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Galanin and epilepsy

J. T. Lerner, R. Sankar and A. M. Mazarati*

Dept. of Pediatrics, Neurology Division, D. Geffen School of Medicine at the University of California, Los Angeles, BOX 951752, 22-474 MDCC, Los Angeles, California 90095-1752 (USA), Fax: +1 310 825 5834, e-mail: mazarati@ucla.edu

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Abstract. Neuroanatomical localization and physiological properties of galanin suggest that the peptide may be involved in the regulation of seizures. Indeed, administration of galanin receptor agonists into brain areas pertinent to the initiation and propagation of epileptic activity attenuated seizure responses under conditions of animal models of epilepsy; pharmacological blocking of galanin receptors exerted proconvulsant effects. Functional deletion of both galanin and galanin type 1 receptor genes produced transgenic mice with either spontaneous seizure phenotype, or with enhanced susceptibility to seizure stimuli. At the same time, overexpression of galanin in seizure pathways, using both transgenic and virus vector transfection techniques, hindered the epileptic process. Galanin exerts anticonvulsant effects through both type 1 and type 2 receptors, with distinct downstream signaling cascades. Several synthetic agonists of galanin receptors with optimized bioavailability have been synthesized and inhibited experimental seizures upon systemic administration, thus opening an opportunity for the development of galanin-based antiepileptic drugs. (Part of a Multi-author Review)

Keywords. Galanin, galanin receptors, epilepsy, animal models, hippocampus.

Introduction

Initial experimental evidence implicating galanin in the regulation of seizure activity came a decade after the peptide had been discovered by Tatemoto and colleagues [1]. First, Mazarati et al. described anticonvulsant effects of intrahippocampal administration of galanin under conditions of the kindling model of epilepsy [2]. Afterwards, Zini et al. showed that bath application of galanin receptor (GalR) agonists inhibited depolarization-induced glutamate release from rat hippocampal slices, thus suggesting that galanin presynaptically inhibited excitatory glutamatergic neurotransmission [3]. Since then, the accumulation of data on the involvement of galanin in the epileptic process paralleled progress in understanding the physiology, biochemistry and pharmacology of galanin and its receptors. Today, galanin firmly occupies its place among other anticonvulsant neuropeptides.

Galanin in temporal lobe epilepsy

Temporal lobe epilepsy (TLE), which is primarily associated with hippocampal pathology, is one of the most commonly occurring forms of the disease. TLE bears several features that make it a particularly important subject of epilepsy research. TLE develops after a variety of precipitating insults, such as status epilepticus (SE), traumatic brain injury, infection, prolonged febrile seizures in childhood and stroke; it is not linked to any definite genetic trait, and thus might affect practically anyone. Most importantly, TLE is frequently refractory, that is resistant to currently available antiepileptic drugs; drug resistance represents a major challenge in managing of the disease in at least one-third of patients. These features of TLE dictate the importance of studies pursuing

* Corresponding author.

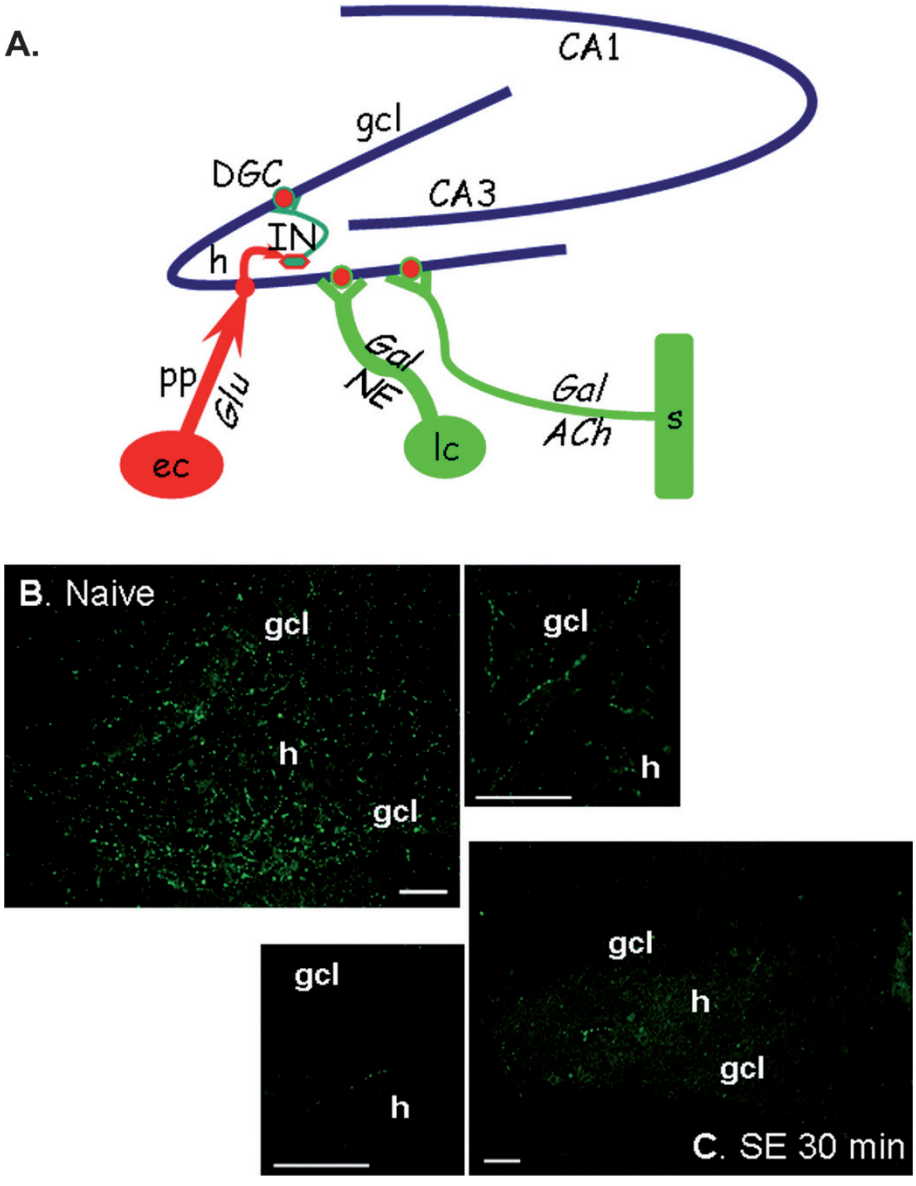

Figure 1. Galaninergic innervation of the rat hippocampus under normal and seizure conditions. (*A*) Anatomical substrate of anticonvulsant effects of galanin in the hippocampus. Dentate granule cells (red circles in the granule cell layer) receive excitatory glutamatergic input from the entorhinal cortex in a form of a perforant path. In the hilus dentate granule cells excite GABAergic interneurons, which in turn inhibit dentate granule cells. At the same time, the dentate gyrus receives galaninergic innervation, which comes with noradrenergic fibers from locus coeruleus and cholinergic fibers from the septum/diagonal band complex. **ec**, entorhinal cortex; **pp**, perforant path; **gcl**, granule cell layer; **h**, hilus; **lc**, locus coeruleus; **s**, septum/diagonal band; DGC, dentate granule cells; IN, interneuron; Glu, glutamate; Gal, galanin; NE, norepinephrine; Ach, acetylcholine. Excitatory projections are shown by red and inhibitory by green lines. (*B*) Galanin immunoreactivity in naïve rat dentate gyrus. **gcl**, granule cell layer; **h**, hilus. Scale bar: 50 micron. (*C*) Perforant path stimulation-induced seizures lead to rapid depletion of galanin in the dentate gyrus. Immunohistochemical galanin labeling in the animal 30 min after the end of perforant path stimulation. Reproduced from [15], © Elsevier with permission.

both its mechanisms and the development of new, more effective therapies.

Within the hippocampus, dentate gyrus represents a major gateway in the propagation of seizure activity [4]. At the same time, dentate gyrus receives galaninergic innervation from two major sources: catecholaminergic input from the locus coeruleus [5, 6], and cholinergic projection from the septum/diagonal band complex [7–9]. The highest density of galanin-immunoreactive fibers is found in the dentate granule cell layer (Fig. 1B). Dentate granule cells, in turn, receive excitatory glutamatergic input from the entorhinal cortex [10]. Seizures can be easily evoked in both rats and mice by brief electrical stimulation of the entorhinal cortex-dentate gyrus projection, known as perforant path [11, 12]. Neuroanatomical localization of galanin and its inhibitory effects on glutamatergic transmission suggest that the shift in the balance between glutamatergic excitation and galaninergic inhibition in the dentate gyrus in favor of the former may contribute to the progression of seizures. Conversely, inhibition of seizures originating from the hippocampus may be achieved through the activation of GalR.

The first compelling evidence that galanin indeed regulates seizures emerged from experiments involving SE in rodents. Experimental limbic SE is a convenient model for studying the effects of neuropeptides on seizure activity. In fact, SE may be regarded as a model of acute epileptogenesis, with broader implications for the mechanisms of limbic epilepsy.

SE consists of two phases – initiation, which depends on the initial epileptogenic stimulus (such as electrical stimulation of perforant path, activation of cholinergic transmission by pilocarpine or activation of AMPA/kainate receptors by kainic acid), and maintenance, which does not depend on initial epileptogenic stimulation [13]. The mechanisms of the transition from the initiation to the maintenance phase are complex and are far from being understood; however, it is agreed that this transition, as well as self-perpetuation of seizures once they have been established, depends on the activation of N-methyl-D-Aspartate receptors [13].

Mazarati et al. [14] reported that SE leads to the profound depletion of galaninergic innervation of the dentate gyrus as early as after 3 h of self-sustaining seizures. Our recent observations [15] found that such depletion occurs even earlier – after only 30 min of seizures, i.e. roughly coincides with the transition from the initiation to the maintenance phase of SE (Fig. 1C). Furthermore, intrahippocampal administration of GalR agonists during this transition time rapidly and irreversibly aborted seizures [14, 16]. Taken together these data suggested that galanin may indeed act as an anticonvulsant peptide, and that fatigue of galaninergic innervation of the hippocampus may contribute to the progression of seizures.

Direct evidence that endogenous galanin is capable of counteracting seizure activity came from experiments involving mutant animals (Fig. 2A–D). Mice with the functional disruption of the galanin gene showed higher seizure susceptibility than their wild-type littermates, evidenced as shorter time needed to induce seizures and higher severity of the convulsions [11]. In contrast, mice which overexpressed galanin under dopamine beta hydroxylase promoter, i.e. in catecholaminergic pathways, showed enhanced resistance to seizure induction [11]. Overexpression of galanin under platelet-derived growth factor B promoter (thus not confined to the catecholaminergic system) inhibited the progression of kindled seizures, and increased after-discharge threshold upon hippocampal stimulation [17, 18], thus confirming the anti-kindling action of galanin.

An elegant approach further exploring the anticonvulsant role of galanin was applied in studies which used adeno-associated virus (AAV) vector carrying the galanin gene to transfect neurons in rats *in vivo*. Haberman et al. [19] cloned a coding sequence of galanin into the AAV vector, which also contained fibronectin secretory signal sequence (FIB), to ensure not only expression but also active secretion of galanin into the extracellular space. *In vivo* injection of AAV-FIB-galanin vector into the inferior colliculus of the rat significantly attenuated seizures induced by focal electrical stimulation of this brain area. In contrast, administration of AAV-galanin vector, which led to the overexpression of galanin but not to the increased secretion of the peptide, had no anticonvulsant effects. Later, McCown [20] found that the administration of AAV-FIB-galanin in to the pirifirm cortex attenuated seizures induced by kainic acid. At the same time Lin et al. [21] found that simple overexpression of galanin induced by AAV-Neuron Specific Enolase (NSE)-galanin vector in the rat dentate gyrus neurons (both hilar interneurons and dentate granule cells) was sufficient to mitigate the severity of focal convulsions induced by intrahippocampal administration of kainate. Using the same experimental paradigm to achieve overexpression of galanin in neurons, Kanter-Schlifke et al. [22] reported that animals with focal overexpression of galanin in the hippocampus resulted in the overall decrease of hippocampal excitability, evidenced as the shortening of hippocampal after-discharge duration and delayed onset of kindled seizures. The result suggested that the secretion of galanin, which was presumably induced by seizures [23] from neurons expressing galanin *de novo*, was sufficient to inhibit focal epileptic activity. In the light of these data, it is worth mentioning that seizures themselves led to the *de novo* expression of galanin in the hippocampal inhibitory interneurons [14, 24, 25]. The latter phenomenon may represent a mechanism purposed to compensate for the above-mentioned fatigue of galaninergic innervation resulting from seizures, and to form an intrinsic hippocampal galanin inhibitory circuit.

Insight in how exactly galanin might inhibit seizures was obtained from galanin transgenic mice. It happened that the altered susceptibility to seizures was in direct correlation with glutamate release from hippocampal slices obtained from these animals (Fig. 2 E, F). Thus, depolarization of hippocampal slices induced by bath application of 60 mM K^+ resulted in significantly larger glutamate release in galanin-knockout mice, as compared with wild-type littermates, while hippocampal slices obtained from galanin-overexpressing animals failed to show any increase in the release of glutamate [11]. These data suggested that the anticonvulsant effects of galanin occurred through presynaptic inhibition of excitatory neurotransmission.

Differential involvement of galanin receptor subtypes in the modulation of seizure activity

Out of three GalR subtypes cloned to date, two subtypes, GalR1 and GalR2 are expressed in the hippocampus [6, 26–30]. Early studies attempted to

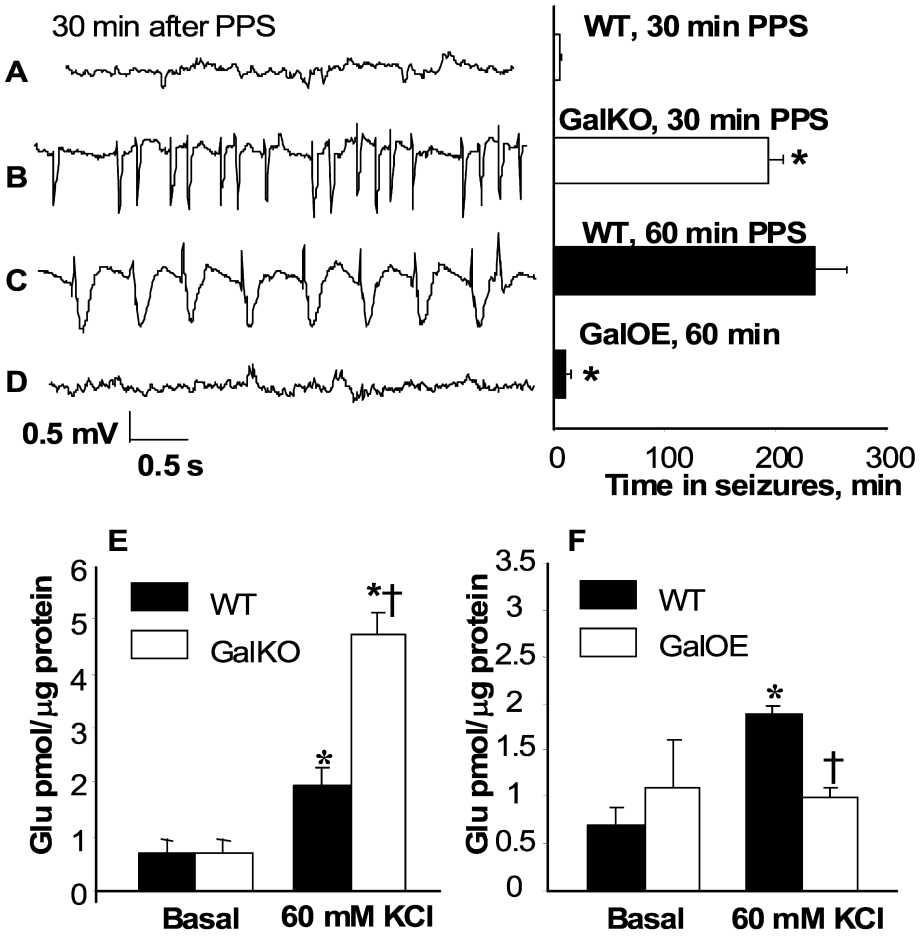

Figure 2. Altered seizure susceptibility and excitability of the hippocampus in galanin transgenic mice. (*A*–*D*) Seizures induced by perforant path stimulation (PPS) in wild-type (WT), galanin knockout (GalKO) and galanin-overexpressing (GalOE) mice. On the left, examples of electrographic activity 1 h after the end of PPS. On the right, duration of self-sustaining seizures upon cessation of 30 min (*A*, *B*) or 60 min (*C*, *D*) PPS. In WT animals 30 min PPS failed to produce self-sustaining seizures (*A*). GalKO developed seizures which lasted between 4 and 5 h, upon 30 min PPS (*B*). 60 min PPS, which was sufficient to induce self-sustaining seizures in WT animals (*C*), produced only brief seizures in GalOE (*D*). (*E*, *F*) Glutamate release from hippocampal slices of WT, GALKO (*E*) and GalOE (*F*) mice. While no differences in glutamate release were observed under resting conditions (Basal), GalKO showed 6–9-fold increase of glutamate release upon 60 mM K^+-induced depolarization, in contrast to WT, which showed 2.5–3-fold increase. GalOE failed to respond to the depolarization with the increase of glutamate release. *$p < 0.054$ vs. basal, +$p < 0.05$ vs. WT. Reproduced from [11, 14], © Society for Neuroscience, with permission.

identify receptor subtype specificity of anticonvulsant effects of galanin. Thus, Zini et al. [3, 31] found that glutamate release from hippocampal slices was inhibited not only by galanin (1–29), but also by galanin (1–16), which later was shown to prefer GalR2 over GalR1 [32]. Mazarati et al. [14, 33] showed that preferential GalR2 agonists Ala-2-galanin (1–29), D-Trp-2-galanin (1–29), and galanin (2–11) [32, 34–36] were as effective as galanin (1–29) in inhibiting seizures. At the same time, anticonvulsant effects of non-selective synthetic GalR agonist galnon were attenuated by *in vivo* pretreatment with anti-GalR1 antisense [37], thus suggesting the involvement of GalR1 in the inhibition of seizures.

The development of GalR1 knockout mice [38, 39] afforded further understanding the role of galanin receptor subtypes in epileptogenesis. Remarkably, GalR1 knockout mice of C57bl/j6 background exhibited spontaneous seizures with 25% penetrance. These animals showed a cascade of the changes in the expression of neuropeptides, similar to that observed in models of limbic epilepsy [40]. Furthermore, spontaneously seizing GalR1 knockout mice exhibited reduced frequency of miniature inhibitory postsynaptic currents (IPSCs) in hippocampal CA1 pyramidal neurons, thus implicating impaired synaptic inhibition in seizure phenotype [41]. Mazarati et al. [42] showed that even spontaneous seizure-free subpopulations of GalR1 knockout animals developed more severe and longer-lasting seizure activity, and

more profound seizure-induced hippocampal neuronal injury under conditions of pilocarpine and perforant path stimulation- induced SE.

The role of GalR2 in seizures was addressed by Mazarati et al. [33], who used an antisense approach, particularly peptide nucleic acid (PNA) antisense, to downregulate GalR2 in the hippocampus *in vivo*. PNA is a DNA or RNA mimic, which binds to DNA or RNA in complementary antiparallel fashion, thus inhibiting transcription or translation [43]. PNA targeted at mRNA encoding GalR2 at positions 18–38 was administered into the dentate gyrus of the rat over a 1-week period. This resulted in a 50% decrease of GalR2 binding in the infused hippocampus, without affecting GalR1 binding. While GalR2 PNA did not modify the threshold for seizure induction, it dramatically increased both the severity and the duration of SE induced by hippocampal stimulation, as compared to the missense-treated controls. These results supported the idea that GalR2 inhibited SE during its maintenance phase, and did not affect the initiation phase, as suggested for GalR1.

At the same time, Gottsch et al. [44] observed neither spontaneous seizures nor altered response to pentyleneterazole and flurothyl-induced convulsions in GalR2 knockout mice.

In contrast to the dramatic changes in galanin expression and distribution in the hippocampus induced by seizures, seizure activity did not significantly alter the expression of hippocampal galanin receptors. Thus, pilocarpine-induced SE led to a 30% downregulation of GalR2, and did not affect GalR1 [45]. Such preservation of target for the action of galanin in epileptic hippocampus further justifies the utility of prospective galanin-based antiepileptic drugs.

Signaling cascades that mediate anticonvulsant effects of galanin

The first plausible scenario explaining why and how galanin might inhibit seizures was proposed by Zini et al. [3, 31], who showed that galanin inhibited depolarization-induced glutamate release from hippocampal slices, and that this effect was prevented by co-application of glybenclamide, a blocker of ATP-dependent K^+ channels (KATP). These results implied that in the hippocampus galanin opens KATP, which in turn leads to membrane hyperpolarization and ultimately to the inhibition of glutamate release from presynaptic terminals. Furthermore, galanin was shown to directly close voltage-gated Ca^{2+} channels [46], which would also hyperpolarize presynaptic membrane and impede glutamate release.

Using kindling as a model of epileptogenesis, Mazarati et al. [47] explored signaling cascades that might mediate antiepileptic effects of galanin. Thus chimeric peptide M617 (galanin(1–13)-Gln14-bradykinin(2–9)-amide) [48]) delayed but did not block acquisition of hippocampal kindling in rats. The anticonvulsant effect of GalR1 agonist depended on the pathway involving G_i protein (the effect was pertussis toxin-sensitive) and further G-protein-coupled inwardly rectifying K+ channels (GIRK), since the effect was reversed by a selective GIRK blocker, tertiapin Q. At the same time, GalR2 agonist galanin 2–11 facilitated kindling development in a manner that depended on $G_{q/11}$ protein (since the effect of the peptide was abolished in the presence of $G_{q/11}$ blocker [D-Arg1,D-Trp5,7,9,Leu11]-substance P) and downstream on the mobilization of intracellular Ca^{2+} (as the effect was sensitive to dantrolene).

Extrahippocampal galanin as a modifier of limbic seizures

In addition to direct action on hippocampal receptors, galanin is capable of modulating limbic seizures through interaction with outside neuronal populations that project to the hippocampus. Thus, in dorsal raphe, which represents a major source of serotonergic innervation of the hippocampus [49, 50], local activation of GalR1 augmented the severity of limbic seizures both in rats and in mice, and concurrently reduced serotonin concentration both in dorsal raphe and in the hippocampus. In contrast, local raphe activation of GalR2 mitigated the severity of seizures in both species and increased serotonin concentration in both areas [51]. Both proconvulsant effects of GalR1 and anticonvulsant effects of GalR2 were absent in the animals in which serotonin had been depleted by para-chloroamphetamine. Thus, the effect of galanin on epileptic activity is not always anticonvulsant; depending on the targeted neuronal population, the type of neurotransmission involved, and the subtype of galanin receptor, galanin may both inhibit and facilitate seizures (Fig. 3).

A prospect for galanin-based antiepileptic drugs

Anticonvulsant effects of galanin, as well as its physiological activity in other neurological disorders of memory, mood and appetite, make it an attractive target for therapeutic interventions. Peptides are generally poor candidates for therapeutic agents due to their low bioavailability (degradation by peptidases

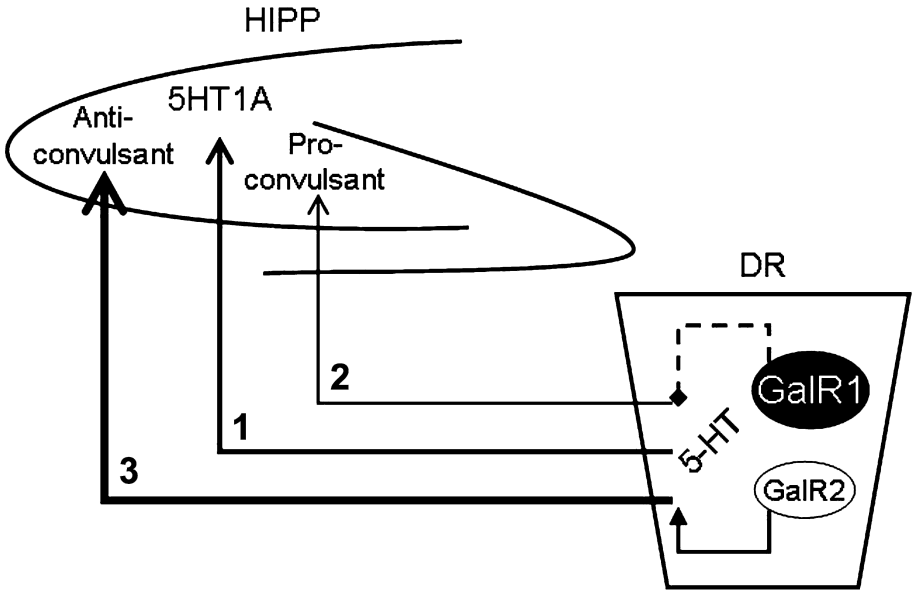

Figure 3. Modulation of the serotonergic raphe-hippocampal pathway by galanin receptors: implication for seizures. The raphe-hippocampal serotonergic pathway originates from serotonergic neurons in the dorsal raphe (DR) and targets 5HT1A receptors in the hippocampus (HIPP). Activation of hippocampal 5-HT1A receptors inhibits glutamate release from pyramidal neurons and inhibits seizures (1). Activation of raphe GalR1 receptors inhibits serotonin (5-HT) innervation of the hippocampus and exacerbates limbic seizures in an animal model of status epilepticus (2). In contrast, activation of GalR2 receptors stimulates 5-HT release along the pathway, and exerts anticonvulsant effects (3) [51].

and low permeability through the blood-brain barrier).

The first successful attempt in overcoming these weaknesses was undertaken by Saar et al. [37]. Screening of galanin sequences established that Trp-2, Asn-5, Tyr-9 were pharmacophores, responsible for the biochemical action of the peptide. Based on this finding, and by applying a combinatorial approach, Saar et al. [37] synthesized the first non-peptide low molecular weight GalR agonist, galnon (Fmoc-Cha-Lys-amidomethylcoumarin), which penetrated the blood-brain barrier. Galnon showed potent anticonvulsant effects on pentylenetetrazole seizures upon systemic administration, and inhibited perforant path stimulation-induced SE in rats [37]. Another non-peptide GalR agonist, galmic, was also effective in inhibiting seizures induced by both perforant path stimulation [52] and pentylenetetrazole upon systemic administration.

Moderate binding affinity of non-peptide galanin receptor ligands, such as galnon and galmic, prompted a search for alternative strategies for the development of galanin-based antiepileptic drugs. Such efforts led to the synthesis of NAX-5055, a rationally designed analog of truncated galanin neuropeptide that contains lipo-amino acid and basic residues at the C-terminus [53]. The compound was shown to be active after systemic administration, under conditions of several seizure models, including kindling epileptogenesis [54].

Conclusions

During the past decade galanin emerged as a powerful endogenous inhibitor of epileptic activity. Despite the novelty of the research field, significant progress has been made in collecting the evidence that galanin is a potent endogenous anticonvulsant peptide which works in different systems and seizure models; in understanding the mechanisms and the contribution of galanin receptor subtypes in its anticonvulsant effects; and, finally, in approaching clinical implementation of research data through the synthesis of stable, blood-brain-permeable GalR agonists.

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