P < 0.01, \text{FLX plus ACSF vs. FLX plus M40})\) (Fig. 4B).

**Galanon, a Galanin Receptor Agonist, Shows Behavioral Effects in Forced Swim Test and Open Field Test.** The systemically active, nonpeptide galanin receptor agonist, galnon, exhibited moderate affinity for both GalR1 (39) and GalR2. We observed that galnon displaces \([^{125}I]\)galanin from both GalR1 and GalR2 receptors with \(Kᵢ\) of 11.7 \(\mu\)M and 34.1 \(\mu\)M, respectively (data not shown).

Galonon, in doses ranging from 1 to 40 mg/kg i.p., was tested in the forced swim test with desipramine (15 mg/kg, i.p.) as a positive control (Fig. 5). The data from the forced swim test after galnon administration were subjected to an ANOVA, with drug treatment as the factor. Results of the ANOVA indicated a significant main effect of drug treatment \((P < 0.01)\). Desipramine exhibited an 46% increase in time spent active in the forced swim test compared to saline-pretreated rats; this effect was significant \((P < 0.05, \text{Fisher's least significant difference (LSD) test})\). This effect of FLX pretreatment was completely reversed by i.c.v. administration of M40. **, Significance of FLX/ACSF vs. FLX/M40, \(P < 0.01\), Fisher’s LSD.

**Activity was measured during a 10 min test. Data represent group means (± SEM) of percentage of time spent active in forced swim test. *, Significance between saline/ACSF vs. FLX/ACSF. Rats pretreated for 14 days with FLX (10 mg/kg) or saline and given single i.c.v. infusion of the galanin receptor antagonist M40 or vehicle (ACSF) 15 min before testing in the forced swim test. Activity was measured during a 10 min test. Data represent group means (± SEM) of percentage of time spent active in forced swim test. **, Significance of FLX/ACSF vs. FLX/M40, \(P < 0.01\), Fisher’s LSD.**
effect of drug treatment \([F(7,61) = 3.35, P < 0.01]\). Post hoc analysis indicated that increasing doses of galnon tended to decrease activity in the open field, as activity was significantly reduced in the two higher doses of 20 mg/kg \((P < 0.01)\) and 40 mg/kg \((P < 0.05)\) galnon. Desipramine also produced a significant reduction \((P < 0.05)\) of locomotor activity, an effect that has been consistently described in the literature \((40)\).

Discussion

In the present study, we have confirmed previous reports that the DRN and LC express high levels of galanin-LI \((9, 10)\) and that galanin-LI is colocalized partially with tryptophan hydroxylase immunoreactivity in the DRN and almost completely with dopamine \(\beta\)-hydroxylase immunoreactivity in the LC \((1)\). We observed a 100% increase in galanin mRNA in the DRN after two antidepressant treatments: electroconvulsive shock for 2 days and FLX treatment for 14 days \((2)\). A similar large increase in galanin mRNA in the LC was induced after treatment with FLX \((14\) days) and sleep deprivation \((24\) h). Previous studies have demonstrated that increase in galanin mRNA expression is associated with an increase in galanin synthesis and release \((41–43)\). The finding that, three different antidepressant treatments strongly elevated galanin mRNA levels at time points when clinically relevant antidepressant effect become effective, suggests that their antidepressant-like activity might be associated with induction of galanin synthesis and release.

Concurrent with the increase in galanin peptide expression, we have also detected an increase in GalR2-binding sites after FLX treatment \((3)\). Simultaneous increases in galanin expression and GalR2 binding may indicate that the GalR2 is not a readily desensitizing receptor subtype. GalR1 receptor sites in DRN were unaltered by chronic FLX treatment and remained at pretreatment levels, whereas GalR2 receptor-binding sites were elevated by 50%, resulting in a relative shift in the effects of galanin on DRN neurons toward a greater influence exerted through GalR2. As GalR1 acts through the G\(_\text{i1}\)-mediated inhibition of adenyl cyclase, whereas GalR2 acts through G\(_\text{q11}\)-mediated increase in inositol phosphate and intracellular Ca\(^{2+}\) concentrations \((30)\), we predict that the FLX treatment induced "bias" toward increased GalR2-mediated influence on DRN neurons would result in increased firing rates in these neurons. Indeed, excitatory effects of galanin, exerted probably through GalR2, on neurotransmitter release have been reported in some brain regions \((44, 45)\). Furthermore, activation of dorsal hippocampal GalR2 receptors facilitates cognition whereas activation of GalR1 in the ventral hippocampus impairs cognitive performance \((45)\), suggesting that a change in the overall action of galanin would occur when the balance between GalR1 and GalR2 is altered.

The relevance of the above findings, which links increases in galanin mRNA and GalR2 receptors in the DRN to the antidepressant-like effect of FLX, was underscored by the findings that the galanin receptor antagonist, M40 \((46)\), attenuated the antidepressant-like effect of FLX in the forced swim test \((4)\). These data suggest that the increased galanin mRNA and GalR2 are relevant for the antidepressant-like effect of this widely used antidepressant drug.

Galanin, the low molecular weight, galanin receptor agonist, displayed antidepressant-like effect in the forced swim test \((5)\). Galnon, in similar doses has been shown to inhibit signs of opiate withdrawal \((16)\) and to increase the latency of phenylethanolamine-induced seizures \((39)\). Galnon is a nonselective agonist for GalR1/ GalR2, and thus its acute antidepressant-like effect is probably similar to the effects of increased galanin release, enhanced by antidepressant treatment. In the acute forced swim test, GalR2 levels are unlikely to be adjusted as they are after 14 days treatment with FLX because of the short time available between injection and testing, and thus, a change in GalR1:GalR2 ratio in the DRN is not likely to explain the antidepressant-like effect of galnon. Other aspects of galanin actions might contribute to the acute effect, i.e., through a direct postsynaptic effect on the prefrontal area, where galanin mRNA levels were also elevated after antidepressant treatments \((2)\). A synergistic effect between galanin and 5-HT1A receptor activation has been reported \((47)\). In addition, the effects of galanin in hypothalamus may contribute to antidepressant-like effect as galanin regulates rapid eye movement sleep \((22, 48, 49)\), feeding \((50)\), and hormone release \((50)\), all of which are disturbed in depression \((17)\). It has been reported that galanin exerts antidepressant-like effect through suppression of REM sleep \((22, 48, 49, 51)\). Other hypothalamically abundant neuropeptides such as corticotrophin-releasing factor \((52)\) and arginine vasopressin \((53)\) have also been implicated in mood regulation. Double immunolabeling studies have showed that whereas the coexistence between corticotrophin-releasing factor and galanin is very low \((54)\), the coexistence between arginine vasopressin and galanin is very common in the paraventricular nucleus \((55)\), and it has been shown that the arginine vasopressin release can be regulated by galanin \((56)\). On the other hand, a recent study showed that REM sleep deprivation selectively up-regulates galanin expression without affecting the mRNA levels of either corticotrophin-releasing
factor or arginine vasopressin (51), suggesting that a galaninergic antidepressant-like effect might be achieved without the influence of these additional neuropeptide systems.

In our study, the three antidepressant treatments, similar to the numerous studies on the mechanisms of action of antidepressant treatments, were carried out on naive, “nondepressed” animals. Despite the differences in the neurochemistry of “depressed” and “naive” brain, antidepressant drug screening in naive animals has been proven useful (57). In agreement with our data from naive animals, an antidepressant effect of i.v. applied galanin in depressed patients was found (22).

In summary, our study demonstrated that galaninergic transmission in the DRN and LC is enhanced by several different antidepressant treatments within time frames that are relevant to their therapeutic effects. In addition, a galanin receptor antagonist, M40, blocked the antidepressant-like effect of FLX in the rat forced swim test, suggesting that the galaninergic system contributes to the antidepressant-like effect of FLX. Finally, a galanin receptor agonist produced an antidepressant-like effect in the same behavioral test. The combined data suggests that the galaninergic system is a putative target for antidepressant therapies, and further studies on various animal models and clinical studies by using selective galanin receptor ligands will be needed to further validate our conclusion.

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