Neuropeptide receptor positive allosteric modulation in epilepsy: Galanin modulation revealed

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The therapeutic potential of neuropeptides has long been recognized; some successes have been registered, for example, in the tachykinin, endothelin, somatostatin, angiotensin, calcitonin gene-related peptide (CGRP), orexin, or glucagon-like peptide 1 (GLP1) fields (1), primarily in peripheral applications. However, given the nature of receptor–ligand interactions at the orthosteric site of G protein-coupled receptors (GPCRs), which seem to involve multiple pharmacophores that are hard to display on low-molecular-weight (LMW) ligands, it is not surprising that the number of such ligands, especially agonists, is very limited, particularly considering brain-penetrant ones. This is not because large pharmaceutical companies have neglected modern high throughput screening technology to find such drug candidates. Many synthetic neuropeptide analogs exist, but most of them are peptides, with inherent limitations in biovailability, plasma half-life/proteolytic degradation, brain penetration, and cost of goods. More recently, alternate approaches in the GPCR field emerged with negative or positive allosteric modulators (PAM), acting as antagonists or agonists, but only in the presence of the endogenous agonist. Galanin, which acts on three GPCRs (GalR1–3), has long been proposed to play a role in seizures; however, with few exceptions, the tools described are still peptides or peptidomimetics. Now, Lu et al. (2) report in PNAS on the discovery of CYM2503, an LMW, potent, selective, and in vivo active GalR2 PAM, effective in several modalities linked to epilepsy in rodents.

This is a major contribution to the galanin and GPCR fields in that a PAM of GalR2 is described: CYM2503 does not interact with the orthosteric site of GalR2 and is devoid of effect on GalR1 binding or function. CYM2503 is effective in vivo in various models of mouse or rat seizures and epileptogenesis and is as active as a reference antiepileptic, making at least two points. (*i*) GalR2 PAM seems to be a valid principle in the treatment of seizures/epileptogenesis. (*ii*) The study validates the fact that galanin, which like other neuropeptides [somatostatin, neuropeptide Y (NPY)] is released after seizure induction and can indeed be modulated to provide protection against seizures.

Galanin and Epilepsy

There is ample evidence that galanin, like NPY or somatostatin, plays a role in epilepsy, because when applied to the brain/hippocampus these neuropeptides or their analogs have antiepileptic properties (2–4). Further, knockout or knockdown of their receptors result in increased sensitivity to seizures, as reported for NPY2, sst2/sst4 receptors (depending on species), and GalR1/GalR2. GalR1 and GalR2 are expressed in the hippocampus, whereas GalR3 is not. It is known that galanin, NPY, or somatostatin are released during epileptogenesis, and one may argue that such a release may contribute to seizures; however, this is not so, because mice constitutively overexpressing the neuropeptides or subjected to lentivirus- or recombinant adeno-associated virus-mediated overexpression are also less susceptible to seizures (2, 5). Lu et al. (2) now make the point that increased galanin release plays a protective role, although not enough galanin is released to provide full protection. However, the GalR2 PAM (which per se has no activating effect), by modulating endogenous peptide induces a large degree of protection in different models of seizure and epileptogenesis. Their article also suggests that targeting GlaR2 receptor may suffice, although previous work also suggested a role for GalR1. It remains to be seen whether a combined effect at GalR1 and GalR2 may improve the outcome, although this may be difficult to achieve; it is assumed that allosteric sites among GPCR subfamily members are highly different. In other words, to achieve a PAM for both GalR1 and GalR2 seems rather unrealistic.

Lu et al. describe an LMW PAM that is reasonably potent at and selective for a neuropeptide receptor.

Allosteric Modulators of Peptide Receptors

The present work highlights another major advantage of the PAM approach, because neuropeptide receptor agonists are largely peptide analogs. Lu et al. describe an LMW PAM that is reasonably potent at and selective for a neuropeptide receptor. There are a few examples of allosteric modulators at corticotropin-releasing factor, CGRP, GLP1, or ghrelin receptors, primarily antagonists (6). When it comes to agonists, almost no neuropeptide receptor PAMs are described. More generally, LMW, centrally acting, bioavailable, and metabolically stable agonists acting at neuropeptide receptors are rare. L-692,429 is a ghrelin receptor PAM, one of the very few peptide receptor PAMs to be described (7). Additionally, LMW ago-allosteric compounds acting on GLP1 receptors exist (8). Ghrelin and GLP1 are not commonly accepted as neuropeptides, although this may change as central effects are being investigated. Allosteric modulation of GPCRs offers great opportunities, especially for receptors for which the chemical space is limited (e.g., glutamate, $GABA_B$, calcium sensing, and now neuropeptides); it is also expected that orthosteric and allosteric sites are very different (although ago-allosteric ligands may act on both), and it seems that allosteric sites with a receptor subfamily are rather divergent, a good basis for selectivity. An allosteric modulator is expected to be largely devoid of (side) effects (if selective enough) because it is inoperant in the absence of the endogenous ligand (6, 9). In addition, it is not expected to induce desensitization, an issue with some neuropeptides. Negative allosteric modulators (antagonists) are in clinical development (10), and a few are on the market (9); it remains to be seen whether neuropeptide receptor PAMs will be as successful, but the data presented here are very promising. Finally, whether a GalR2 PAM has peripheral effects is to be investigated. Interestingly, Fallarino et al. (11) report on the effects of an MgluR4 PAM in animal models of multiple sclerosis [experimental autoimmune encephalomyelitis (EAE)]; that article also concludes that endogenous glutamate is released as a protective mechanism in rodent EAE models and that an mGluR4 PAM, just

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like the GalR2 PAM by Lu et al. (2), is able to confer additional protection.

This represents the near-culmination of a long search. Tamas Bartfai received galanin from Viktor Mutt in late 1986 and since then has published more than 140 papers on galanin, galanin receptors, and their functional and pathophysiological roles, with emphasis on epilepsy, pain, and other CNS disorders, the ultimate aim being to discover an LMW, systemically active, selective and brain-penetrant galanin receptor agonist (12–17). Numerous attempts in that direction were made: galmic and galnon are two LMW nonpeptide GalR agonists; however, their affinity for GalRs and a number of other GPCRs is in the high micromolar range, thus a more selective compound was needed. Interestingly, although galnon is unselective, it is a good tool for screening modulators or antagonists at recombinant cell lines, galnon being more stable than galanin. A few galanin peptide analogs (e.g., NAX5055) that have antiepileptic effects when applied systemically have since been described; however, NAX5055 has a relatively short half-life in vivo and, being a peptide, will lack bioavailability (18); furthermore, cost of goods is not to be neglected with peptides. Thus, CYM2503 seems to be very close to the goal set by Bartfai and his collaborators, and many others, some time ago.

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