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Neuropeptides in Epilepsy

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Abstract: Neuropeptides are signaling molecules participating in the modulation of synaptic transmission. Neuropeptides are stored in dense core synaptic vesicles, the release of which requires profound excitation. Only in the extracellular space, neuropeptides act on G-protein coupled receptors to exert a relatively slow action both pre- and postsynaptically. Consequently, neuropeptide modulators are ideal candidates to influence epileptic tissue overexcited during seizures. Indeed, a number of neuropeptides have been implicated in epilepsy and many of them are considered to have endogenous neuroprotective actions. Neuropeptides, present in the hippocampus, the most frequent focus of seizures in temporal lobe epilepsy, received the largest attention as potential anti-epileptic substances. Hippocampal neuropeptides, somatostatin, neuropeptide Y, galanin, dynorphin, enkephalin, substance P, cholecystokinin, vasoactive intestinal polypeptide, and some neuropeptides, which are also hormones such as ghrelin, angiotensins, corticotropin-releasing hormone, adrenocorticotropin, thyrotropin-releasing hormone, oxytocin and vasopressin involved in epilepsy are discussed in the review article. Oral application of neuropeptides as drugs is typically not efficient because of low bioavailability: rapid degradation and insufficient penetration through the blood-brain barrier. Recent progress in the development of non-peptide agonists and antagonists of neuropeptide receptors as well as gene therapeutic approaches leading to the local production of neuropeptides within the central nervous system will also be discussed.

Keywords: Neuromodulation, neuronal plasticity, neuropeptide receptors, seizure, temporal lobe epilepsy, therapeutic application.

1. INTRODUCTION

Epilepsy is a group of disorders characterized by seizures, excessive neuronal activity in the brain. Epilepsy affects 4-6 of out 10000 individuals each year. Approximately 50 million people are affected worldwide according to a report by World Health Organization (www.who.int/mediacentre/factsheets/fs999/en/). A number of drugs are available for the treatment of patients suffering from the different types of epilepsies. However, about 30-35% of patients do not properly respond to any drugs and continue to have seizures with a frequency that seriously hinders working ability, and reduces intellectual capabilities and life quality [1].

All forms of epilepsy result from shifting the excitatory/inhibitory balance of nerve cells toward excitation causing hyperexcitability, which can spread in the central nervous system. Increasing the inhibitory tone or decreasing the excitatory tone is the purpose of medications [2]. Most of the current medications achieve this goal by inhibiting ion channels or glutamatergic neurotransmission, or alternatively promote GABA-ergic neurotransmission [3]. The fast transmitters as Glu and GABA are major factors in epileptic seizure genesis but all drugs targeting their receptors also influence an immense number of other cells, which results in a significant spectrum of side-effects [4]. Because the receptors and ion channels in neurons have a complex interacting network of membrane proteins linking the external influences to intracellular signaling systems and alterations in gene transcription, theoretically, the epileptic cells can be controlled by many different ways. Optimal fine tuning of excitatory-inhibitory balance of neurons cannot be achieved by drugs acting on GABA or Glu receptors as even physiologically, these roles are achieved by co-transmitters and modulators. Neuromodulator substances do not transmit fast information in synapses but rather modulate the efficacy of neurotransmission directly or indirectly using various signal transduction mechanisms [5]. Indeed, different neuromodulatory systems including neuropeptides, nitric-oxide, nucleosides, steroids, cytokines, growth factors, cannabinoids have been suggested to affect the formation and propagation of seizures and, therefore, might represent target candidates for drug development [6]. The present review focuses on neuropeptides and we tried to make a collection of information useful for drug discovery and for understanding epilepsy at neurochemical level.

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2. NEUROPEPTIDES

Neuropeptides consist of 3-50 amino acids (aa) encoded by the genome. Thus, some peptides, synthesized enzymatically such as glutathione or aspartyl-glutamate, do not belong to neuropeptides. Neuropeptides are always cleaved enzymatically by prehormone convertases in the endoplasmic reticulum and the Golgi apparatus, and often undergo additional posttranslational modifications such as amidation, pyroglutamate formation, etc. [7]. Neuropeptides are then packed into so-called dense core vesicles and transported to the presynaptic terminals [8]. Importantly, these vesicles are not released by single action potentials or low-frequency stimulation even though small vesicles containing neurotransmitters are released by this stimulus at the same synapse [9]. Thus, neuropeptides typically have no effect on the basal function of the network [10]. In turn, high-frequency stimulation leads to the secretion of a large amount of neuropeptides, which can act locally or more distantly by diffusion. Neuropeptides always act *via* high-affinity G-protein coupled receptors [11]. There is not a single neuropeptide the receptor of which is a ligand-gated ion channel in vertebrates. Some proteins considered neuropeptides by some researchers, e.g. prolactin and leptin, have single transmembrane domain tyrosine-kinase receptors. However, it is more logical to not consider these signal proteins as neuropeptides. Thus, all neuropeptides possess 7 transmembrane domain receptors, which belong to 2 subgroups [12]. Typically, the receptors of small neuropeptides (3-30 aa) are class I, rhodopsin-like G-protein coupled receptors while larger neuropeptides (25-50 aa) possess class II, secretin-like G-proteins coupled receptors [13]. Neuropeptides are eliminated from the extracellular space by proteolytic degradation. After neuropeptides are released from the presynaptic terminal, they are not taken back by the terminals and the large dense core vesicles cannot be replenished [8]. Instead, newly synthesized neuropeptide packages arrive from the cell body, a process, which takes a significant amount of time, several hours or even days [14]. These properties of neuropeptides have to be taken into account when assessing their role during and after seizures. The high frequency discharge of neurons participating in the development and propagation of seizures leads to the release of neuropeptides [15]. On the other hand, neuropeptides are not available after being depleted, which may contribute to the maintenance of epileptic seizures, and the rearrangement and stabilization of the epileptic focus [16].

3. NEUROPEPTIDE SYSTEMS AS DRUG TARGETS

There are over 100 different neuropeptides identified so far in the human brain. They often have highly circumscribed localization restricted to a few cell types. Even the distribution of the most widespread neuropeptides is considerably more restricted than that of fast neurotransmitter systems. Thus, we can claim that drugs acting on neuropeptide receptors have the potential for small side-effects not only because of the lack of interference with the basal function of the network but also because of restricted localization of their receptors. On the other hand, there are some inherent difficulties in drug development in the neuropeptide field. Namely, neuropeptides are metabolized in the gastrointestinal system, have a short half-life in the circulation because of endogenous plasma protease activity, and typically do not cross the blood-brain barrier [17]. Still, there are a number of clinical trials to investigate the presence of neuropeptides as biomarkers (e.g. in migraine, pain, Parkinson's disease, maternal obesity, behavioral and mood disorders) or their potential therapeutic effects (e.g. in mood disorders, autism, pain). To increase the bioavailability of neuropeptides for better utilization of their therapeutic potentials, non-peptide agonists and antagonists are needed, which often represents a difficult task as the binding site of the receptor is suited to accept the larger peptide as ligand. Nevertheless, some progress has been made in this field and small molecule ligands have been developed for some neuropeptide receptors as discussed below [18]. Furthermore, the fact that neuropeptides are genetically coded can be utilized by gene transfer methods [19]. Indeed, constitutive or even regulated expression of a number of neuropeptides has been tested in recent years in clinical trials [20]. Engineering artificial neuropeptides is also relatively straightforward. In particular, changing a handful of the amino acids within neuropeptides can result in increased receptor specificity, or the opposite: antagonists can be generated this way [21]. The introduction of these artificial agonists and antagonists is also possible by gene transfer tools [22]. Using viral tools, the expression of neuropeptide agonists and antagonists can be restricted either by local injections or by using molecular biological techniques for the infection and action in specific cell types. Recent progress in this field will be discussed for neuropeptides tested to be delivered by gene transfer.

4. NEUROPEPTIDE SYSTEMS INVOLVED IN EPILEPSY

Epilepsies can be divided on a phenomenological basis into two major categories: generalized and partial epilepsies depending on whether both hemispheres of the brain is involved with a loss of consciousness or only circumscribed brain regions are affected during the seizure. One of the most common epileptic focuses in partial epilepsies is the temporal lobe, particularly the hippocampus. Therefore, no wonder that neuropeptides present in the hippocampus received the largest attention in epilepsy research. In addition, it is a key clinical observation that a relatively high percentage of patients with temporal lobe epilepsy do not respond to even combined medication (therapy refracter epilepsy, where the complete or partial surgical removal of the affected lobe might be necessary) warranting the need for novel approaches. The hippocampus proper (the dentate gyrus and the Ammon´s horn) receives its major input from another part of the hippocampal formation, the enthorhinal cortex (the perforant pathway to the dentate gyrus), and provides its major output to the subiculum, through which the hippocampus proper is connected with other cortical areas. Additional subcortical connections of the hippocampus include the amygdala, the septum, the thalamic reuniens nucleus, and the mamillary region of the hypothalamus. Several neuropeptides are located in the intrinsic neurons of the hippocampus proper supplementing glutamate in excitatory projection neurons and GABA in inhibitory interneurons. The projection neuron of the dentate gyrus is the granule cell, which contains dynorphin. The other 2 layers of the dentate gyrus, the molecular layer and the hilus also contain different neu-

ropeptides. The pyramidal cells in the pyramidal layer of the CA1-CA3 regions of the hippocampus do not contain neuropeptides under normal circumstances, but some neuropeptides could be induced in them by seizures. In turn, a number of different types of interneurons have been identified with diverse neuropeptide content in other layers of the hippocampus, including the stratum oriens, the stratum radiatum, and the stratum lacunosum/molecular [23]. Somatostatin, neuropeptide Y, galanin, opioids, substance P, cholecystokinin, and vasoactive intestinal polypeptide are the best known neuropeptides present in the hippocampus [24]. The Ammon´s horn and the CA1 region in particular, are most affected histopathologically in patients with temporal lobe epilepsy. Degeneration of this region known as hippocampal sclerosis is often evident in the pyramidal cell layers. Reorganization of interneurons is characteristic of epilepsy histopathology in both the dentate gyrus and the Ammon´s horn [25], which is accompanied by changes in neuropeptide expression and function. Hippocampal neuropeptides, their distribution, involvement in seizures, receptors, as well as their current status in anti-epileptic drug development will be the subject of the current review.

In addition to focal epilepsies, neuropeptides might also be involved in generalized epilepsies, e.g. absence epilepsy. In absence epilepsy, the seizures are based on activation of T-type Ca^{2+} channels [26], which open at slightly hyperpolarized membrane potential. The sustained hyperpolarization is probably the result of an increase in GABAergic tone. Absence seizures are generated in the thalamo-cortical circuits and involve thalamic relay neurons, GABAergic neurons of the reticular thalamic nucleus, cortical pyramidal cells and interneurons. In principle, all neuropeptides present in the thalamo-cortical absence seizure genesis loop are potential participants of absence epilepsy mechanism. For example, neuropeptide Y was suggested as a putative anti-epileptic peptide in the thalamo-cortical loop and suppressed absence seizures [27, 28]. Several other neuropeptides are also involved in the generation and maintenance of seizures, and therefore, their receptors represent potential drug targets. These neuropeptides, -many of them having additional hormonal functions, too, such as ghrelin, angiotensins, corticotropin-releasing hormone, adrenocorticotropin, thyrotropin-releasing hormone, oxytocin, and vasopressin-, will also be discussed in the paper.

5. SPECIFIC NEUROPEPTIDE SYSTEMS AS POTENTIAL DRUG TARGETS IN EPILEPSY

5.1. Somatostatin

The 28 aa somatostatin is produced in the digestive system, the stomach, the intestine and the delta cells of the pancreas whereas the 14 aa somatostatin is dominant in the brain [29]. Here, somatostatin has a relatively widespread distribution including the periventricular hypothalamic nucleus inhibiting growth hormone secretion. Its expression level is also remarkably high in the cerebral cortex [30]. In the hippocampus, somatostatin is produced in a variety of inhibitory neurons including hilar interneurons, bistratified cells, and O-LM interneurons in the Ammon´s horn [24]. Somatostatin cells in the hilus make up to 16% of total inhibitory interneurons in the dentate gyrus [31]. The terminals of these cells are in the outer molecular layer and presumably affect perforant path excitatory synapses [32]. Bistratified cells have axon terminals in both the stratum oriens and radiatum and may reduce the excitatory influence of Schaffer collaterals on the dendrites of pyramidal cells [33]. The cell bodies and horizontal dendrites of O-LM cells are located in the stratum oriens and provide inhibition to the distal dendrites of pyramidal cells in the stratum lacunosum-moleculare [34]. Somatostatin is released from hippocampal neurons after seizures [35] and its expression was enhanced by seizures in animal models [36]. However, a loss of somatostatin interneurons was found in the hilus in human temporal lobe epilepsy and in animal models of epilepsy while somatostatin usually remained in the Ammon´s horn even in sclerotic hippocampus [37, 38]. From these data, as well as a number of animal experiments on the effect of seizure activity of somatostatin, it was concluded that somatostatin has neuroprotective function and anti-epileptic action [39]. Indeed, somatostatin inhibits spontaneous and evoked epileptiform bursting *in vitro* [40] while reducing glutamate release in the hippocampus [41]. Somatostatin, injected intracerebroventricularly [42] or overexpressed in the hippocampus using viral tools [43] decreased the severity of seizures *in vivo*. In turn, sequestering endogenous somatostatin by infusion of an anti-somatostatin antibody had pro-convulsant effects [44].

Somatostatin can act on 5 different somatostatin receptors (sst_1-sst_5), which are all coupled to $G_{i/o,}$ and of which sst_1-sst_4 are expressed in the hippocampus [45]. Sst_1 receptors are often autoreceptors and probably do not participate in the action of somatostatin on epileptic activity [46, 47]. Sst_2-sst_4 receptors are located in granule cells of the dentate gyrus and in pyramidal cells of the Ammon´s horn [45]. Recently, some somatostatin receptor subtype-selective small molecule drugs were developed using combinatorial libraries constructed on the basis of molecular modeling of known peptide agonists [48]. These studies relied on using sugar or benzodiazepinone non-peptide mimics of betaII´ turn around the biologically essential Trp-Lys dipeptide at the 8 and 9 position of somatostatin. In subsequent studies, these agonists (Fig. **1**) have also been characterized *in vivo* [49]. Later, selective antagonists of sst_1 [50], sst_2 [51], and sst_3 [52] were also developed, which can be useful experimental tools, although the latter two are peptide derivatives.

Sst_2-sst_4 all participate in the mediation of the anti-convulsant action of somatostatin although with some species-dependence regarding sst_2 in particular, which appears to have an anti-epileptic action in rats but not in mice [53]. The selective sst_2 agonist L-779,976, the sst_3 agonist L-796,778, and the sst_4 agonist L-803,087 all protected rats against focal pilocarpine-induced seizures [54]. Apart from pharmacological tools, transgenic animals lacking somatostatin receptors also suggest the role of these receptor subtypes in epilepsy [46]. Despite the extensive results of animal experimentation available, however, human data are largely missing as far as the anti-epileptic activity of somatostatin. Therefore, further progress in this field is eagerly awaited.

5.2. Neuropeptide Y

Neuropeptide Y (NPY) is a 36 aa neuropeptide expressed in the nervous system and in a variety of secretory cells in

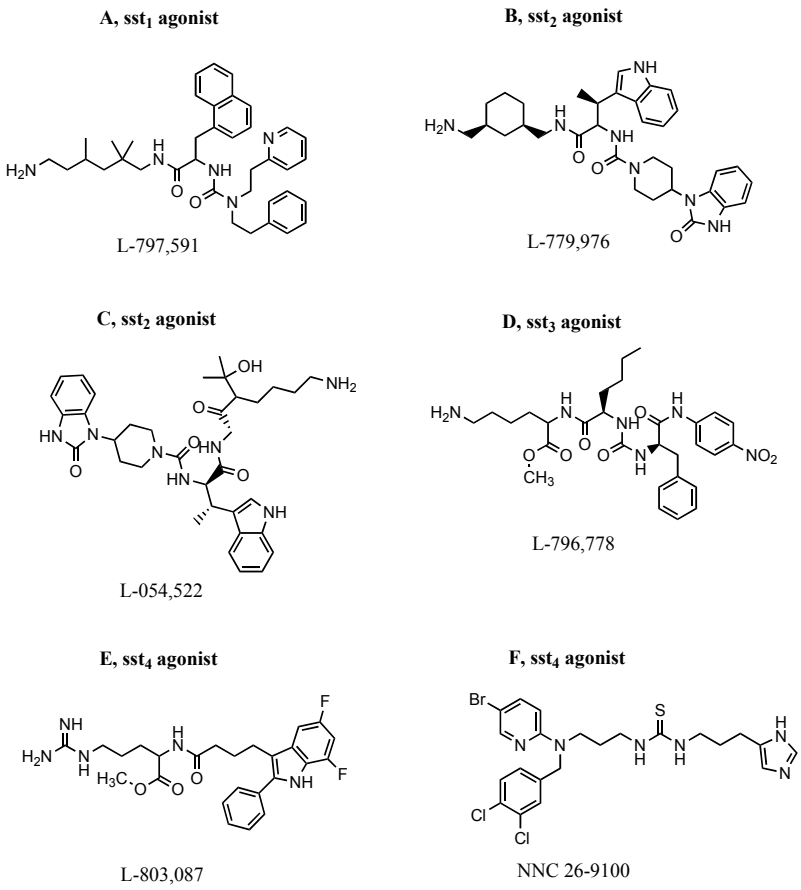


Fig. (1). Non-peptide agonists acting selectively on sst_1-sst_4 receptors.

the periphery. Within the central nervous system, NPY is most abundant in the cerebral cortex, the hippocampus, the hypothalamus, the striatum and the reticular nucleus of the thalamus [55]. Within the hippocampus, NPY is expressed in bistratified GABA-ergic interneurons [23] and inhibits the excitatory input onto pyramidal cells in the Ammon´s horn [56]. NPY levels are increased in epilepsy models [57] but prolonged overstimulation in animal models or in human epilepsy ablates NPY [38, 58]. Additional evidence for the involvement of NPY in epilepsy was provided by the finding that valproate, a widely used anti-epileptic drug elevated NPY levels in the hippocampus [59].

A large body of information provided evidence that exogenously applied NPY has anti-epileptic activity in both *in vitro* models (bursting without Mg^{2+}, picrotoxin and stimulus-train induced bursting in hippocampal slices), and *in vivo* models of partial seizures such as different kindling models and kainate-induced seizures reviewed previously [60, 61]. Although less extensively studied, NPY may also be beneficial in models of generalized seizures, the pentylenetetrazole [62] and the electroconvulsive seizures [63], and models of absence epilepsy [28]. Studies using transgenic animals also suggest a role of NPY in the control of seizures. In mice lacking a functional NPY gene, spontaneous seizures were observed and their threshold for pentylenetetrazole was reduced [64]. In turn, rats overexpressing NPY showed diminished kainate and kindling-induced seizures [65]. Gene transfer studies in the field of epilepsy are also pioneered using the neuropeptide Y system. Overexpression of NPY in the hippocampus using adeno-associated viral vectors suppressed the consequences of kainate- and kindling-induced seizures [66]. A similar gene transfer method reduced spontaneous seizure frequency in a progressive and spontaneous seizure model of temporal lobe epilepsy induced by electrical stimulation of the temporal pole of the hippocampus [67]. At present, however, neuropeptide Y is used in clinical trials of a number of disorders like mood disorders, post traumatic stress disorder but not in epilepsy [68].

There are 4 different neuropeptide receptors identified in human, Y1, Y2, Y4 and Y5, of which Y1, Y2 and Y5 may be involved in epilepsy [61]. These receptors signal through G_i proteins that inhibit adenylate cyclase and decrease intracellular Ca^{2+} levels [69]. Y2 has predominantly presynaptic localization while Y1 and Y5 reside on postsynaptic neurons [70] possibly with different excitatory or inhibitory nature. Y1 receptor overexpression aggravated kainate-induced seizures [71] while Y5 receptor overexpression together with NPY overexpression had stronger seizure-suppressant effect than NPY overexpression alone [72]. In addition, mice lacking the Y5 receptor had enhanced sensitivity for kainate-induced seizures [73]. Experiments with Y2 knockout mice and overexpression of the selective Y2 ligand NPY13-36 suggest that Y2 receptor is also involved in the suppression of seizures [74, 75]. In line with these studies, Y1 antagonists, and Y2 and Y5 agonists were suggested to have anti-epileptic potential [76]. Indeed, a large number of different

peptide derivatives of NPY were tested in animal models of epilepsy supporting this suggestion [60]. However, the development of selective drugs acting on NPY receptors lags behind the understanding of their importance. Some non-peptide Y1 antagonists are available and a selective peptide-derivative Y5 agonist (BWX-46; ((CYS31,NVA34)-Neuropeptide Y (27-36))2) has been reported [77] while selective small molecule Y2 agonists are not available yet. The selective Y1 receptor antagonists BIBP 3225 and BIBO 3304 decreased seizure susceptibility in rats [62]. Another Y1 receptor antagonist, BIBP-3226 (Fig. **2A**) was also investigated in structure-activity studies. Replacing the benzylamino by a tetrahydrobenzazepinyl group preserves most of the Y1 activity while combination with a NG-phenylpropyl arginine and a Na-p-biphenylylacetyl moiety shifted the NPY receptor selectivity towards Y5 [78]. Using BIBO-3226 as a lead compound resulted in some other Y1 antagonist but these ligands were not superior to the parent compound [79, 80].

5.3. Galanin

Galanin consists of 29 amino acids in rodents and 30 amino acids in human. It is expressed in the gastrointestinal tract, and the nervous system. In the central nervous system, galanin is abundant in the hypothalamus, the cortex, the hippocampus, and the brainstem. Galanin-immunoreactive nerve terminals have a particularly high density in the granule cell layer of the dentate gyrus. Some of these terminals originate in the septum and also contain acetylcholine [81]. Other galanin-containing terminals in the dentate gyrus are catecholaminergic and arise from the locus coeruleus [82]. In addition, seizures lead to the expression of galanin in hippocampal inhibitory neurons [83, 84]. Furthermore, galanin receptors were identified on serotonergic raphe neurons projecting to the hippocampus [85]. Thus, galanin was suggested to also influence limbic seizures indirectly *via* these brainstem galanin receptors [86].

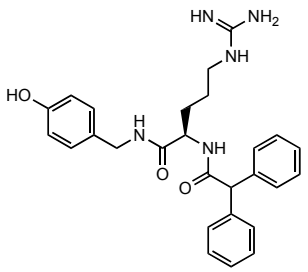
Galanin leads to the inhibition of glutamate release in the hippocampus [87], which probably is the reason of the anti-seizure effect of intrahippocampal galanin injection [88]. The anti-convulsant activity of galanin was also supported using transgenic mice lacking galanin as these mice demonstrated an increased seizure susceptibility and higher severity of convulsions [89]. In turn, mice overexpressing galanin showed an increased discharge threshold and a reduced progression of kindled seizures [90]. Similarly, viral overexpression of galanin also suppressed seizure development [91, 92].

There exist 3 subtypes of galanin receptors (GalR) in mammals. These receptors are inhibitory G-protein coupled receptors. Two of them, GalR1 and GalR2 are expressed in the hippocampus [93]. Mice lacking GalR1 showed spontaneous seizures and were highly sensitive in induced epilepsy models [94]. Timing of the effects of antisense DNA for GalR2 suggested that GalR1 affects the initiation phase while GalR2 affects the maintenance phase of status epilepticus [95]. Selective chimeric peptides for the GalR1 (M617: Galanin(1-13)-Gln^{14}-bradykinin(2-9)amide) and for GalR2 (M1145: (RG)(2)-N-galanin(2-13)-VL-(P)(3)-(AL)(2)-A-amide) were developed [96, 97]. To produce GalR agonists with increased stability and blood-brain barrier penetration capability, truncated galanin analogues, in which nonessential amino acid residues were replaced by cationic and/or lipoamino acid residues, were designed. That way, a non-selective GalR1-GalR2 peptide NAX 5055 was produced, which contains the -Lys-Lys-Lys(palmitoyl)-Lys-NH(2) motif and exhibited high affinity for galanin receptors. Structure-activity-relationship analysis suggested that cationization combined with position-specific lipidization was critical for improving the systemic activity of the analogues [98]. These, and some other peptides with various degrees of galanin receptor selectivity, had anti-convulsant properties in a variety of epilepsy models suggesting the involvement of both GalR1 and GalR2 in epilepsy [86]. Therefore, the development of small molecule agonists on galanin receptors became a goal of many pharmaceutical companies. Instead of true agonists, however, some positive allosteric modulators were developed. Galmic (Fig. **2B**) and galnon (Fig. **2C**) bind to both GalR1 and GalR2 with similar and unfortunately low affinities [99-101]. Still, both substances possess the advantage of allosteric modulators: they activate the receptor only in the presence of the endogenous agonist, that is, when the receptor is supposed to be activated thereby reducing the side-effects resulting from non-physiological activity of the receptor [102]. More recently, a modified version of galnon, CYM2503 (Fig. **2D**) was produced as a high affinity allosteric modulator [103], the utilization of which is promising in future research on the anti-convulsant action of galanin receptors [104].

5.4. Dynorphin and Enkephalin

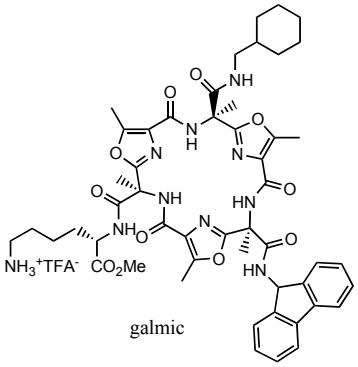
Dynorphins and enkephalins are opioid peptides, that is, endogenous ligands of the opiate receptors, through which morphine exerts its anti-nociceptive actions [105]. Dynorphin A and B are both 13 aa peptides encoded by the preprodynorphin gene. They act on the kappa-opioid receptors (KOR). Enkephalins also have 2 forms, met-, and leu-enkephalin, which are both 5 aa peptides differing in one amino acid. They act on the mu- and delta-opioid receptors (MOR and DOR). Met-enkephalin peptide sequence is coded for by the preproenkephalin gene; the leu-enkephalin peptide sequence is coded for by both the preproenkephalin gene and the preprodynorphin gene [106]. The third family of opioid peptides, the endorphins, has not been significantly implicated in the generation and control of seizures even though all opioid peptides are present in several brain regions [107]. In the hippocampus, enkephalins are present in mossy fibers, the perforant path, and in scattered GABAergic interneurons while dynorphins are present in granule cells, as well as perforant path and supramammillary afferents [108]. Kindling and seizures were shown to increase the release of enkephalin [109, 110]. In turn, the expression level and concentration of enkephalins are increased during and after seizures. Generalized clonic-tonic seizures increased the preproenkephalin gene expression in the dentate gyrus [111, 112]. In a spontaneously epileptic strain, a strong upregulation of enkephalin was found in the granule cells and mossy fibers [113]. An enhancement of proenkephalin expression was found after pentylenetetrazol treatment, too [114]. A single dose of kainic acid elevates the levels of enkephalins in the hippocampus for up to 1 year [115]. Furthermore, an

A, Y1 NPY receptor antagonist



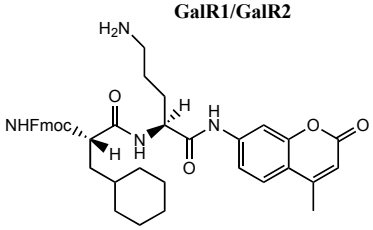
BIBP-3226

B, Positive allosteric modulator of GalR1/GalR2



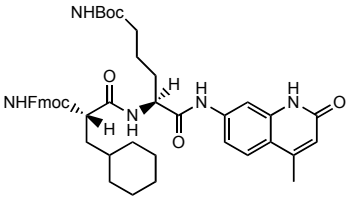
galmic

C, Positive allosteric modulator of GalR1/GalR2



galnon

D, Positive allosteric modulator of GalR1/GalR2



CYM2503

Fig. (2). Low molecular weight anti-convulsant drugs acting on NPY and galanin receptors.

increase in enkephalin-like immunoreactivity was found in human hippocampi with generalized epilepsy [116]. The induction of preproenkephalin is also in line with the activation of enkephalin-synthesizing neurons during seizures [117]. In contrast, most studies reported a decreased level of dynorphins following seizures. Generalized clonic-tonic seizures decreased the preprodynorphin gene expression in the dentate gyrus [111] and the Ammon´s horn [112]. Dynorphin mRNA levels were decreased in a spontaneously epileptic mouse strain [113]. Status epilepticus induced by intermittent stimulation of the perforant path also decreased the dynorphin-like immunoreactivity in the dentate gyrus and CA3 [118]. However, an up-regulation of prodynorphin transcription was found in human temporal lobe epilepsy [119]. The acute changes in the level of opioid peptides may contribute to increased excitation during induction of seizures, while the chronic changes may contribute directly to persistently increased excitability in the hippocampus and possibly other brain regions, too [112].

Apart from the different alterations in their level during seizures, enkephalin and dynorphin had opposite effects in their action on seizure susceptibility [120, 121]. DOR agonists produce convulsions similar to the effect of pentylenetetrazol [122] while MOR agonists also evoked EEG epileptiform activity [123-125]. Also, MOR agonists intensified the neurotoxic effects of kainate in the hippocampus [126]. In turn, MOR and DOR antagonists raised seizure threshold [127], depressed pentylenetetrazol-induced kindling in rats [128, 129], and prevented wet dog shaking and status epilepticus following perforant path stimulation [118, 130]. Electroshock seizure threshold was also increased by MOR antagonists [131]. Bicuculline-induced convulsions were potentiated by intracerebroventricularly administered enkephalin derivatives while selective MOR as well as DOR antagonists abolished the enhancement of the bicuculline-induced convulsions [132]. In contrast, selective KOR agonist suppressed bicuculline-induced convulsions [132] and showed a marked anti-convulsant profile in different animal models of epilepsy [133]. These findings are consistent with the neuromodulatory actions of opioid peptides. Enkephalin inhibited GABA release from inhibitory interneurons, resulting in increased excitability of hippocampal pyramidal cells and dentate gyrus granule cells. The role of enkephalins as pro-convulsive agents is also consistent with the seizure-inducing side-effect of high concentration morphine treatment [134, 135]. Prodynorphin-derived peptides primarily acted at presynaptic kappa opioid receptors to inhibit excitatory amino acid release from perforant path and mossy fiber terminals [108, 136]. Furthermore, direct injection of a DOR receptor antagonists or dynorphin-A into the hilus of the dentate gyrus prevented the development of status epilepticus by perforant path stimulation while perihilar administration of a DOR agonist enkephalin derivative or a kappa-antagonist facilitated the establishment of self-sustaining status epilepticus suggesting actions in the hippocampus [118]. Altogether, these data suggest that kappa-opioids in the hippocampus counteract but delta-opioids promote initiation and maintenance of status epilepticus. In addition to limbic seizures, dynorphin may also suppress encephalitis-induced seizures [137, 138] and absence eplileptic-like seizures [139] suggesting that its anti-convulsive action is not restricted to specific epilepsies connected to the hippocampus. In fact, opioids could have indirect actions on the hippocampus, too, as medial septal injection of the opioid antagonist naloxone elevated acetylcholine release in the hippocampus and induced seizures in the rat [140]. Additional strong evidence on the involvement of dynorphin in seizure activity came from studies using mice that lack functional

prodynorphin gene. These mice displayed a reduced seizure threshold in response to pentylenetetrazole. This phenotype could be entirely rescued by kappa receptor-specific agonist while a delta-specific agonist decreased seizure threshold in both wild-type and knockout mice [141]. Moreover prodynorphin knockout mice showed faster seizure onset and a prolonged time of seizure activity after kainate injection, which was accompanied by an increased extent of neuronal loss in the hippocampus [142]. The involvement of dynorphin in the etiology of epilepsy was also strengthened by a functional polymorphism in the prodynorphin gene promotor associated with temporal lobe epilepsy. In a case control association study, the prodynorphin promotor low-expression L-alleles conferred an increased risk for temporal lobe epilepsy. Irrespective of the familial background, L-homozygotes displayed a higher risk for secondarily generalized seizures and status epilepticus [143].

Primary opioid receptors, MOR, DOR and KOR, and the nociceptin/orphanin FQ peptide receptor, have been further classified pharmacologically. However, cloning and knockout technologies did not support the existence of the pharmacologically classified receptor subtypes [144]. Opioid receptors can couple to G_i, G_o, G_q, and possible $G_{z/x}$. Opioids inhibit adenylate cyclase and Ca^{2+} channels, and stimulate K^+ channels by G_i and G_o, while coupling with G_q leads to the activation of phospholipase C [145]. The pharmacology of opioid receptors is exceptionally well elaborated. Several different KOR agonists are available and used as analgesics in clinical practice [146]. Non-peptide, selective KOR agonists include asimadoline, bremazocine, butophanol, BRL-52537, cyclazocine, enadoline, GR-89696, HZ-2, ICI-199,441, ketazocin, LPK-26, salvinorin B methoxymethyl ether, spiradoline, tifluadom, U-50488, U-69593, which belong to different chemical classes of molecules. Some of these drugs including asimadoline ICI-204,448 do not cross the blood brain barrier, and therefore, are not suitable to test as anti-epileptics. Some other compounds including butophanol (Fig. **3A**), cyclazocine (Fig. **3B**), ketacyclazocine (Fig. **3C**), salvinorin A (Fig. **3D**), and U-50488 (Fig. **3E**) have been tested in animal models and demonstrated anticonvulsant properties [147-151]. Other potential antiepileptic compounds based on their above described actions are MOR and DOR antagonists [152]. Selective non-peptide MOR antagonists include cyprodime (Fig. **3F**), and beta-Funaltrexamine (Fig. **3G**). Stable peptide analogues, such as [Dmt1, d-2-Nal4]endomorphin-2, designated as antanal-2, have also been developed. These drugs were tested in epilepsy models and were found to be anti-convulsant [130, 132, 153-156]. Selective non-peptide DOR antagonists include naltrindole (Fig. **3H**) and naltriben (Fig. **3I**), which showed anti-convulsant properties in different animal models of epilepsy [118, 128, 129, 132]. Therefore, KOR agonists, MOR and DOR antagonists should be further investigated as anti-epileptics despite potential major side-effects including dysphoria.

5.5. Substance P

Substance P belongs to the tachykinin family of peptides and consists of 11 amino acids [157]. It has a relatively widespread distribution in the brain including the hippocampus. Some substance P-expressing neurons are found in the subiculum and enthorhinal cortex. The granule cells, and some interneurons of the hilus, stratum oriens and stratum radiatum of the Ammon's horn also contain substance P [158, 159]. However, the substance P-containing fibers densely innervating the hippocampal formation [158-160] derive to a large extent from extrahippocampal sources, the supramammillary nucleus [161] and the medial septum [162]. Furthermore, substance P expression within the hippocampus is increased by seizures [113, 163].

Substance P plays a role in sustaining status epilepticus [16]. In addition, a resistance to excitotoxin-induced seizures and neuronal death was found in mice lacking the preprotachykinin A gene, which encodes substance P [164]. Furthermore, the involvement of substance P in other types of seizures, e.g. seizures in neurocysticercosis has also been firmly established [165]. The mechanisms of the proconvulsant effect of substance P are not known yet. Substance P modulates synaptic transmission [166] and can depolarize cortical neurons to elicit an increase in glutamate release at excitatory synapses [167]. Substance P typically affects interneurons, therefore, a facilitation of inhibitory drive to GABA-ergic interneurons may be the mechanism of overexcitation of the epileptic tissue [168].

Substance P acts relatively specifically on neurokinin-1 (NK-1) receptor [157]. This receptor can couple to $G_{q/11}$, G_s and G_0 proteins [169]. Stimulation of NK1 receptor activates phospholipase C and also adenylate cyclase without cross-talk between the signaling pathways [170]. Immunostaining for substance P receptor labels GABA-ergic interneurons with distinct termination patterns in the hippocampus [171, 172]. The morphology and synaptic input of substance P receptor-immunoreactive interneurons alters in epileptic human hippocampus [173]. Substance P receptor-immunopositive cells possess significantly larger numbers of dendritic branches in the epileptic CA1 region, and the synaptic input of their dendrites increases [174]. Consistent with this, an increased neurokinin-1 receptor availability was found in temporal lobe epilepsy by positron emission tomography study using a substance P receptor ligand [175] suggesting an increased density of substance P receptors in the hippocampus.

Selective peptide agonists of NK-1 receptor have been developed. However, these drugs do not possess major therapeutic potential [157]. In contrast, NK-1 receptor antagonists are intensively investigated for a variety of neuronal diseases including epilepsy. Consequently, non-peptide small molecule antagonists are available including CP-122,721 [176], GR205171 [177], and MK-869 [178] as shown in (Fig. **4**). MK-869 (Aprepitant) is used at present as an anti-emetic agent [179]. NK-1 receptor antagonists have been tested in animal models of epilepsy and were found effective. CP-122,721-1, attenuated kainate induced seizure activity [180] while GR205171 potentiated the anticonvulsant efficacy of sodium channel inhibitors [181]. Thus, research using NK-1 receptor antagonist in epilepsy is expected to intensify in the future [182].

5.6. Cholecystokinin

Cholecystokinin (CCK) includes peptides with different lengths but overlapping sequences cleaved from the same

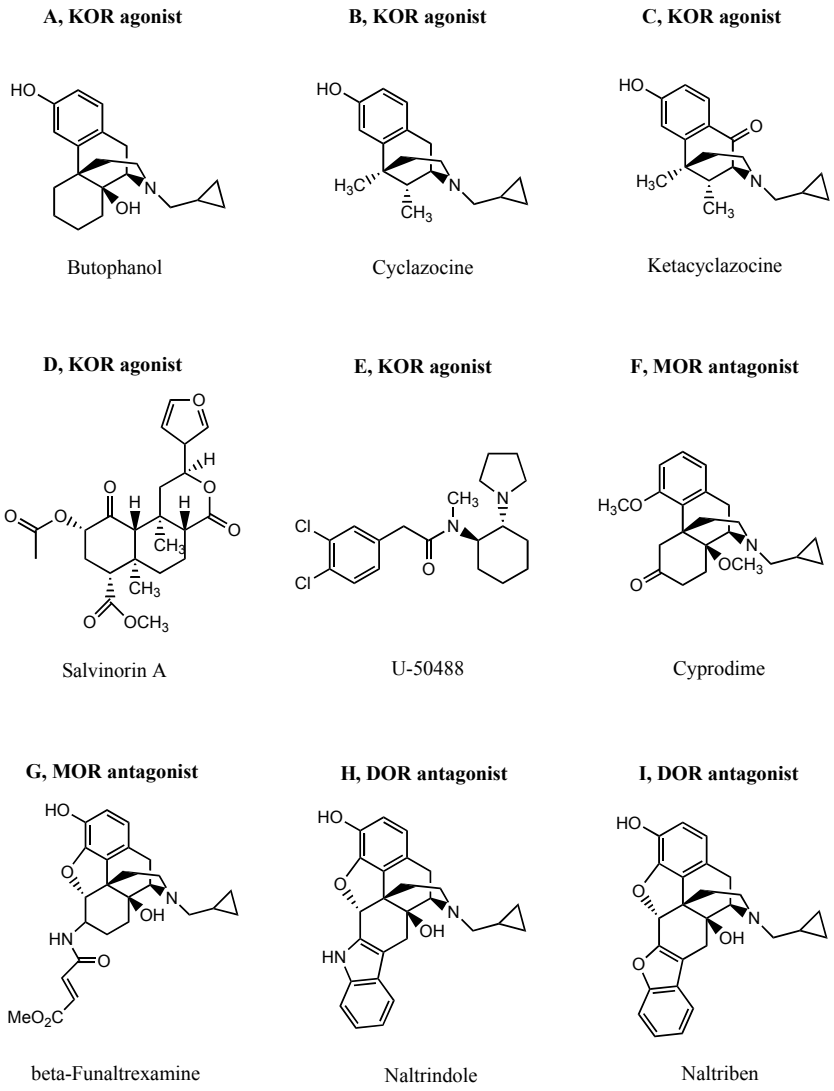


Fig. (3). Low molecular weight anti-convulsant drugs acting on opioid receptors.

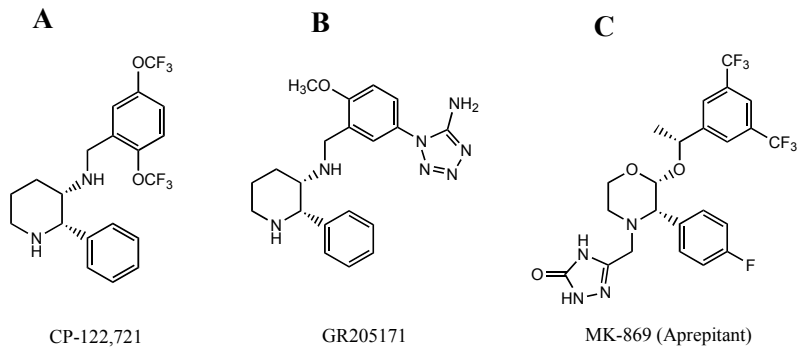


Fig. (4). Low molecular weight antagonists of the NK-1 receptor with anti-epileptic potential.

propeptide. Longer forms are secreted from I cells of the epithelium of the small intestine and act as hormones to increase the amount of digestive enzymes and bile in the duodenum [183]. In the brain, the 8 aa CCK is one of the most abundant neuropeptides. It is produced in the cerebral cortex, the hippocampus, the amygdala, the caudate putamen, the hypothalamus, and also in some neurons of additional brain regions [184]. In the hippocampus, CCK is present in granule cells of the dentate gyrus, basket cells, and Schaffer collateral associated and lacunosum-moleculare perforant path associated interneurons in the Ammon´s horn [24]. In addition, seizure-induced appearance of CCK in pyramidal cells has also been reported [185].

The anti-convulsant action of CCK is controversial. CCK inhibited seizures induced by picrotoxin and electroshock in rats [186] and in mice [187]. Also, CCK increased vigaba-

trin-induced anti-convulsant effects [188] and reduced pentylenetetrazole-induced kindling [189]. However, pro-convulsant actions of CCK have also been reported in animal models of epilepsy [83]. In addition, CCK produces a well-documented depolarizing effect on hippocampal neurons that leads to the release of glutamate [190, 191].

CCK has 2 different receptors CCK1 (previously CCK-A) and CCK2 (previously CCK-B) receptors, which activate phospholipase C via G_q proteins [192]. CCK2 receptor is the dominant form in the brain as well as in the hippocampus. Selective and potent CCK receptor agonists and antagonists have been developed. The first selective CCK2 antagonists were modified peptides (Cl-988 and CI-1015). Small molecule CCK2 antagonists (L-365,260, L-369,293, YF-476, RP-69758, LY-288,513, PD-145,942) and inverse agonist (L-740,093) have been developed [193, 194], but are typically considered in anxiety disorders, panic attack, schizophrenia, and alcohol withdrawal, but not for anti-epileptogenic potential [17, 195-197].

5.7. Vasoactive Intestinal Peptide

Vasoactive intestinal peptide (VIP) is a 28 aa neuropeptide expressed in a variety of brain regions [198] including all parts of the hippocampal formation. In the hippocampus proper, VIP is expressed by interneurons innervating pyramidal and granule cell bodies and proximal dendrites (basket cells) and by interneurons innervating selectively other interneurons [199, 200]. Pentylenetetrazol-induced seizures resulted in short term decrease in the level of VIP [57, 201]. VIP has an overall excitatory action on synaptic transmission in the hippocampus [202], in which the cAMP-mediated activation of NMDA receptor plays a role [203]. Still, VIP was neuroprotective in some models of neurodegeneration including neuroinflammation [204] and beta-amyloid-induced neurodegeneration [205] providing the possible neuroprotective role in epileptic sclerosis.

VIP actions are exerted by G-protein coupled receptors VPAC1-R and VPAC2-R [198], which belong to class II of G-protein coupled receptors, recognize VIP and also the related neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP). These receptors were shown to be upregulated in hippocampal surgical samples of patients suffering from human temporal lobe epilepsy [206]. Some subtype-specific peptide agonists and antagonists for VIP receptors are available. Studies using these peptides suggest that VPAC2-R but not VPAC1-R is involved in the VIP enhancement of population spikes in the hippocampus [207]. Altogether, we can conclude that although the VIP system is ideally situated to affect epileptic seizures, data are scarce as to their involvement in seizure generation and propagation. Development of small molecule drugs acting selectively on VIP receptors would be useful in exploring the role of these receptors in epilepsy. Thus, while there were a number of studies in human subjects with different forms of headache where VIP was assessed as a biomarker [208], its utilization in epilepsy is not imminent.

5.8. Ghrelin

Ghrelin, a 28 aa peptide, was identified as the endogenous ligand of the growth hormone secretagogue receptor (GHSR) using reverse pharmacology [209]. Accordingly, ghrelin increases the secretion of growth hormone from the pituitary. Ghrelin has 2 forms depending whether its serine-3 residue is acetylated with octanoate, performed by a recently identified specific enzyme in about 90% of circulated ghrelin [210]. Ghrelin is most abundant in the stomach from which it is secreted to the circulation as a hormone. Its serum levels increase before meals and decrease after meals as it participates in the regulation of food intake. Ghrelin is also known to be expressed in the hypothalamus while its expression in other brain regions is debated [211]. Therefore, it is questioned sometimes if ghrelin qualifies as a neuropeptide or it should be considered only a hormone. Since its neurochemistry and anti-epileptic actions are similar to those of other neuropeptides, we include the discussion of ghrelin in this review.

The ghrelin receptor gene encodes GHSR1a and GHSR1b, of which GHSR1a is the functional ghrelin receptor while GHSR1b might be a negative modulator of GHSR1a by dimerization [212]. Activation of GHSR1a activates the phospholipase C pathway *via* G_{11}/G_{q11} [213]. GHSR is abundant in widespread brain regions including the hippocampus [214] although its precise localization within the hippocampus is not revealed yet. Similarly, the origin of endogenous ghrelin activating these receptors remains to be established. A peripheral source is possible as ghrelin can penetrate the blood-brain barrier [215]. Despite the uncertainties regarding the neurochemistry of ghrelin, its anti-epileptic activity has been demonstrated recently [216] among other potential therapeutic actions [217]. Ghrelin, injected intraperitoneally, reduced the severity of pentylenetetrazol-induced seizures [218]. Furthermore, ghrelin attenuated kainic acid-induced neuronal cell death in the mouse hippocampus [219] and protected hippocampal neurons in pilocarpine-induced seizures of rats by inhibiting cell apoptosis [220]. In turn, another study suggested that only des-acylated ghrelin is beneficial in hippocampal seizures [221]. Different mechanisms of action were suggested for ghrelin action on seizures and its neuroprotective actions. Since ghrelin leads to neuropeptide Y and GABA release in the hypothalamus, it may have a similar action in the hippocampus [214]. Another possibility is that ghrelin induces the proliferation of hippocampal neural stem cells [222]. Alternatively, it acts as an anti-inflammatory agent, as demonstrated in the periphery [223]. Recently, another interesting hypothesis was suggested based on the finding that the GHSR1a receptor has significant basal activity [224] and that the GHSR knockout mice had a higher seizure threshold than their wild-type littermates [225]: an endogenous agonist can have anticonvulsant action by reducing the activity of the constitutively active receptor with a combination of inverse agonism and desensitization/internalization of the receptor [225]. This hypothesis should be tested by newly developed inverse agonists of the GHSR1a receptor [226, 227].

To utilize actions *via* the ghrelin receptor, more stable analogues were recently developed. BIM-28131, a small peptide ghrelin agonist, and BIM-28163, a full-length ghrelin analog antagonist were used in some studies on hypothalamic actions of ghrelin [228]. Also, some peptidomimetics have been produced including the pseudotripeptide EP01572 (H-Aib-(D)-Trp-(D)-gTrp-formyl) with increased stability

and oral bioavailability [229] even in human [230], and JMV 1843 [231] shown in (Fig. **5A**).

Subsequently, it was found that the C-terminal of JMV 1843 is not necessary for ligand binding and can be omitted [232], which resulted in JMV 2215 (Fig. **5B**). Based on the structure of this pseudo-dipeptide, peptidomimetics were developed by rigidifying the structure using different heterocycles [233]. Triazoles including JMV 2683 (Fig. **5C**) were selected as lead compounds. Further structure-activity relationship studies produced the potent GHSR1a receptor antagonist JMV 2959 (Fig. **5D**), which could be used for *in vivo* studies to further establish the mechanism of anti-convulsant action of ghrelin [233]. In turn, non-peptide agonists have also been developed [234, 235], including NN703 (Fig. **5E**), MK-677 (Fig. **5F**), and L-692,429. These agonists of the GHSR1a receptor should be used in the future to test their anti-convulsant properties.

5.9. Angiotensins

The renin-angiotensin system (RAS) has long been known as a peripheral regulator of several functions including excretion and blood pressure. By now, the components of the RAS expressed in the brain are also identified [236]. Angiotensinogen is cleaved by renin to form the 10 aa angiotensin (Ang) I, from which angiotensin converting enzyme (ACE) produces the 8 aa AngII. Additional cleavages lead to the 7 aa AngIII, and the 6 aa AngIV. Originally described in the hypothalamus related to the control of fluid intake [237], the RAS has also been identified in other brain regions, too, including the cortex and the hippocampus [238-240]. Furthermore, the levels of components of the RAS were changed in the hippocampus following pilocarpine treatment of rats [241] and audiogenic seizures [242] as well as in human temporal lobe epilepsy [243]. Initial studies of a research group suggested anti-convulsant actions of Ang peptides against pentylenetetrazol-induced seizures [244] reviewed recently [245]. Emerging new data, however, suggest a more complex effect of angotensins on seizure activity [246]. The anti-convulsant action of AngIV has been confirmed in the pilocarpine model of epilepsy [247]. However, results using inhibition of ACE and antagonists of AngII receptors suggest a pro-convulsant action of AngII in the brain. AngII receptor antagonists showed anti-convulsant affects in pentylenetetrazol treated rats [248] and both AngII receptor antagonists and ACE antagonists attenuated audiogenic seizures [242]. Moreover, the effects of known anti-epileptics were potentiated by AngII receptor antagonists in electroshock seizures [249, 250] and by ACE inhibitors in audiogenic seizures [251]. The pro-convulsive effect of AngII could be related to its inhibitory action on GABA release [252] and its excitatory action on hippocampal pyrami-

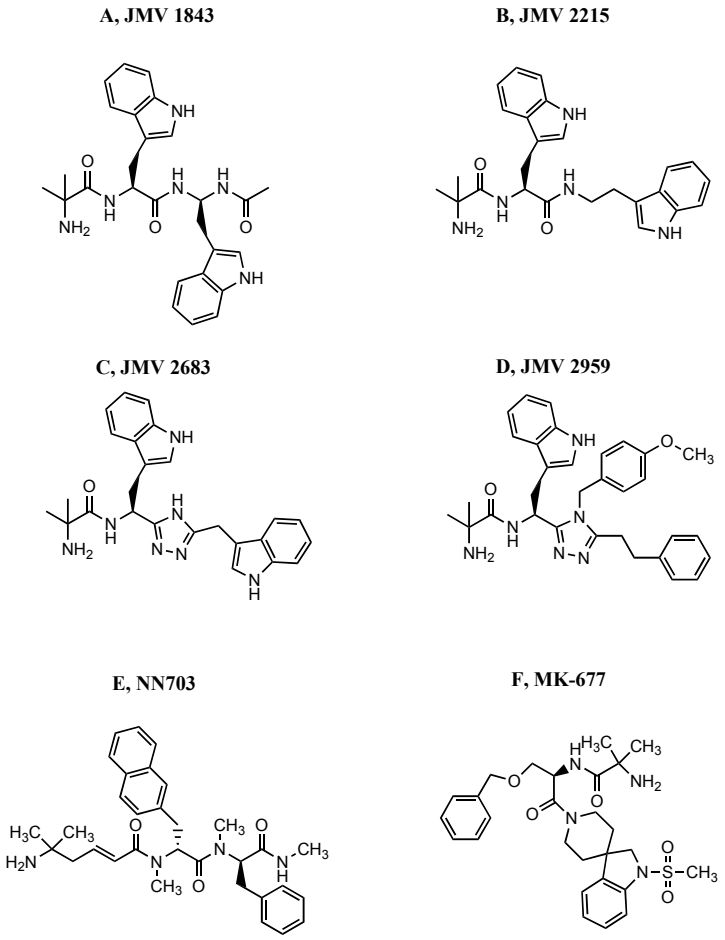


Fig. (5). Drugs acting on the ghrelin receptor. **A-D**: Steps of the development of a non-peptide antagonist JMV 2959. **E**, **F**: Non-peptide agonists of the ghrelin receptor.

dal cells [240]. The interaction of AngII with other neuromodulator systems, particularly adenosine has also been suggested [253]. Given the recent description of distribution of nucleosides in human [254, 255], the interaction of AngII with nucleosides promises interesting findings in the future.

The angiotensin II receptor type 1 (AT1) is the most widespread receptor recognizing AngII and AngIII. AT2 has a similar affinity for angiotensins as AT1 with some preference for AngIII while AT4 is activated selectively by AngIV. AT1 is coupled to $G_{q/11}$ and $G_{i/o}$ while AT2 to $G_{i2/3}$ [236]. The existence of AT3 became questionable while the identity of AT4 is a matter of debate, however, it most likely not a G-protein coupled receptor [256]. The candidates for AT4 include insulin-regulated aminopeptidase, hepatocyte growth factor, and the type 1 tyrosine kinase receptor c-Met [257]. All 3 Ang receptors are present in several brain regions. AT4 is the most abundant in the cortex and the hippocampus [236]. Elevated mRNA expression was shown for AT1 but not for AT2 following audiogenic seizures [242] and in temporal lobe epilepsy [243], while no data are available for AT4. Furthermore, specific AT1 antagonists had anti-convulsant properties in animal models of epilepsy [242, 248-250]. These findings support that the AT1 and possibly the AT4 receptors may be involved in the pathophysiology of epilepsy. Non-peptide AT1 antagonists (e.g. losartan) and ACE inhibitors (e.g. captopril) are available and widely used to reduce blood pressure. Their effects in animal models of epilepsy suggest the necessity of careful analysis for their anti-convulsive actions in human. As for AT4, the identification of small but critically important peptide fragments [258] and their N- and C-terminal modifications led to the development of the selective AT4 agonist N-hexanoic-Tyr-Ile-(6) aminohexanoic amide. This molecule was sufficiently blood-brain barrier permeable to have precognitive effects in Morris water maze performance [259]. Experiments using selective AT4 agonists are needed in the future to address their anti-convulsive actions.

5.10. Corticotropin-releasing Hormone and Adrenocorticotropin

Corticotropin-releasing hormone (CRH), a 41 aa peptide, and adrenocorticotropin (ACTH), a 39 aa peptide are pivotal elements of the classical stress effector pathway. During stress response, CRH synthesized in the paraventricular hypothalamic nucleus leads to the release of ACTH from the pituitary, which in turn leads to the secretion of glucocorticoids, e.g. cortisol in human and corticosterone in rat, from the adrenal gland. In turn, ACTH and glucocorticoids exert a negative feed-back to the activity of CRH [260]. More recently, ACTH, CRH and their receptors identified in different limbic brain regions were found to be involved in affective responses [261-263]. Data are available on the changes of CRH levels during development and in epilepsy models [264]. Interestingly, CRH concentrations in limbic areas are the highest during early postnatal development [265]. CRH gene expression was up-regulated after pilocarpine induced status epilepticus in the strata oriens and pyramidale of CA1 area and in the stratum pyramidale of CA3 area [266] while amygdala-kindled seizures and electroconvulsive shock increased the expression of CRH and CRH-binding protein in interneurons of the dentate hilus [267, 268]. A day after seizures, hippocampal areas undergoing neurodegeneration exhibited increased CRH immunoreactivity. In fact, networks of CRH fibers closely surrounded moribund neurons [269]. Elevations in CRH mRNA and immunoreactivity were identified not only in the hippocampus but also in the bed nucleus of the stria terminalis, the globus pallidus, the piriform cortex, and the central nucleus of the amygdala following kainate-induced seizures [270, 271]. However, physically stressful stimuli, e.g. cold, which activate hypothalamic CRH neurons, were not effective in the hippocampus [272].

CRH administered into the cerebral ventricles of rats during the first postnatal week caused a specific and stereotyped behavior sequence: rhythmic chewing and licking (jaw myoclonus) followed by 'limbic'-type seizures [273], which resembled kainate and amygdala kindled convulsions [274]. Furthermore, death of hippocampal pyramidal cells occurred following CRH-induced status epilepticus in infant rats [275]. The pro-convulsant actions of corticotropin-releasing hormone in the hippocampus of infant rats are consistent with the stimulatory activity of CRH on incoming excitatory signals reaching pyramidal cells [276]. However, CRH-produced age-specific seizures correlated with rhythmic amygdala discharges while paroxysmal hippocampal and cortical discharges developed suggesting that CRH-induced electrographic and behavioral seizures may originate in the amygdala in infant rats [277]. Reduction of CRH effects, e.g. by CRH-saporin, reduced handling-induced seizure susceptibility in a seizure-prone strain [278]. Furthermore, astressin, a novel and potent CRH antagonist delayed the onset of CRH-induced seizures after infusion into the cerebral ventricles of infant rats [279]. In addition, astressin inhibited kainate-induced seizures and also had neuroprotective features [280]. The involvement of CRH in epilepsy was also supported by genetic studies. Nocturnal frontal lobe epilepsy was correlated with a nucleotide variation in the promoter of CRH [281], which could result in reduced levels of CRH secretion in response to stressful stimuli [282].

As opposed to the pro-convulsant activity of CRH, ACTH is anti-epileptic. Its efficacy in primary infantile spasms is long known [283]. Different protocols are used in clinical practice. High-dose, short-duration versus low-dose, long-duration ACTH therapies and low-dose alternate-day corticotropin therapy could all be effective [284, 285]. ACTH clearly increases plasma corticosteroid levels providing the possibility for indirect actions. However, ACTH treatment is superior to steroid (e.g. predonsone) treatment for infantile spasms, as assessed by both clinical and EEG criteria [286]. There is a substantial amount of evidence available that ACTH acts by reducing CRH function in different brain sites, similar to its action in the neuroendocrine hypothalamus. ACTH, administered peripherally as well as centrally, down-regulated the expression of CRH and CRH-binding protein in infant rat hippocampus and amygdala [287, 288]. ACTH does not control neonatal seizures induced by administration of exogenous CRH [289]. Moreover, selective blocking of ACTH receptors prevented ACTH-induced down-regulation of CRH expression [287]. Based on these findings, the CRH-excess theory was formulated: the many etiologies in early childhood seizures, particularly West syndrome, all lead to the production and secretion of CRH as part of a central stress response. These effects of CRH are restricted to the infancy period because the recep-

tors for CRH, which mediate its action on neurons, are most abundant during this developmental period [290]. ACTH administration is known to inhibit production and release of CRH *via* a negative feedback mechanism [291]. However, other actions of ACTH, such as a direct potentiation of inhibitory interneurons have also been suggested [292].

There are 2 CRH receptors, designated as type 1 and 2 (CRHR1 and CRHR2). These receptors belong to class II of G-protein coupled receptors. Both use G_s and G_q/G_{11} proteins to increase adenylate cyclase and phospholipase C activity, respectively [293]. Elevated CRHR1 but not CRHR1 expression was found in *post mortem* brain obtained from children with generalized epilepsy suggesting the role of CRHR1 in seizure generation [294]. Furthermore, selective CRH1R but not CRH2R antagonists increased the latency and decreased the duration of CRH-induced seizures [295]. Thus, CRHR1 antagonists could be useful in the treatment of early childhood seizures. ACTH treatment has some side-effects, including potential hypertrophic cardiomyopathy [296]. Therefore, drugs which block the actions of CRH on its receptors may provide a better therapy for early childhood seizures. There are some small molecule non-peptide selective antagonists of the CRHR1 available [297] including pexacerfont (BMS-562,086), antalarmin, and CP-154,526 (Fig. **6**). These and similar drugs are being tested for depression, alcoholism, anxiety disorders, etc.

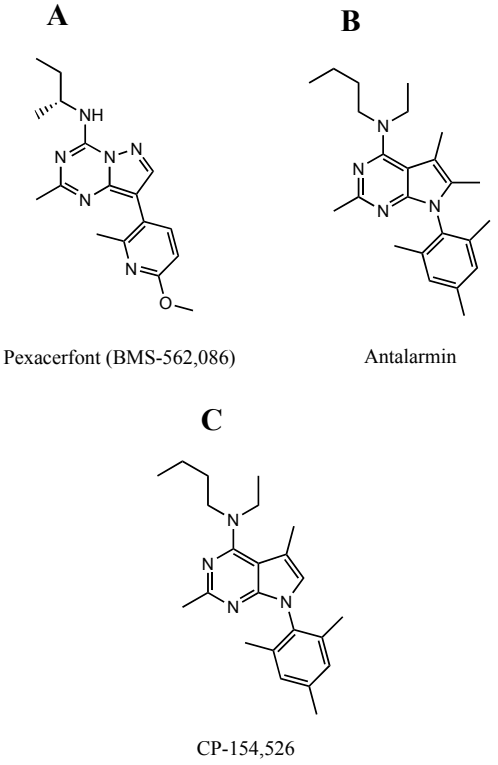


Fig. (6). Selective non-peptide antagonists of the CRHR1.

The receptor of ACTH is the melanocortin-2 receptor (MC2R) coupled to G_s proteins to activate adenylate cyclase. Other melanocortin receptors whose ligands are melanocyte-stimulating hormones, do not recognize ACTH [298]. MC2R agonists could be useful alternatives of ACTH treatment in early childhood seizures, however, such drugs are not available at present.

5.11. Thyrotropin-releasing Hormone

The hypothalamic paraventricular nucleus synthesizes the tripeptide thyrotropin-releasing hormone (TRH) to affect thyroid hormone secretion from the thyroid gland. In addition, TRH is also produced in different brain regions including the cerebral cortex, the hippocampus, the amygdala, the striatum, and the brainstem while experimental models of epilepsy induce the largest increase in the hippocampal TRH expression [299]. Furthermore, the hippocampal release of TRH by seizures has also been reported [300]. In turn, TRH inhibits glutamate release but increases GABA release in the hippocampus [301, 302], which might be the basis of its anti-epileptic action.

TRH was anti-convulsant in experimental models of temporal [303, 304] and absence epilepsies [305, 306]. TRH is safe in children and effective in some cases of West syndrome and Lennox-Gastaut syndrome [307] and in progressive myoclonic epilepsy [308]. TRH was suggested to be used if side-effects of ACTH are too disturbing [309]. To overcome the problems with bioavailability of TRH, different approaches were tested. A TRH was attached to a biodegradable polyanhydride copolymer as a sustained-release carrier and implanted into the amygdala of kindling rats. The anti-convulsant effects provided evidence in support of *in situ* microdisk pharmacotherapy for potential neuropeptide delivery in intractable epilepsy [310]. Intranasal delivery of 3-methyl-histidine TRH was demonstrated to attenuate seizures in amygdala-kindled rats [311]. Furthermore, intranasal application of microdisc nanoparticle containing TRH was also effective [312].

TRH acts through 2 different G-protein coupled receptors, TRH-R1 and TRH-R2. Both receptors elevate phospholipase C *via* G_{q11}. TRH-R1 is the major form in the hypothalamo-pituitary axis while TRH-R2 plays the major role in extrahypothalamic brain sites [313]. Although TRH itself was shown to have anti-epileptic activity, its short half-life and poor penetration through the blood-brain barrier does not allow its widespread application [314]. Consequently, a number of modified peptide analogues of TRH were synthesized. Although many of these have improved stability, none of them is particularly selective for the TRH receptor subtypes. Still, they represent a tool to affect convulsions, and were indeed successfully applied in a variety of experimental models and in human [315]. YM-14673 (N alpha-[(S)-4-oxo-2-azetidinyl]-carbonyl]-L-prolinamide dihydrate) shortened afterdischarge duration following kindling [303]. CNK-602A (N-[(6-methyl-5-oxo-3-thiomorpholinyl) carbonyl]-L-histidyl-L-prolinamide) inhibited absence-like seizure and tonic convulsion in spontaneously epileptic rats [316, 317]. NP-355 (L-pGlu-(1-benzyl)-L-His-L-ProNH(2); Fig. **7**) and NP-647 (L-pGlu-(2-propyl)-L-His-L-ProNH2) were effective anti-convulsants in a number of different models of epilepsy [318-320]. Clearly, selective non-peptide molecules acting on TRH-R2 would have great anti-epileptic potential and their development is awaited.

5.12. Oxytocin and Vasopressin

Oxytocin and vasopressin are structurally related 9 aa hormones released from the terminals of magnocellular hypothalamic neurons in the pituitary [321]. However, they cannot readily penetrate the blood-brain barrier. Therefore, their cen-

tral actions are probably exerted as neuropeptides synthesized in the paraventricular hypothalamic nucleus and possibly other brain regions, from which oxytocin- and vasopressin-containing terminals reach a variety of brain areas [322] including the hippocampus [323, 324]. Both oxytocin and vasopressin mRNA expressions are elevated in the paraventricular hypothalamic nucleus during seizures [325]. There is an increasing amount of evidence - albeit sometimes contradictory - on the effects of oxytocin and vasopressin on seizure activity. Intraperitoneally injected oxytocin was shown to exacerbate pentylenetetrazol-induced seizures in mice [326] while a higher concentration of oxytocin was anti-convulsive in the same epilepsy model in rats [327]. The enhancement of hippocampal inhibitory transmission by oxytocin also supports an anti-convulsive action of oxytocin [328, 329]. In contrast, subcutaneously applied vasopressin had pro-convulsive effects [330] in agreement with the excitatory potential of this neuropeptide [331, 332]. For concerns on the penetration of oxytocin and vasopressin through the blood-brain-barrier, they have also been successfully applied intranasally [333-335].

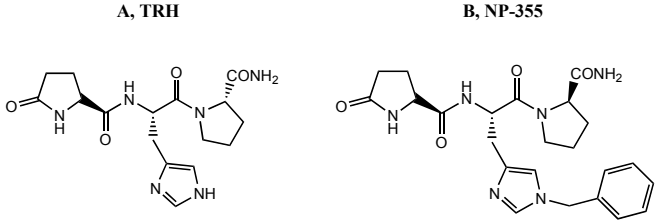


Fig. (7). The structure of TRH and its analogue NP-355.

There are 4 mammalian receptors of oxytocin and vasopressin: oxytocin receptor (OTR), and vasopressin receptors 1a, 1b, and 2 (V_{1a}, V_{1b}, and V_2). A significant cross-reactivity characterizes these ligand-receptor systems. Vasopressin acts on all 4 receptors while oxytocin recognizes vasopressin receptors albeit with less affinity than OTR [336]. OTR, and possibly vasopressin receptors, too, can bind to $G_{q/11}$, G_i, and G_s initiating different signaling pathways. The predominant neural forms are OTR and V_{1a}, which have widespread but complementary expressions in the brain including the hippocampus: OTRs are found in the CA1 region and the subiculum, while V_1 in the dentate gyrus, CA2 and CA3 regions [328, 337].

There is a considerable amount of evidence that the pro-convulsive effects of oxytocin and vasopressin are exerted *via* V_{1a} receptor while OTR could be responsible for anti-convulsive actions [326, 330, 338]. These findings suggest that OTR agonists and V_{1a} receptor antagonists could be potential drug targets in epilepsy. The pharmacology of oxytocin and vasopressin receptors is well elaborated [339]. There are selective non-peptide OTR agonists, such as compound 39 (Fig. **8A**) and WAY-267,464 (Fig. **8B**) and selective V_{1a} receptor antagonists including the orally active relcovaptan (Fig. **8C**) available [340-342]. These drugs represent important tools to further define the involvement of oxytocin and vasopressin receptors in epilepsy by means of their usage in animal experimentation.

6. COMPARISON OF NEUROPEPTIDE SYSTEMS REGARDING THEIR ROLE IN EPILEPSY AND THEIR THERAPEUTIC POTENTIAL

Neuropeptide systems possess inherent similarities because of their similar character and neurochemical nature. On the other hand, their modulatory actions also differ from each other. They have different distributions in the brain and also within specific brain regions, e.g. the hippocampus, which was best studied for their importance in epilepsy. Neuropeptides with well-established profile in the hippocampus are studied for their role in temporal lobe epilepsies. Obviously, such a role does not exclude their involvement in other types of epilepsies. Still, the focus of research for peptides such as CRH and ACTH, where the latter is used for the treatment of early childhood epilepsies is understandably different. The role of none of the neuropeptides is so firmly established that some types of epilepsies should be excluded from their investigations. Some neuropeptides, e.g. NPY [28], dynorphin [139], TRH [305, 306] are known to be involved in absence epilepsy, too. However, many other neuropeptides have not been thoroughly investigated in animal models of absence epilepsies.

The neuropeptides described in this chapter are all implicated to some degree with seizures (Table **1**). However, the strength of evidence supporting their involvement is different. Most of the peptides demonstrate altered levels during or after seizures, which is, however, only indicative for their role in seizure, and an indirect evidence at best. The peptides discussed were all tested for their effects in convulsion, too. Most of the neuropeptides have anti-convulsive actions. However, some of them, such as enkephalin, substance P, CCK, AngII, CRH, and vasopressin were generally pro-convulsive. For peptides, the role of which in seizure generation or maintenance is best established, pharmacological antagonism or transgenic studies supported the results of peptide injections. E.g. mice lacking somatostatin receptors [46], functional NPY [64] or galanin gene or receptor [89] were prone to seizures and showed increased sensitivity for convulsive agents. On the other hand, for CCK and VIP, the evidence supporting their role in any seizure activity is relatively weak. Even for peptides with firmly established anti- or pro-convulsive effect, the mechanisms behind the actions remain obscure. The exact localization of their receptors (excitatory or inhibitory neurons, cell body, axon terminal, proximal, or distal dendrite, etc.), their signal transduction and electrophysiological analysis has to be clarified for many neuropeptides and cell types in the future. Furthermore, these properties may change in the course of seizures. Neuropeptides are released during overstimulation and their subsequent absence in the tissue may contribute to the maintenance of seizures, an event, which is incompletely understood. Still, it is critically important for the treatment of epilepsy if a neuropeptide receptor can interrupt this maintenance phase as shown for galanin and dynorphin as opposed to only influences the initiation of seizures [2, 343].

Another critically important issue using neuropeptide systems in epilepsy therapy is the advancement of their pharmacology. This means developing drugs acting on their receptors as their synthesizing and degrading enzymes are not known yet or are not sufficiently specific to be exploited, except for angiotensin (ACE blockers). It is particularly challenging to produce stable, small molecule drugs acting on neuropeptide receptors that cross the blood-brain barrier. Such pharmacology is the most progressed for opioid peptides, angiotensin II, agonists and antagonists of which are used in clinical practice but were still not suitable for

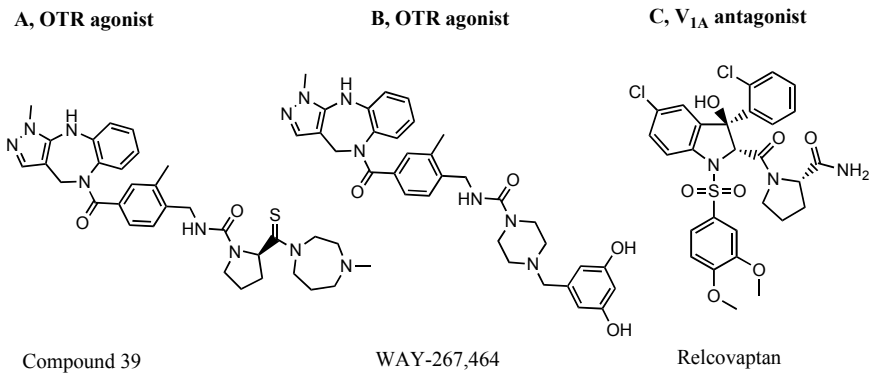


Fig. (8). Non-peptide molecules acting selectively on oxytocin and vasopressin receptor subtypes.

Table 1. The Comparison of the State of Research Regarding the Receptors of Neuropeptides Potentially Involved in Seizures and Selective Drugs Acting on the Receptor Subtypes that are Potentially Involved in Epilepsy

Neuropeptid	Size (aa)	Receptors	Receptor Types Involved in Seizures	Feature of Potentially Anti-convulsive Drugs	Selective, Non-peptide, Potentially Anti-convulsive Compounds
Somatostatin	14	sst_1-sst_5	sst_2, sst_3, sst_4	sst_2 agonists	L779.97[illegible] L-054,522
				sst_3 agonists	L-796,778
				sst_4 agonists	L-803,087; NNC 26-9100
NPY	36	Y1, 2, 4, 5	Y1, Y2, Y5	Y1 antagonists	BIBP-3226, BIBO-3226
				Y2, Y5 agonists	
Galanin	29	GalR1, 2, 3	GalR1, GalR2	GalR1/2 positive allosteric modulators	Galmic, Galnon, CYM2503
Enkephalin	5	MOR (μ) DOR (δ)	MOR, DOR	MOR antagonists	Cyprodime; beta-Funaltrexamine
				DOR antagonists	Naltrindol, Naltriben
Dynorphin	13	KOR (κ)	KOR	KOR agonists	Butophanol; Cyclazocine; U-50488
Substance P	11	NK-1	NK-1	NK-1 antagonists	CP-122,721; GR205171; MK-869
CCK	8	CCK1, 2	CCK2 ?	CCK2 antagonists	L-365,260; YF-476; RP-69758
				CCK2 inverse agonists	L-740,093
VIP	28	VPAC1,2-R	VPAC2-R ?		
Ghrelin	28	GHSR1a	GHSR1a	GHSR1a agonists	NN703; MK-677; L-692,429
AngII	8	AT1, 2	AT1	AT1 antagonists	Losartan
				ACE inhibitors	Captopril
AngIV	6	AT4	AT4	AT4 agonists	
CRH	41	CRHR1, 2	CRHR1	CRHR1 antagonists	Pexacerfont; Antalarmin; CP-154,526
ACTH	39	MC2R	MC2R	MC2R agonists	
TRH	3	TRH-R1, 2	TRH-R2	TRH-R2 agonists	
Oxytocin	9	OTR	OTR	OTR agonists	Compound 39, WAY-267,464
Vasopressin	9	OTR, V_{1a} V_{1b}, V_2	V_{1a}	V_{1a} antagonists	Relcovaptan

epilepsy. In fact, there are no drugs acting on neuropeptide receptors used for the treatment of epilepsy except for ACTH and to some degree TRH for early childhood seizures. Recent progress is, however promising for galanin, NPY, ghre-

lin, and to some degree somatostatin and substance P (Table **1**). A potential approach to tackle the difficulty of developing small molecules that bind the large binding sites of peptides is using allosteric modulators, which was most successful for galanin receptors [102]. In addition, solutions are tested that can possibly circumvent the problem of developing orally available drugs. Tools for subcutaneous delivery are becoming more convenient. The stability of peptides can be enhanced by reducing their degradation after better understanding of this process. Furthermore, attempts are promising for using existent transport mechanisms to support the penetration of therapeutic peptides through the blood-brain barrier [17, 344]. Another possible solution is gene transfer delivery of neuropeptides, e.g. by using viral tools [20, 22]. These approaches are most advanced for NPY [66].

7. CONCLUSION

We demonstrated here that epileptic activity of all forms of epilepsy can be modulated by activating and inhibiting different neuropeptide receptors that shifts the excitatory/inhibitory balance out of hyperexcitability range. Thus, neuropeptide receptors represent very promising novel targets for drug development in general because of the potentially low side-effects and because they possess the pharmacologically least exploited G-protein receptors. In particular, the endogenous functions of neuropeptides, their sustained and specific influence on neuronal excitation and gene transcription make them appealing for the development of anti-epileptic drugs. However, the difficulties for their therapeutic applications are also significant, as proteases quickly degrade peptides, and the large binding sites make is difficult to develop small agonists and antagonist of neuropeptide receptors. In fact, the majority of the currently available drugs listed in the manuscript are suitable only for basic research with limited therapeutic potential. However, recent progress in the field makes us to believe that neuropeptide systems will be utilized clinically for the treatment of epilepsy in the near future *via* developing peptidomimetics or other non-peptide agonists, antagonists, allosteric regulators, etc., and even gene transfer methods. In particular, recent progress with neuropeptide Y and ghrelin receptor drugs are promising. Our conclusive message is that investigation of neuropeptides in epilepsy must be boosted up to get more targets for drug development.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

aa	=	Amino acid
ACE	=	Angiotensin converting enzyme
ACTH	=	Adrenocorticotropin
Ang	=	Angiotensin
AT1	=	Angiotensin II receptor type 1
CCK	=	Cholecystokinin
CCK1	=	Cholecystokinin 1 receptor
CRH	=	Corticotropin-releasing hormone
DOR	=	Delta-opioid receptor
GalR	=	Galanin receptor
GHSR	=	Growth hormone secretagogue receptor
KOR	=	Kappa-opioid receptor
MC2R	=	Melanocortin-2 receptor
MOR	=	mu-opioid receptor
NK-1	=	Neurokinin-1 receptor
NPY	=	Neuropeptide Y
OTR	=	Oxytocin receptor
PACAP	=	Pituitary adenylate cyclase-activating polypeptide
RAS	=	Renin-angiotensin system
sst	=	Somatostatin receptors
TRH	=	Thyrotropin-releasing hormone
V	=	Vasopressin receptor
VIP	=	Vasoactive intestinal peptide
Y	=	Neuropeptide Y receptor

REFERENCES

[1] Kwan, P.; Brodie, M.J. Drug treatment of epilepsy: When does it fail and how to optimize its use? *CNS Spectr.,* **2004,** *9*(2), 110-119.

[2] Chen, J.W.; Naylor, D.E.; Wasterlain, C.G. Advances in the pathophysiology of status epilepticus. *Acta Neurol. Scand.,* **2007,** *115*(4 Suppl), 7-15.

[3] Kobow, K.; Auvin, S.; Jensen, F.; Loscher, W.; Mody, I.; Potschka, H.; Prince, D.; Sierra, A.; Simonato, M.; Pitkanen, A.; Nehlig, A.; Rho, J.M. Finding a better drug for epilepsy: Antiepileptogenesis targets. *Epilepsia,* **2012,** *53*(11), 1868-1876.

[4] de Lanerolle, N.C.; Brines, M.; Williamson, A.; Kim, J.H.; Spencer, D.D. Neurotransmitters and their receptors in human temporal lobe epilepsy. *Epilepsy Res. Suppl.,* **1992,** *7*, 235-250.

[5] Devlin, A.; Odell, M.; Charlton, J.; Koppel, S. Epilepsy and driving: Current status of research. *Epilepsy Res.,* **2012,** *102*(3), 135-152.

[6] Casillas-Espinosa, P.M.; Powell, K.L.; O'Brien, T.J. Regulators of synaptic transmission: Roles in the pathogenesis and treatment of epilepsy. *Epilepsia,* **2012,** *53 Suppl 9*, 41-58.

[7] Hokfelt, T.; Broberger, C.; Xu, Z.Q.; Sergeyev, V.; Ubink, R.; Diez, M. Neuropeptides--an overview. *Neuropharmacology,* **2000,** *39*(8), 1337-1356.

[8] Bean, A.J.; Zhang, X.; Hokfelt, T. Peptide secretion: What do we know? *FASEB J.,* **1994,** *8*(9), 630-638.

[9] Hokfelt, T.; Bean, A.; Ceccatelli, S.; Dagerlind, A.; Elde, R.P.; Goldstein, M.; Meister, B.; Melander, T.; Nicholas, A.P.; Pelto-Huikko, M. Neuropeptides and classical transmitters. Localization and interaction. *Arzneimittelforschung,* **1992,** *42*(2A), 196-201.

[10] Hokfelt, T.; Millhorn, D.; Seroogy, K.; Tsuruo, Y.; Ceccatelli, S.; Lindh, B.; Meister, B.; Melander, T.; Schalling, M.; Bartfai, T.; Terenius, L. Coexistence of peptides with classical neurotransmitters. *Experientia,* **1987,** *43*(7), 768-780.

[11] Baraban, S.C.; Tallent, M.K. Interneuron diversity series: Interneuronal neuropeptides--endogenous regulators of neuronal excitability. *Trends Neurosci.,* **2004,** *27*(3), 135-142.

[12] Fredholm, B.B.; Hokfelt, T.; Milligan, G. G-protein-coupled receptors: An update. *Acta Physiol. (Oxf.),* **2007,** *190*(1), 3-7.

[13] Jacoby, E.; Bouhelal, R.; Gerspacher, M.; Seuwen, K. The 7 tm g-protein-coupled receptor target family. *ChemMedChem,* **2006,** *1*(8), 761-782.

[14] Van den Pol, A.N. Neuropeptide transmission in brain circuits. *Neuron,* **2012,** *76*(1), 98-115.

[15] Werner, F.M.; Covenas, R. Classical neurotransmitters and neuropeptides involved in generalized epilepsy: A focus on antiepileptic drugs. *Curr. Med. Chem.,* **2011,** *18*(32), 4933-4948.

[16] Wasterlain, C.G.; Liu, H.; Mazarati, A.M.; Baldwin, R.A.; Shirasaka, Y.; Katsumori, H.; Thompson, K.W.; Sankar, R.; Pereira de Vasconselos, A.; Nehlig, A. Self-sustaining status epilepticus: A condition maintained by potentiation of glutamate receptors and by plastic changes in substance p and other peptide neuromodulators. *Epilepsia,* **2000,** *41 Suppl 6*, S134-143.

[17] McGonigle, P. Peptide therapeutics for cns indications. *Biochem. Pharmacol.,* **2012,** *83*(5), 559-566.

[18] Pitkanen, A. New pharmacotherapy for epilepsy. *IDrugs,* **2004,** *7*(5), 471-477.

[19] Boison, D. Cell and gene therapies for refractory epilepsy. *Curr. Neuropharmacol.,* **2007,** *5*(2), 115-125.

[20] Riban, V.; Fitzsimons, H.L.; During, M.J. Gene therapy in epilepsy. *Epilepsia,* **2009,** *50*(1), 24-32.

[21] Weinberg, M.S.; McCown, T.J. Current prospects and challenges for epilepsy gene therapy. *Exp. Neurol.,* **2011**.

[22] McCown, T.J. The future of epilepsy treatment: Focus on adeno-associated virus vector gene therapy. *Drug News Perspect.,* **2010,** *23*(5), 281-286.

[23] Freund, T.F.; Buzsaki, G. Interneurons of the hippocampus. *Hippocampus,* **1996,** *6*(4), 347-470.

[24] Somogyi, P.; Klausberger, T. Defined types of cortical interneurone structure space and spike timing in the hippocampus. *J. Physiol.,* **2005,** *562*(Pt 1), 9-26.

[25] De Lanerolle, N.C.; Lee, T.S.; Spencer, D.D. Histopathology of human epilepsy. In: *Jasper's basic mechanisms of the epilepsies;* Noebels, J.L.; Avoli, M.; Rogawski, M.A.; Olsen, R.W.; Delgado-Escueta, A.V., Eds.; Bethesda (MD), **2012**.

[26] Vitko, I.; Chen, Y.; Arias, J.M.; Shen, Y.; Wu, X.R.; Perez-Reyes, E. Functional characterization and neuronal modeling of the effects of childhood absence epilepsy variants of cacna1h, a t-type calcium channel. *J. Neurosci.,* **2005,** *25*(19), 4844-4855.

[27] Van Raay, L.; Jovanovska, V.; Morris, M.J.; O'Brien, T.J. Focal administration of neuropeptide y into the s2 somatosensory cortex maximally suppresses absence seizures in a genetic rat model. *Epilepsia,* **2012,** *53*(3), 477-484.

[28] Morris, M.J.; Gannan, E.; Stroud, L.M.; Beck-Sickinger, A.G.; O'Brien, T.J. Neuropeptide y suppresses absence seizures in a genetic rat model primarily through effects on y receptors. *Eur. J. Neurosci.,* **2007,** *25*(4), 1136-1143.

[29] Efendic, S.; Hokfelt, T.; Luft, R. Somatostatin. *Adv. Metab. Disord.,* **1978,** *9*, 367-424.

[30] Viollet, C.; Lepousez, G.; Loudes, C.; Videau, C.; Simon, A.; Epelbaum, J. Somatostatinergic systems in brain: Networks and functions. *Mol. Cell. Endocrinol.,* **2008,** *286*(1-2), 75-87.

[31] Kosaka, T.; Wu, J.Y.; Benoit, R. Gabaergic neurons containing somatostatin-like immunoreactivity in the rat hippocampus and dentate gyrus. *Exp. Brain Res.,* **1988,** *71*(2), 388-398.

[32] Leranth, C.; Malcolm, A.J.; Frotscher, M. Afferent and efferent synaptic connections of somatostatin-immunoreactive neurons in the rat fascia dentata. *J. Comp. Neurol.,* **1990,** *295*(1), 111-122.

[33] Buhl, E.H.; Halasy, K.; Somogyi, P. Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the number of synaptic release sites. *Nature,* **1994,** *368*(6474), 823-828.

[34] Maccaferri, G.; Roberts, J.D.; Szucs, P.; Cottingham, C.A.; Somogyi, P. Cell surface domain specific postsynaptic currents evoked by identified gabaergic neurones in rat hippocampus *in vitro*. *J. Physiol.,* **2000,** *524 Pt 1*, 91-116.

[35] Marti, M.; Bregola, G.; Morari, M.; Gemignani, A.; Simonato, M. Somatostatin release in the hippocampus in the kindling model of epilepsy: A microdialysis study. *J. Neurochem.,* **2000,** *74*(6), 2497-2503.

[36] Hashimoto, T.; Obata, K. Induction of somatostatin by kainic acid in pyramidal and granule cells of the rat hippocampus. *Neurosci. Res.,* **1991,** *12*(4), 514-527.

[37] Kobayashi, M.; Buckmaster, P.S. Reduced inhibition of dentate granule cells in a model of temporal lobe epilepsy. *J. Neurosci.,* **2003,** *23*(6), 2440-2452.

[38] De Lanerolle, N.C.; Kim, J.H.; Robbins, R.J.; Spencer, D.D. Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. *Brain Res.,* **1989,** *495*(2), 387-395.

[39] Tallent, M.K.; Qiu, C. Somatostatin: An endogenous antiepileptic. *Mol. Cell. Endocrinol.,* **2008,** *286*(1-2), 96-103.

[40] Tallent, M.K.; Siggins, G.R. Somatostatin acts in ca1 and ca3 to reduce hippocampal epileptiform activity. *J. Neurophysiol.,* **1999,** *81*(4), 1626-1635.

[41] Boehm, S.; Betz, H. Somatostatin inhibits excitatory transmission at rat hippocampal synapses via presynaptic receptors. *J. Neurosci.,* **1997,** *17*(11), 4066-4075.

[42] Mazarati, A.M.; Telegdy, G. Effects of somatostatin and anti-somatostatin serum on picrotoxin-kindled seizures. *Neuropharmacology,* **1992,** *31*(8), 793-797.

[43] Zafar, R.; King, M.A.; Carney, P.R. Adeno associated viral vector-mediated expression of somatostatin in rat hippocampus suppresses seizure development. *Neurosci. Lett.,* **2012,** *509*(2), 87-91.

[44] Monno, A.; Rizzi, M.; Samanin, R.; Vezzani, A. Anti-somatostatin antibody enhances the rate of hippocampal kindling in rats. *Brain Res.,* **1993,** *602*(1), 148-152.

[45] Csaba, Z.; Dournaud, P. Cellular biology of somatostatin receptors. *Neuropeptides,* **2001,** *35*(1), 1-23.

[46] Qiu, C.; Zeyda, T.; Johnson, B.; Hochgeschwender, U.; de Lecea, L.; Tallent, M.K. Somatostatin receptor subtype 4 couples to the m-current to regulate seizures. *J. Neurosci.,* **2008,** *28*(14), 3567-3576.

[47] De Bundel, D.; Aourz, N.; Kiagiadaki, F.; Clinckers, R.; Hoyer, D.; Kastellakis, A.; Michotte, Y.; Thermos, K.; Smolders, I. Hippocampal sst(1) receptors are autoreceptors and do not affect seizures in rats. *Neuroreport,* **2010,** *21*(4), 254-258.

[48] Rohrer, S.P.; Birzin, E.T.; Mosley, R.T.; Berk, S.C.; Hutchins, S.M.; Shen, D.M.; Xiong, Y.; Hayes, E.C.; Parmar, R.M.; Foor, F.; Mitra, S.W.; Degrado, S.J.; Shu, M.; Klopp, J.M.; Cai, S.J.; Blake, A.; Chan, W.W.; Pasternak, A.; Yang, L.; Patchett, A.A.; Smith, R.G.; Chapman, K.T.; Schaeffer, J.M. Rapid identification of subtype-selective agonists of the somatostatin receptor through combinatorial chemistry. *Science,* **1998,** *282*(5389), 737-740.

[49] Rohrer, S.P.; Schaeffer, J.M. Identification and characterization of subtype selective somatostatin receptor agonists. *J. Physiol. Paris,* **2000,** *94*(3-4), 211-215.

[50] Hoyer, D.; Nunn, C.; Hannon, J.; Schoeffter, P.; Feuerbach, D.; Schuepbach, E.; Langenegger, D.; Bouhelal, R.; Hurth, K.; Neumann, P.; Troxler, T.; Pfaeffli, P. Sra880, *in vitro* characterization of the first non-peptide somatostatin sst(1) receptor antagonist. *Neurosci Lett.,* **2004,** *361*(1-3), 132-135.

[51] Feniuk, W.; Jarvie, E.; Luo, J.; Humphrey, P.P. Selective somatostatin sst(2) receptor blockade with the novel cyclic octapeptide, cyn-154806. *Neuropharmacology,* **2000,** *39*(8), 1443-1450.

[52] Reubi, J.C.; Schaer, J.C.; Wenger, S.; Hoeger, C.; Erchegyi, J.; Waser, B.; Rivier, J. Sst3-selective potent peptidic somatostatin receptor antagonists. *Proc. Natl. Acad. Sci. U.S.A.,* **2000,** *97*(25), 13973-13978.

[53] Moneta, D.; Richichi, C.; Aliprandi, M.; Dournaud, P.; Dutar, P.; Billard, J.M.; Carlo, A.S.; Viollet, C.; Hannon, J.P.; Fehlmann, D.; Nunn, C.; Hoyer, D.; Epelbaum, J.; Vezzani, A. Somatostatin receptor subtypes 2 and 4 affect seizure susceptibility and hippocampal excitatory neurotransmission in mice. *Eur. J. Neurosci.,* **2002,** *16*(5), 843-849.

[54] Aourz, N.; De Bundel, D.; Stragier, B.; Clinckers, R.; Portelli, J.; Michotte, Y.; Smolders, I. Rat hippocampal somatostatin sst3 and sst4 receptors mediate anticonvulsive effects *in vivo*: Indications of functional interactions with sst2 receptors. *Neuropharmacology,* **2011,** *61*(8), 1327-1333.

[55] Pedrazzini, T.; Pralong, F.; Grouzmann, E. Neuropeptide y: The universal soldier. *Cell. Mol. Life Sci.,* **2003,** *60*(2), 350-377.

[56] Colmers, W.F.; Lukowiak, K.; Pittman, Q.J. Neuropeptide y action in the rat hippocampal slice: Site and mechanism of presynaptic inhibition. *J. Neurosci.,* **1988,** *8*(10), 3827-3837.

[57] Marksteiner, J.; Sperk, G.; Maas, D. Differential increases in brain levels of neuropeptide y and vasoactive intestinal polypeptide after kainic acid-induced seizures in the rat. *Naunyn Schmiedebergs Arch. Pharmacol.,* **1989,** *339*(1-2), 173-177.

[58] Sloviter, R.S. Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in the rat: The "dormant basket cell" hypothesis and its possible relevance to temporal lobe epilepsy. *Hippocampus,* **1991,** *1*(1), 41-66.

[59] Lagrange, A.H. Valproate enhances neuropeptide y expression: Modulating the modulators. *Epilepsy Curr.,* **2007,** *7*(4), 107-109.

[60] Woldbye, D.P.; Kokaia, M. Neuropeptide y and seizures: Effects of exogenously applied ligands. *Neuropeptides,* **2004,** *38*(4), 253-260.

[61] Colmers, W.F.; El Bahh, B. Neuropeptide y and epilepsy. *Epilepsy Curr.,* **2003,** *3*(2), 53-58.

[62] Vezzani, A.; Moneta, D.; Mule, F.; Ravizza, T.; Gobbi, M.; French-Mullen, J. Plastic changes in neuropeptide y receptor subtypes in experimental models of limbic seizures. *Epilepsia,* **2000,** *41 Suppl 6*, S115-121.

[63] Husum, H.; Gruber, S.H.; Bolwig, T.G.; Mathe, A.A. Extracellular levels of npy in the dorsal hippocampus of freely moving rats are markedly elevated following a single electroconvulsive stimulation, irrespective of anticonvulsive y1 receptor blockade. *Neuropeptides,* **2002,** *36*(5), 363-369.

[64] Erickson, J.C.; Clegg, K.E.; Palmiter, R.D. Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide y. *Nature,* **1996,** *381*(6581), 415-421.

[65] Vezzani, A.; Michalkiewicz, M.; Michalkiewicz, T.; Moneta, D.; Ravizza, T.; Richichi, C.; Aliprandi, M.; Mule, F.; Pirona, L.; Gobbi, M.; Schwarzer, C.; Sperk, G. Seizure susceptibility and epileptogenesis are decreased in transgenic rats overexpressing neuropeptide y. *Neuroscience,* **2002,** *110*(2), 237-243.

[66] Richichi, C.; Lin, E.J.; Stefanin, D.; Colella, D.; Ravizza, T.; Grignaschi, G.; Veglianese, P.; Sperk, G.; During, M.J.; Vezzani, A. Anticonvulsant and antiepileptogenic effects mediated by adeno-associated virus vector neuropeptide y expression in the rat hippocampus. *J. Neurosci.,* **2004,** *24*(12), 3051-3059.

[67] Noe, F.; Pool, A.H.; Nissinen, J.; Gobbi, M.; Bland, R.; Rizzi, M.; Balducci, C.; Ferraguti, F.; Sperk, G.; During, M.J.; Pitkanen, A.; Vezzani, A. Neuropeptide y gene therapy decreases chronic spontaneous seizures in a rat model of temporal lobe epilepsy. *Brain,* **2008,** *131*(Pt 6), 1506-1515.

[68] Eaton, K.; Sallee, F.R.; Sah, R. Relevance of neuropeptide y (npy) in psychiatry. *Curr. Top. Med. Chem.,* **2007,** *7*(17), 1645-1659.

[69] Walther, C.; Morl, K.; Beck-Sickinger, A.G. Neuropeptide y receptors: Ligand binding and trafficking suggest novel approaches in drug development. *J. Pept. Sci.,* **2011,** *17*(4), 233-246.

[70] Cabrele, C.; Beck-Sickinger, A.G. Molecular characterization of the ligand-receptor interaction of the neuropeptide y family. *J. Pept. Sci.,* **2000,** *6*(3), 97-122.

[71] Olesen, M.V.; Christiansen, S.H.; Gotzsche, C.R.; Nikitidou, L.; Kokaia, M.; Woldbye, D.P. Neuropeptide y y1 receptor hippocampal overexpression via viral vectors is associated with modest anxiolytic-like and proconvulsant effects in mice. *J. Neurosci. Res.,* **2012,** *90*(2), 498-507.

[72] Gotzsche, C.R.; Nikitidou, L.; Sorensen, A.T.; Olesen, M.V.; Sorensen, G.; Christiansen, S.H.; Angehagen, M.; Woldbye, D.P.; Kokaia, M. Combined gene overexpression of neuropeptide y and its receptor y5 in the hippocampus suppresses seizures. *Neurobiol. Dis.,* **2012,** *45*(1), 288-296.

[73] Marsh, D.J.; Baraban, S.C.; Hollopeter, G.; Palmiter, R.D. Role of the y5 neuropeptide y receptor in limbic seizures. *Proc. Natl. Acad. Sci. U.S.A.,* **1999,** *96*(23), 13518-13523.

[74] Foti, S.; Haberman, R.P.; Samulski, R.J.; McCown, T.J. Adeno-associated virus-mediated expression and constitutive secretion of npy or npy13-36 suppresses seizure activity *in vivo*. *Gene Ther.,* **2007,** *14*(21), 1534-1536.

[75] Lin, E.J.; Young, D.; Baer, K.; Herzog, H.; During, M.J. Differential actions of npy on seizure modulation via y1 and y2 receptors: Evidence from receptor knockout mice. *Epilepsia,* **2006,** *47*(4), 773-780.

[76] Meurs, A.; Clinckers, R.; Ebinger, G.; Michotte, Y.; Smolders, I. Clinical potential of neuropeptide y receptor ligands in the treatment of epilepsy. *Curr. Top. Med. Chem.,* **2007,** *7*(17), 1660-1674.

[77] Balasubramaniam, A.; Sheriff, S.; Zhai, W.; Chance, W.T. Bis(31/31')[[cys(31), nva(34)]npy(27-36)-nh(2)]: A neuropeptide y (npy) y(5) receptor selective agonist with a latent stimulatory effect on food intake in rats. *Peptides,* **2002,** *23*(8), 1485-1490.

[78] Aiglstorfer, I.; Hendrich, I.; Moser, C.; Bernhardt, G.; Dove, S.; Buschauer, A. Structure-activity relationships of neuropeptide y y1 receptor antagonists related to bibp 3226. *Bioorg. Med. Chem. Lett.,* **2000,** *10*(14), 1597-1600.

[79] Weiss, S.; Keller, M.; Bernhardt, G.; Buschauer, A.; Konig, B. Modular synthesis of non-peptidic bivalent npy y1 receptor antagonists. *Bioorg. Med. Chem.,* **2008,** *16*(22), 9858-9866.

[80] Weiss, S.; Keller, M.; Bernhardt, G.; Buschauer, A.; Konig, B. N(g)-acyl-argininamides as npy y(1) receptor antagonists: Influence of structurally diverse acyl substituents on stability and affinity. *Bioorg. Med. Chem.,* **2010,** *18*(17), 6292-6304.

[81] Melander, T.; Staines, W.A.; Rokaeus, A. Galanin-like immunoreactivity in hippocampal afferents in the rat, with special reference to cholinergic and noradrenergic inputs. *Neuroscience,* **1986,** *19*(1), 223-240.

[82] Xu, Z.Q.; Shi, T.J.; Hokfelt, T. Galanin/gmap- and npy-like immunoreactivities in locus coeruleus and noradrenergic nerve terminals in the hippocampal formation and cortex with notes on the galanin-r1 and -r2 receptors. *J. Comp. Neurol.,* **1998,** *392*(2), 227-251.

[83] Arabadzisz, D.; Antal, K.; Parpan, F.; Emri, Z.; Fritschy, J.M. Epileptogenesis and chronic seizures in a mouse model of temporal lobe epilepsy are associated with distinct eeg patterns and selective neurochemical alterations in the contralateral hippocampus. *Exp. Neurol.,* **2005,** *194*(1), 76-90.

[84] Mazarati, A.M.; Liu, H.; Soomets, U.; Sankar, R.; Shin, D.; Katsumori, H.; Langel, U.; Wasterlain, C.G. Galanin modulation of seizures and seizure modulation of hippocampal galanin in animal models of status epilepticus. *J. Neurosci.,* **1998,** *18*(23), 10070-10077.

[85] Mazarati, A.M.; Baldwin, R.A.; Shinmei, S.; Sankar, R. *In vivo* interaction between serotonin and galanin receptors types 1 and 2 in the dorsal raphe: Implication for limbic seizures. *J. Neurochem.,* **2005,** *95*(5), 1495-1503.

[86] Lerner, J.T.; Sankar, R.; Mazarati, A.M. Galanin and epilepsy. *Cell. Mo.l Life Sci.,* **2008,** *65*(12), 1864-1871.

[87] Zini, S.; Roisin, M.P.; Langel, U.; Bartfai, T.; Ben-Ari, Y. Galanin reduces release of endogenous excitatory amino acids in the rat hippocampus. *Eur. J. Pharmacol.,* **1993,** *245*(1), 1-7.

[88] Mazarati, A.M.; Halaszi, E.; Telegdy, G. Anticonvulsive effects of galanin administered into the central nervous system upon the picrotoxin-kindled seizure syndrome in rats. *Brain Res.,* **1992,** *589*(1), 164-166.

[89] Mazarati, A.M.; Hohmann, J.G.; Bacon, A.; Liu, H.; Sankar, R.; Steiner, R.A.; Wynick, D.; Wasterlain, C.G. Modulation of hippocampal excitability and seizures by galanin. *J. Neurosci.,* **2000,** *20*(16), 6276-6281.

[90] Kokaia, M.; Holmberg, K.; Nanobashvili, A.; Xu, Z.Q.; Kokaia, Z.; Lendahl, U.; Hilke, S.; Theodorsson, E.; Kahl, U.; Bartfai, T.; Lindvall, O.; Hokfelt, T. Suppressed kindling epileptogenesis in mice with ectopic overexpression of galanin. *Proc. Natl. Acad. Sci. U.S.A.,* **2001,** *98*(24), 14006-14011.

[91] Lin, E.J.; Richichi, C.; Young, D.; Baer, K.; Vezzani, A.; During, M.J. Recombinant aav-mediated expression of galanin in rat hippocampus suppresses seizure development. *Eur. J. Neurosci.,* **2003,** *18*(7), 2087-2092.

[92] Haberman, R.P.; Samulski, R.J.; McCown, T.J. Attenuation of seizures and neuronal death by adeno-associated virus vector galanin expression and secretion. *Nat. Med.,* **2003,** *9*(8), 1076-1080.

[93] Branchek, T.A.; Smith, K.E.; Gerald, C.; Walker, M.W. Galanin receptor subtypes. *Trends Pharmacol. Sci.,* **2000,** *21*(3), 109-117.

[94] Mazarati, A.; Lu, X.; Shinmei, S.; Badie-Mahdavi, H.; Bartfai, T. Patterns of seizures, hippocampal injury and neurogenesis in three models of status epilepticus in galanin receptor type 1 (galr1) knockout mice. *Neuroscience,* **2004,** *128*(2), 431-441.

[95] Mazarati, A.; Lu, X.; Kilk, K.; Langel, U.; Wasterlain, C.; Bartfai, T. Galanin type 2 receptors regulate neuronal survival, susceptibility to seizures and seizure-induced neurogenesis in the dentate gyrus. *Eur. J. Neurosci.,* **2004,** *19*(12), 3235-3244.

[96] Runesson, J.; Saar, I.; Lundstrom, L.; Jarv, J.; Langel, U. A novel galr2-specific peptide agonist. *Neuropeptides,* **2009,** *43*(3), 187-192.

[97] Lu, X.; Lundstrom, L.; Langel, U.; Bartfai, T. Galanin receptor ligands. *Neuropeptides,* **2005,** *39*(3), 143-146.

[98] Bulaj, G.; Green, B.R.; Lee, H.K.; Robertson, C.R.; White, K.; Zhang, L.; Sochanska, M.; Flynn, S.P.; Scholl, E.A.; Pruess, T.H.; Smith, M.D.; White, H.S. Design, synthesis, and characterization of high-affinity, systemically-active galanin analogues with potent anticonvulsant activities. *J. Med. Chem.,* **2008,** *51*(24), 8038-8047.

[99] Saar, K.; Mazarati, A.M.; Mahlapuu, R.; Hallnemo, G.; Soomets, U.; Kilk, K.; Hellberg, S.; Pooga, M.; Tolf, B.R.; Shi, T.S.; Hokfelt, T.; Wasterlain, C.; Bartfai, T.; Langel, U. Anticonvulsant activity of a nonpeptide galanin receptor agonist. *Proc. Natl. Acad. Sci. U.S.A.,* **2002,** *99*(10), 7136-7141.

[100] Ceide, S.C.; Trembleau, L.; Haberhauer, G.; Somogyi, L.; Lu, X.; Bartfai, T.; Rebek, J., Jr. Synthesis of galmic: A nonpeptide galanin receptor agonist. *Proc. Natl. Acad. Sci. U.S.A.,* **2004,** *101*(48), 16727-16732.

[101] Bartfai, T.; Lu, X.; Badie-Mahdavi, H.; Barr, A.M.; Mazarati, A.; Hua, X.Y.; Yaksh, T.; Haberhauer, G.; Ceide, S.C.; Trembleau, L.; Somogyi, L.; Krock, L.; Rebek, J., Jr. Galmic, a nonpeptide galanin receptor agonist, affects behaviors in seizure, pain, and forced-swim tests. *Proc. Natl. Acad. Sci. U.S.A.,* **2004,** *101*(28), 10470-10475.

[102] Hoyer, D. Neuropeptide receptor positive allosteric modulation in epilepsy: Galanin modulation revealed. *Proc. Natl. Acad. Sci. U.S.A.,* **2010,** *107*(34), 14943-14944.

[103] Lu, X.; Roberts, E.; Xia, F.; Sanchez-Alavez, M.; Liu, T.; Baldwin, R.; Wu, S.; Chang, J.; Wasterlain, C.G.; Bartfai, T. Galr2-positive allosteric modulator exhibits anticonvulsant effects in animal models. *Proc. Natl. Acad. Sci. U.S.A.,* **2010,** *107*(34), 15229-15234.

[104] Sagi, V.N.; Liu, T.; Lu, X.; Bartfai, T.; Roberts, E. Synthesis and biological evaluation of novel pyrimidine derivatives as sub-micromolar affinity ligands of galr2. *Bioorg. Med. Chem. Lett.,* **2011,** *21*(23), 7210-7215.

[105] Holden, J.E.; Jeong, Y.; Forrest, J.M. The endogenous opioid system and clinical pain management. *AACN Clin. Issues,* **2005,** *16*(3), 291-301.

[106] LaForge, K.S.; Nyberg, F.; Kreek, M.J. Primary structure of guinea pig preprodynorphin and preproenkephalin mrnas: Multiple transcription initiation sites for preprodynorphin. *Brain Res. Bull.,* **2004,** *63*(2), 119-126.

[107] Feng, Y.; He, X.; Yang, Y.; Chao, D.; Lazarus, L.H.; Xia, Y. Current research on opioid receptor function. *Curr. Drug Targets,* **2012,** *13*(2), 230-246.

[108] Drake, C.T.; Chavkin, C.; Milner, T.A. Opioid systems in the dentate gyrus. *Prog. Brain Res.,* **2007,** *163*, 245-263.

[109] Hong, J.S.; McGinty, J.F.; Grimes, L.; Kanamatsu, T.; Obie, J.; Mitchell, C.L. Seizure-induced alterations in the metabolism of hippocampal opioid peptides suggest opioid modulation of seizure-related behaviors. *NIDA Res. Monogr.,* **1988,** *82*, 48-66.

[110] Asai, M.; Matamoros-Trejo, G.; Linares, G. Long-term pharmacological kindling increases *in vitro* release of ir-met and ir-leu-enkephalin from amygdala. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.,* **1998,** *120*(2), 269-275.

[111] Przewlocki, R.; Kaminska, B.; Lukasiuk, K.; Nowicka, D.Z.; Przewlocka, B.; Kaczmarek, L.; Lason, W. Seizure related changes in the regulation of opioid genes and transcription factors in the dentate gyrus of rat hippocampus. *Neuroscience,* **1995,** *68*(1), 73-81.

[112] Harrison, M.B.; Shumate, M.D.; Lothman, E.W. Opioid peptide expression in models of chronic temporal lobe epilepsy. *Neuroscience,* **1995,** *65*(3), 785-795.

[113] Fetissov, S.O.; Jacoby, A.S.; Brumovsky, P.R.; Shine, J.; Iismaa, T.P.; Hokfelt, T. Altered hippocampal expression of neuropeptides in seizure-prone galr1 knockout mice. *Epilepsia,* **2003,** *44*(8), 1022-1033.

[114] Rocha, L.; Cano, A.; Cruz, C.; Omana-Zapata, I.; Villalobos, R.; Maidment, N.T. Opioid peptide systems following a subconvulsant dose of pentylenetetrazol in rats. *Epilepsy Res.,* **1999,** *37*(2), 141-150.

[115] Bing, G.; Wilson, B.; Hudson, P.; Jin, L.; Feng, Z.; Zhang, W.; Bing, R.; Hong, J.S. A single dose of kainic acid elevates the levels of enkephalins and activator protein-1 transcription factors in the hippocampus for up to 1 year. *Proc. Natl. Acad. Sci. U.S.A.,* **1997,** *94*(17), 9422-9427.

[116] Rees, H.; Ang, L.C.; Shul, D.D.; George, D.H.; Begley, H.; McConnell, T. Increase in enkephalin-like immunoreactivity in hippocampi of adults with generalized epilepsy. *Brain Res.,* **1994,** *652*(1), 113-119.

[117] Pretel, S.; Applegate, C.D.; Piekut, D.T. Seizure-induced activation of enkephalin- and somatostatin-synthesizing neurons. *Peptides,* **1995,** *16*(5), 951-957.

[118] Mazarati, A.; Liu, H.; Wasterlain, C. Opioid peptide pharmacology and immunocytochemistry in an animal model of self-sustaining status epilepticus. *Neuroscience,* **1999,** *89*(1), 167-173.

[119] Pirker, S.; Gasser, E.; Czech, T.; Baumgartner, C.; Schuh, E.; Feucht, M.; Novak, K.; Zimprich, F.; Sperk, G. Dynamic up-regulation of prodynorphin transcription in temporal lobe epilepsy. *Hippocampus,* **2009,** *19*(11), 1051-1054.

[120] Tortella, F.C. Endogenous opioid peptides and epilepsy: Quieting the seizing brain? *Trends Pharmacol. Sci.,* **1988,** *9*(10), 366-372.

[121] Simonato, M.; Romualdi, P. Dynorphin and epilepsy. *Prog. Neurobiol.,* **1996,** *50*(5-6), 557-583.

[122] Comer, S.D.; Hoenicke, E.M.; Sable, A.I.; McNutt, R.W.; Chang, K.J.; De Costa, B.R.; Mosberg, H.I.; Woods, J.H. Convulsive effects of systemic administration of the delta opioid agonist bw373u86 in mice. *J. Pharmacol. Exp. Ther.,* **1993,** *267*(2), 888-895.

[123] Cain, D.P.; Boon, F.; Corcoran, M.E. Involvement of multiple opiate receptors in opioid kindling. *Brain Res.,* **1990,** *517*(1-2), 236-244.

[124] Tortella, F.C.; Robles, L.; Mosberg, H.I. Evidence for mu opioid receptor mediation of enkephalin-induced electroencephalographic seizures. *J. Pharmacol. Exp. Ther.,* **1987,** *240*(2), 571-577.

[125] Haffmans, J.; Dzoljic, M.R. Differential epileptogenic potentials of selective mu and delta opiate receptor agonists. *J. Neural Transm.,* **1983,** *57*(1-2), 1-11.

[126] Lason, W.; Simpson, J.N.; McGinty, J.F. Mu but not delta opioid receptor stimulation intensifies kainic acid-induced neurotoxicity in rat hippocampus. *Neuropeptides,* **1988,** *12*(2), 89-94.

[127] Tortella, F.C.; Robles, L.E.; Holaday, J.W.; Cowan, A. Ici 154,129, a delta-opioid receptor antagonist raises seizure threshold in rats. *Eur. J. Pharmacol.,* **1984,** *97*(1-2), 141-144.

[128] Grecksch, G.; Becker, A.; Schroeder, H.; Hollt, V. Involvement of delta-opioid receptors in pentylenetetrazol kindling development and kindling-related processes in rats. *Naunyn Schmiedebergs Arch. Pharmacol.,* **1999,** *360*(2), 151-156.

[129] Schroeder, H.; Becker, A.; Grecksch, G.; Schroeder, U.; Hoellt, V. The effect of pentylenetetrazol kindling on synaptic mechanisms of interacting glutamatergic and opioid system in the hippocampus of rats. *Brain Res.,* **1998,** *811*(1-2), 40-46.

[130] Mitchell, C.L.; Barnes, M.I.; Hudson, P.M.; Hong, J.S. Opioid mu and delta receptor antagonists reduce wet dog shaking elicited by perforant path stimulation. *Pharmacol. Biochem. Behav.,* **1991,** *38*(4), 801-805.

[131] Jackson, H.C.; Nutt, D.J. Differential effects of selective mu-, kappa- and delta-opioid antagonists on electroshock seizure threshold in mice. *Psychopharmacology (Berl),* **1991,** *103*(3), 380-383.

[132] Yajima, Y.; Narita, M.; Takahashi-Nakano, Y.; Misawa, M.; Nagase, H.; Mizoguchi, H.; Tseng, L.F.; Suzuki, T. Effects of differential modulation of mu-, delta- and kappa-opioid systems on bicuculline-induced convulsions in the mouse. *Brain Res.,* **2000,** *862*(1-2), 120-126.

[133] Tortella, F.C.; Robles, L.; Holaday, J.W. U50,488, a highly selective kappa opioid: Anticonvulsant profile in rats. *J. Pharmacol. Exp. Ther.,* **1986,** *237*(1), 49-53.

[134] Saboory, E.; Derchansky, M.; Ismaili, M.; Jahromi, S.S.; Brull, R.; Carlen, P.L.; El Beheiry, H. Mechanisms of morphine enhancement of spontaneous seizure activity. *Anesth. Analg.,* **2007,** *105*(6), 1729-1735.

[135] Shafaroodi, H.; Baradaran, N.; Moezi, L.; Dehpour, S.; Kabiri, T.; Dehpour, A.R. Morphine sensitization in the pentylenetetrazole-induced clonic seizure threshold in mice: Role of nitric oxide and mu receptors. *Epilepsy Behav.,* **2011,** *20*(4), 602-606.

[136] Simmons, M.L.; Chavkin, C. Endogenous opioid regulation of hippocampal function. *Int. Rev. Neurobiol.,* **1996,** *39*, 145-196.

[137] Solbrig, M.V.; Adrian, R.; Baratta, J.; Lauterborn, J.C.; Koob, G.F. Kappa opioid control of seizures produced by a virus in an animal model. *Brain,* **2006,** *129*(Pt 3), 642-654.

[138] Solbrig, M.V.; Koob, G.F. Epilepsy, cns viral injury and dynorphin. *Trends Pharmacol. Sci.,* **2004,** *25*(2), 98-104.

[139] Przewlocka, B.; Lason, W.; Machelska, H.; van Luijtelaar, G.; Coenen, A.; Przewlocki, R. Kappa opioid receptor agonists

suppress absence seizures in wag/rij rats. *Neurosci Lett.,* **1995,** *186*(2-3), 131-134.

[140] Mizuno, T.; Kimura, F. Medial septal injection of naloxone elevates acetylcholine release in the hippocampus and induces behavioral seizures in rats. *Brain Res.,* **1996,** *713*(1-2), 1-7.

[141] Loacker, S.; Sayyah, M.; Wittmann, W.; Herzog, H.; Schwarzer, C. Endogenous dynorphin in epileptogenesis and epilepsy: Anticonvulsant net effect via kappa opioid receptors. *Brain,* **2007,** *130*(Pt 4), 1017-1028.

[142] Schunk, E.; Aigner, C.; Stefanova, N.; Wenning, G.; Herzog, H.; Schwarzer, C. Kappa opioid receptor activation blocks progressive neurodegeneration after kainic acid injection. *Hippocampus,* **2011,** *21*(9), 1010-1020.

[143] Stogmann, E.; Zimprich, A.; Baumgartner, C.; Aull-Watschinger, S.; Hollt, V.; Zimprich, F. A functional polymorphism in the prodynorphin gene promotor is associated with temporal lobe epilepsy. *Ann. Neurol.,* **2002,** *51*(2), 260-263.

[144] Dietis, N.; Rowbotham, D.J.; Lambert, D.G. Opioid receptor subtypes: Fact or artifact? *Br. J. Anaesth.,* **2011,** *107*(1), 8-18.

[145] Jordan, B.; Devi, L.A. Molecular mechanisms of opioid receptor signal transduction. *Br. J. Anaesth.,* **1998,** *81*(1), 12-19.

[146] Kaczor, A.; Matosiuk, D. Non-peptide opioid receptor ligands - recent advances. Part i - agonists. *Curr. Med. Chem.,* **2002,** *9*(17), 1567-1589.

[147] Kaminski, R.M.; Witkin, J.M.; Shippenberg, T.S. Pharmacological and genetic manipulation of kappa opioid receptors: Effects on cocaine- and pentylenetetrazol-induced convulsions and seizure kindling. *Neuropharmacology,* **2007,** *52*(3), 895-903.

[148] Manocha, A.; Mediratta, P.K.; Sharma, K.K. Studies on the anticonvulsant effect of u50488h on maximal electroshock seizure in mice. *Pharmacol. Biochem. Behav.,* **2003,** *76*(1), 111-117.

[149] Manocha, A.; Sharma, K.K.; Mediratta, P.K. Possible mechanism involved in the anticonvulsant action of butorphanol in mice. *Pharmacol. Biochem. Behav.,* **2003,** *74*(2), 343-350.

[150] Bausch, S.B.; Esteb, T.M.; Terman, G.W.; Chavkin, C. Administered and endogenously released kappa opioids decrease pilocarpine-induced seizures and seizure-induced histopathology. *J. Pharmacol. Exp. Ther.,* **1998,** *284*(3), 1147-1155.

[151] Frey, H.H. Effect of mu- and kappa-opioid agonists on the electroconvulsive seizure threshold in mice and antagonism by naloxone and mr 2266. *Pharmacol. Toxicol.,* **1988,** *62*(3), 150-154.

[152] Kaczor, A.; Matosiuk, D. Non-peptide opioid receptor ligands - recent advances. Part ii - antagonists. *Curr. Med. Chem.,* **2002,** *9*(17), 1591-1603.

[153] Fichna, J.; Socala, K.; Nieoczym, D.; Gach, K.; Perlikowska, R.; Janecka, A.; Wlaz, P. The mu-opioid receptor-selective peptide antagonists, antanal-1 and antanal-2, produce anticonvulsant effects in mice. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **2013,** *40*, 126-131.

[154] Liu, H.; Gao, H.M.; Zhang, W.Q.; Tang, Y.Y.; Song, H.S. Effects of chronic administration of pl017 and beta-funaltrexamine hydrochloride on susceptibility of kainic acid-induced seizures in rats. *Sheng Li Xue Bao,* **2004,** *56*(1), 101-106.

[155] Lee, P.H.; Obie, J.; Hong, J.S. Opioids induce convulsions and wet dog shakes in rats: Mediation by hippocampal mu, but not delta or kappa opioid receptors. *J. Neurosci.,* **1989,** *9*(2), 692-697.

[156] Jackson, H.C.; Nutt, D.J. Investigation of the involvement of opioid receptors in the action of anticonvulsants. *Psychopharmacology (Berl.),* **1993,** *111*(4), 486-490.

[157] Harrison, S.; Geppetti, P. Substance p. *Int. J. Biochem. Cell Biol.,* **2001,** *33*(6), 555-576.

[158] Borhegyi, Z.; Leranth, C. Substance p innervation of the rat hippocampal formation. *J. Comp. Neurol.,* **1997,** *384*(1), 41-58.

[159] Roberts, G.W.; Woodhams, P.L.; Polak, J.M.; Crow, T.J. Distribution of neuropeptides in the limbic system of the rat: The hippocampus. *Neuroscience,* **1984,** *11*(1), 35-77.

[160] Lotstra, F.; Mailleux, P.; Vanderhaeghen, J.J. Substance p neurons in the human hippocampus: An immunohistochemical analysis in the infant and adult. *J. Chem. Neuroanat.,* **1988,** *1*(2), 111-123.

[161] Borhegyi, Z.; Leranth, C. Distinct substance p- and calretinin-containing projections from the supramammillary area to the hippocampus in rats; a species difference between rats and monkeys. *Exp. Brain Res.,* **1997,** *115*(2), 369-374.

[162] Peterson, G.M.; Shurlow, C.L. Morphological evidence for a substance p projection from medial septum to hippocampus. *Peptides,* **1992,** *13*(3), 509-517.

[163] Liu, H.; Sankar, R.; Shin, D.H.; Mazarati, A.M.; Wasterlain, C.G. Patterns of status epilepticus-induced substance p expression during development. *Neuroscience,* **2000,** *101*(2), 297-304.

[164] Liu, H.; Cao, Y.; Basbaum, A.I.; Mazarati, A.M.; Sankar, R.; Wasterlain, C.G. Resistance to excitotoxin-induced seizures and neuronal death in mice lacking the preprotachykinin a gene. *Proc. Natl. Acad. Sci. U.S.A.,* **1999,** *96*(21), 12096-12101.

[165] Robinson, P.; Garza, A.; Weinstock, J.; Serpa, J.A.; Goodman, J.C.; Eckols, K.T.; Firozgary, B.; Tweardy, D.J. Substance p causes seizures in neurocysticercosis. *PLoS Pathog.,* **2012,** *8*(2), e1002489.

[166] Ogier, R.; Raggenbass, M. Action of tachykinins in the rat hippocampus: Modulation of inhibitory synaptic transmission. *Eur. J. Neurosci.,* **2003,** *17*(12), 2639-2647.

[167] Stacey, A.E.; Woodhall, G.L.; Jones, R.S. Neurokinin-receptor-mediated depolarization of cortical neurons elicits an increase in glutamate release at excitatory synapses. *Eur. J. Neurosci.,* **2002,** *16*(10), 1896-1906.

[168] Ogier, R.; Wrobel, L.J.; Raggenbass, M. Action of tachykinins in the hippocampus: Facilitation of inhibitory drive to gabaergic interneurons. *Neuroscience,* **2008,** *156*(3), 527-536.

[169] Roush, E.D.; Kwatra, M.M. Human substance p receptor expressed in chinese hamster ovary cells directly activates g(alpha q/11), g(alpha s), g(alpha o). *FEBS Lett.,* **1998,** *428*(3), 291-294.

[170] Takeda, Y.; Blount, P.; Sachais, B.S.; Hershey, A.D.; Raddatz, R.; Krause, J.E. Ligand binding kinetics of substance p and neurokinin a receptors stably expressed in chinese hamster ovary cells and evidence for differential stimulation of inositol 1,4,5-trisphosphate and cyclic amp second messenger responses. *J. Neurochem.,* **1992,** *59*(2), 740-745.

[171] Acsady, L.; Katona, I.; Gulyas, A.I.; Shigemoto, R.; Freund, T.F. Immunostaining for substance p receptor labels gabaergic cells with distinct termination patterns in the hippocampus. *J. Comp. Neurol.,* **1997,** *378*(3), 320-336.

[172] Sloviter, R.S.; Ali-Akbarian, L.; Horvath, K.D.; Menkens, K.A. Substance p receptor expression by inhibitory interneurons of the rat hippocampus: Enhanced detection using improved immunocytochemical methods for the preservation and colocalization of gaba and other neuronal markers. *J. Comp. Neurol.,* **2001,** *430*(3), 283-305.

[173] Toth, K.; Wittner, L.; Urban, Z.; Doyle, W.K.; Buzsaki, G.; Shigemoto, R.; Freund, T.F.; Magloczky, Z. Morphology and synaptic input of substance p receptor-immunoreactive interneurons in control and epileptic human hippocampus. *Neuroscience,* **2007,** *144*(2), 495-508.

[174] Magloczky, Z. Sprouting in human temporal lobe epilepsy: Excitatory pathways and axons of interneurons. *Epilepsy Res.,* **2010,** *89*(1), 52-59.

[175] Danfors, T.; Ahs, F.; Appel, L.; Linnman, C.; Fredrikson, M.; Furmark, T.; Kumlien, E. Increased neurokinin-1 receptor availability in temporal lobe epilepsy: A positron emission tomography study using [(11)c]gr205171. *Epilepsy Res.,* **2011,** *97*(1-2), 183-189.

[176] Rosen, T.J.; Coffman, K.J.; McLean, S.; Crawford, R.T.; Bryce, D.K.; Gohda, Y.; Tsuchiya, M.; Nagahisa, A.; Nakane, M.; Lowe, J.A., 3rd Synthesis and structure-activity relationships of cp-122,721, a second-generation nk-1 receptor antagonist. *Bioorg. Med. Chem. Lett.,* **1998,** *8*(3), 281-284.

[177] Syvanen, S.; Eriksson, J.; Genchel, T.; Lindhe, O.; Antoni, G.; Langstrom, B. Synthesis of two potential nk1-receptor ligands using [1-11c]ethyl iodide and [1-11c]propyl iodide and initial pet-imaging. *BMC Med. Imaging,* **2007,** *7*, 6.

[178] Wahlestedt, C. Reward for persistence in substance p research. *Science,* **1998,** *281*(5383), 1624-1625.

[179] Dos Santos, L.V.; Souza, F.H.; Brunetto, A.T.; Sasse, A.D.; da Silveira Nogueira Lima, J.P. Neurokinin-1 receptor antagonists for chemotherapy-induced nausea and vomiting: A systematic review. *J. Natl. Cancer Inst.,* **2012,** *104*(17), 1280-1292.

[180] Zachrisson, O.; Lindefors, N.; Brene, S. A tachykinin nk1 receptor antagonist, cp-122,721-1, attenuates kainic acid-induced seizure activity. *Brain Res. Mol. Brain Res.,* **1998,** *60*(2), 291-295.

[181] Kalinichev, M.; Bradford, A.; Bison, S.; Lucas, A.; Sartori, I.; Garbati, N.; Andreetta, F.; Bate, S.; Austin, N.E.; Jones, D.N.; Read, K.D.; Alvaro, G.; Large, C.H. Potentiation of the anticonvulsant efficacy of sodium channel inhibitors by an nk1-receptor antagonist in the rat. *Epilepsia,* **2010,** *51*(8), 1543-1551.

[182] Alvaro, G.; Di Fabio, R. Neurokinin 1 receptor antagonists--current prospects. *Curr. Opin. Drug Discov. Devel.,* **2007,** *10*(5), 613-621.
[183] Dockray, G.J. Cholecystokinin. *Curr. Opin. Endocrinol. Diabetes Obes.,* **2012,** *19*(1), 8-12.
[184] Lee, S.Y.; Soltesz, I. Cholecystokinin: A multi-functional molecular switch of neuronal circuits. *Dev. Neurobiol.,* **2011,** *71*(1), 83-91.
[185] Wyeth, M.S.; Zhang, N.; Houser, C.R. Increased cholecystokinin labeling in the hippocampus of a mouse model of epilepsy maps to spines and glutamatergic terminals. *Neuroscience,* **2012,** *202*, 371-383.
[186] Kadar, T.; Pesti, A.; Penke, B.; Telegdy, G. Inhibition of seizures induced by picrotoxin and electroshock by cholecystokinin octapeptides and their fragments in rats after intracerebroventricular administration. *Neuropharmacology,* **1984,** *23*(8), 955-961.
[187] Zetler, G. Anticonvulsant effects of caerulein, cholecystokinin octapeptide (cck-8) and diazepam against seizures produced in mice by harman, thiosemicarbazide and isoniazid. *Neurosci Lett.,* **1981,** *24*(2), 175-180.
[188] Ferraro, G.; Sardo, P. Cholecystokinin-8 sulfate modulates the anticonvulsant efficacy of vigabatrin in an experimental model of partial complex epilepsy in the rat. *Epilepsia,* **2009,** *50*(4), 721-730.
[189] Tirassa, P.; Costa, N.; Aloe, L. Cck-8 prevents the development of kindling and regulates the gaba and npy expression in the hippocampus of pentylenetetrazole (ptz)-treated adult rats. *Neuropharmacology,* **2005,** *48*(5), 732-742.
[190] Deng, P.Y.; Xiao, Z.; Jha, A.; Ramonet, D.; Matsui, T.; Leitges, M.; Shin, H.S.; Porter, J.E.; Geiger, J.D.; Lei, S. Cholecystokinin facilitates glutamate release by increasing the number of readily releasable vesicles and releasing probability. *J. Neurosci.,* **2010,** *30*(15), 5136-5148.
[191] Shinohara, S.; Kawasaki, K. Electrophysiological changes in rat hippocampal pyramidal neurons produced by cholecystokinin octapeptide. *Neuroscience,* **1997,** *78*(4), 1005-1016.
[192] Dufresne, M.; Seva, C.; Fourmy, D. Cholecystokinin and gastrin receptors. *Physiol. Rev.,* **2006,** *86*(3), 805-847.
[193] Noble, F.; Roques, B.P. Cck-b receptor: Chemistry, molecular biology, biochemistry and pharmacology. *Prog Neurobiol,* **1999,** *58*(4), 349-379.
[194] Kalindjian, S.B.; McDonald, I.M. Strategies for the design of non-peptide cck2 receptor agonist and antagonist ligand. *Curr. Top. Med. Chem.,* **2007,** *7*(12), 1195-1204.
[195] Harro, J. Cck and npy as anti-anxiety treatment targets: Promises, pitfalls, and strategies. *Amino Acids,* **2006,** *31*(3), 215-230.
[196] Eser, D.; Leicht, G.; Lutz, J.; Wenninger, S.; Kirsch, V.; Schule, C.; Karch, S.; Baghai, T.; Pogarell, O.; Born, C.; Rupprecht, R.; Mulert, C. Functional neuroanatomy of cck-4-induced panic attacks in healthy volunteers. *Hum. Brain Mapp.,* **2009,** *30*(2), 511-522.
[197] Wilson, J.; Little, H.J. Cck(b) antagonists protect against some aspects of the ethanol withdrawal syndrome. *Pharmacol. Biochem. Behav.,* **1998,** *59*(4), 967-973.
[198] Gozes, I.; Brenneman, D.E. Vip: Molecular biology and neurobiological function. *Mol. Neurobiol.,* **1989,** *3*(4), 201-236.
[199] Papp, E.C.; Hajos, N.; Acsady, L.; Freund, T.F. Medial septal and median raphe innervation of vasoactive intestinal polypeptide-containing interneurons in the hippocampus. *Neuroscience,* **1999,** *90*(2), 369-382.
[200] Hajos, N.; Acsady, L.; Freund, T.F. Target selectivity and neurochemical characteristics of vip-immunoreactive interneurons in the rat dentate gyrus. *Eur. J. Neurosci.,* **1996,** *8*(7), 1415-1431.
[201] Romualdi, P.; Lesa, G.; Donatini, A.; Balboni, G.; Tomatis, R.; Ferri, S. Alterations in vasoactive intestinal polypeptide-related peptides after pentylenetetrazole-induced seizures in rat brain. *Eur. J. Pharmacol.,* **1992,** *229*(2-3), 149-153.
[202] Cunha-Reis, D.; Fontinha, B.M.; Ribeiro, J.A.; Sebastiao, A.M. Tonic adenosine a1 and a2a receptor activation is required for the excitatory action of vip on synaptic transmission in the ca1 area of the hippocampus. *Neuropharmacology,* **2007,** *52*(2), 313-320.
[203] Yang, K.; Lei, G.; Jackson, M.F.; Macdonald, J.F. The involvement of pacap/vip system in the synaptic transmission in the hippocampus. *J. Mol. Neurosci.,* **2010,** *42*(3), 319-326.
[204] Koh, S.W.; Cheng, J.; Dodson, R.M.; Ku, C.Y.; Abbondandolo, C.J. Vip down-regulates the inflammatory potential and promotes survival of dying (neural crest-derived) corneal endothelial cells ex vivo: Necrosis to apoptosis switch and up-regulation of bcl-2 and n-cadherin. *J. Neurochem.,* **2009,** *109*(3), 792-806.
[205] Delgado, M.; Varela, N.; Gonzalez-Rey, E. Vasoactive intestinal peptide protects against beta-amyloid-induced neurodegeneration by inhibiting microglia activation at multiple levels. *Glia,* **2008,** *56*(10), 1091-1103.
[206] De Lanerolle, N.C.; Gunel, M.; Sundaresan, S.; Shen, M.Y.; Brines, M.L.; Spencer, D.D. Vasoactive intestinal polypeptide and its receptor changes in human temporal lobe epilepsy. *Brain Res.,* **1995,** *686*(2), 182-193.
[207] Cunha-Reis, D.; Ribeiro, J.A.; Sebastiao, A.M. Vpac2 receptor activation mediates vip enhancement of population spikes in the ca1 area of the hippocampus. *Ann. N. Y. Acad. Sci.,* **2006,** *1070*, 210-214.
[208] Bellamy, J.L.; Cady, R.K.; Durham, P.L. Salivary levels of cgrp and vip in rhinosinusitis and migraine patients. *Headache,* **2006,** *46*(1), 24-33.
[209] Kojima, M.; Hosoda, H.; Date, Y.; Nakazato, M.; Matsuo, H.; Kangawa, K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature,* **1999,** *402*(6762), 656-660.
[210] Gutierrez, J.A.; Solenberg, P.J.; Perkins, D.R.; Willency, J.A.; Knierman, M.D.; Jin, Z.; Witcher, D.R.; Luo, S.; Onyia, J.E.; Hale, J.E. Ghrelin octanoylation mediated by an orphan lipid transferase. *Proc. Natl. Acad. Sci. U.S.A.,* **2008,** *105*(17), 6320-6325.
[211] Ferrini, F.; Salio, C.; Lossi, L.; Merighi, A. Ghrelin in central neurons. *Curr. Neuropharmacol.,* **2009,** *7*(1), 37-49.
[212] Leung, P.K.; Chow, K.B.; Lau, P.N.; Chu, K.M.; Chan, C.B.; Cheng, C.H.; Wise, H. The truncated ghrelin receptor polypeptide (ghs-r1b) acts as a dominant-negative mutant of the ghrelin receptor. *Cell Signal.,* **2007,** *19*(5), 1011-1022.
[213] Camina, J.P. Cell biology of the ghrelin receptor. *J. Neuroendocrinol.,* **2006,** *18*(1), 65-76.
[214] Cowley, M.A.; Smith, R.G.; Diano, S.; Tschop, M.; Pronchuk, N.; Grove, K.L.; Strasburger, C.J.; Bidlingmaier, M.; Esterman, M.; Heiman, M.L.; Garcia-Segura, L.M.; Nillni, E.A.; Mendez, P.; Low, M.J.; Sotonyi, P.; Friedman, J.M.; Liu, H.; Pinto, S.; Colmers, W.F.; Cone, R.D.; Horvath, T.L. The distribution and mechanism of action of ghrelin in the cns demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron,* **2003,** *37*(4), 649-661.
[215] Banks, W.A.; Tschop, M.; Robinson, S.M.; Heiman, M.L. Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. *J. Pharmacol. Exp. Ther.,* **2002,** *302*(2), 822-827.
[216] Portelli, J.; Michotte, Y.; Smolders, I. Ghrelin: An emerging new anticonvulsant neuropeptide. *Epilepsia,* **2012,** *53*(4), 585-595.
[217] Angelidis, G.; Valotassiou, V.; Georgoulias, P. Current and potential roles of ghrelin in clinical practice. *J. Endocrinol. Invest.,* **2010,** *33*(11), 823-838.
[218] Obay, B.D.; Tasdemir, E.; Tumer, C.; Bilgin, H.M.; Sermet, A. Antiepileptic effects of ghrelin on pentylenetetrazole-induced seizures in rats. *Peptides,* **2007,** *28*(6), 1214-1219.
[219] Lee, J.; Lim, E.; Kim, Y.; Li, E.; Park, S. Ghrelin attenuates kainic acid-induced neuronal cell death in the mouse hippocampus. *J. Endocrinol.,* **2010,** *205*(3), 263-270.
[220] Zhang, R.; Yang, G.; Wang, Q.; Guo, F.; Wang, H. Acylated ghrelin protects hippocampal neurons in pilocarpine-induced seizures of immature rats by inhibiting cell apoptosis. *Mol. Biol. Rep.,* **2013,** *40*(1), 51-58.
[221] Biagini, G.; Torsello, A.; Marinelli, C.; Gualtieri, F.; Vezzali, R.; Coco, S.; Bresciani, E.; Locatelli, V. Beneficial effects of desacyl-ghrelin, hexarelin and ep-80317 in models of status epilepticus. *Eur. J. Pharmacol.,* **2011,** *670*(1), 130-136.
[222] Chung, H.; Li, E.; Kim, Y.; Kim, S.; Park, S. Multiple signaling pathways mediate ghrelin-induced proliferation of hippocampal neural stem cells. *J. Endocrinol.,* **2013,** *218*(1), 49-59.
[223] Baatar, D.; Patel, K.; Taub, D.D. The effects of ghrelin on inflammation and the immune system. *Mol. Cell. Endocrinol.,* **2011,** *340*(1), 44-58.
[224] Holst, B.; Cygankiewicz, A.; Jensen, T.H.; Ankersen, M.; Schwartz, T.W. High constitutive signaling of the ghrelin receptor--identification of a potent inverse agonist. *Mol. Endocrinol.,* **2003,** *17*(11), 2201-2210.
[225] Portelli, J.; Thielemans, L.; Ver Donck, L.; Loyens, E.; Coppens, J.; Aourz, N.; Aerssens, J.; Vermoesen, K.; Clinckers, R.; Schallier, A.; Michotte, Y.; Moechars, D.; Collingridge, G.L.; Bortolotto,

Z.A.; Smolders, I. Inactivation of the constitutively active ghrelin receptor attenuates limbic seizure activity in rodents. *Neurotherapeutics,* **2012,** *9*(3), 658-672.

[226] Els, S.; Schild, E.; Petersen, P.S.; Kilian, T.M.; Mokrosinski, J.; Frimurer, T.M.; Chollet, C.; Schwartz, T.W.; Holst, B.; Beck-Sickinger, A.G. An aromatic region to induce a switch between agonism and inverse agonism at the ghrelin receptor. *J. Med. Chem.,* **2012,** *55*(17), 7437-7449.

[227] Mokrosinski, J.; Frimurer, T.M.; Sivertsen, B.; Schwartz, T.W.; Holst, B. Modulation of constitutive activity and signaling bias of the ghrelin receptor by conformational constraint in the second extracellular loop. *J. Biol. Chem.,* **2012,** *287*(40), 33488-33502.

[228] Hassouna, R.; Labarthe, A.; Zizzari, P.; Videau, C.; Culler, M.; Epelbaum, J.; Tolle, V. Actions of agonists and antagonists of the ghrelin/ghs-r pathway on gh secretion, appetite, and cfos activity. *Front. Endocrinol. (Lausanne),* **2013,** *4*, 25.

[229] Broglio, F.; Boutignon, F.; Benso, A.; Gottero, C.; Prodam, F.; Arvat, E.; Ghe, C.; Catapano, F.; Torsello, A.; Locatelli, V.; Muccioli, G.; Boeglin, D.; Guerlavais, V.; Fehrentz, J.A.; Martinez, J.; Ghigo, E.; Deghenghi, R. Ep1572: A novel peptido-mimetic gh secretagogue with potent and selective gh-releasing activity in man. *J. Endocrinol. Invest.,* **2002,** *25*(8), RC26-28.

[230] Piccoli, F.; Degen, L.; MacLean, C.; Peter, S.; Baselgia, L.; Larsen, F.; Beglinger, C.; Drewe, J. Pharmacokinetics and pharmacodynamic effects of an oral ghrelin agonist in healthy subjects. *J. Clin. Endocrinol. Metab.,* **2007,** *92*(5), 1814-1820.

[231] Guerlavais, V.; Boeglin, D.; Mousseaux, D.; Oiry, C.; Heitz, A.; Deghenghi, R.; Locatelli, V.; Torsello, A.; Ghe, C.; Catapano, F.; Muccioli, G.; Galleyrand, J.C.; Fehrentz, J.A.; Martinez, J. New active series of growth hormone secretagogues. *J. Med. Chem.,* **2003,** *46*(7), 1191-1203.

[232] Peschke, B.; Ankersen, M.; Hansen, T.K.; Hansen, B.S.; Lau, J.; Nielsen, K.K.; Raun, K. New highly potent dipeptidic growth hormone secretagogues with low molecular weight. *Eur. J. Med. Chem.,* **2000,** *35*(6), 599-618.

[233] Moulin, A.; Brunel, L.; Boeglin, D.; Demange, L.; Ryan, J.; M'Kadmi, C.; Denoyelle, S.; Martinez, J.; Fehrentz, J.A. The 1,2,4-triazole as a scaffold for the design of ghrelin receptor ligands: Development of jmv 2959, a potent antagonist. *Amino Acids,* **2013,** *44*(2), 301-314.

[234] Hansen, B.S.; Raun, K.; Nielsen, K.K.; Johansen, P.B.; Hansen, T.K.; Peschke, B.; Lau, J.; Andersen, P.H.; Ankersen, M. Pharmacological characterisation of a new oral gh secretagogue, nn703. *Eur. J. Endocrinol.,* **1999,** *141*(2), 180-189.

[235] Holst, B.; Brandt, E.; Bach, A.; Heding, A.; Schwartz, T.W. Nonpeptide and peptide growth hormone secretagogues act both as ghrelin receptor agonist and as positive or negative allosteric modulators of ghrelin signaling. *Mol. Endocrinol.,* **2005,** *19*(9), 2400-2411.

[236] Wright, J.W.; Harding, J.W. Brain renin-angiotensin--a new look at an old system. *Prog. Neurobiol.,* **2011,** *95*(1), 49-67.

[237] Lenkei, Z.; Palkovits, M.; Corvol, P.; Llorens-Cortes, C. Expression of angiotensin type-1 (at1) and type-2 (at2) receptor mrnas in the adult rat brain: A functional neuroanatomical review. *Front. Neuroendocrinol.,* **1997,** *18*(4), 383-439.

[238] Whiting, P.; Nava, S.; Mozley, L.; Eastham, H.; Poat, J. Expression of angiotensin converting enzyme mrna in rat brain. *Brain Res. Mol. Brain Res.,* **1991,** *11*(1), 93-96.

[239] Sirett, N.E.; Bray, J.J.; Hubbard, J.I. Localization of immunoreactive angiotensin ii in the hippocampus and striatum of rat brain. *Brain Res.,* **1981,** *217*(2), 405-411.

[240] Haas, H.L.; Felix, D.; Celio, M.R.; Inagami, T. Angiotensin ii in the hippocampus. A histochemical and electrophysiological study. *Experientia,* **1980,** *36*(12), 1394-1395.

[241] Gouveia, T.L.; Frangiotti, M.I.; de Brito, J.M.; de Castro Neto, E.F.; Sakata, M.M.; Febba, A.C.; Casarini, D.E.; Amado, D.; Cavalheiro, E.A.; Almeida, S.S.; Manchini, M.T.; Araujo, R.C.; Silva, J.A., Jr.; Naffah-Mazzacoratti Mda, G. The levels of renin-angiotensin related components are modified in the hippocampus of rats submitted to pilocarpine model of epilepsy. *Neurochem. Int.,* **2012,** *61*(1), 54-62.

[242] Pereira, M.G.; Becari, C.; Oliveira, J.A.; Salgado, M.C.; Garcia-Cairasco, N.; Costa-Neto, C.M. Inhibition of the renin-angiotensin system prevents seizures in a rat model of epilepsy. *Clin. Sci. (Lond.),* **2010,** *119*(11), 477-482.

[243] Arganaraz, G.A.; Konno, A.C.; Perosa, S.R.; Santiago, J.F.; Boim, M.A.; Vidotti, D.B.; Varella, P.P.; Costa, L.G.; Canzian, M.; Porcionatto, M.A.; Yacubian, E.M.; Sakamoto, A.C.; Carrete, H., Jr.; Centeno, R.S.; Amado, D.; Cavalheiro, E.A.; Junior, J.A.; Mazzacoratti Mda, G. The renin-angiotensin system is upregulated in the cortex and hippocampus of patients with temporal lobe epilepsy related to mesial temporal sclerosis. *Epilepsia,* **2008,** *49*(8), 1348-1357.

[244] Georgiev, V.P.; Petkova, B.P.; Petkov, V.D.; Kambourova, T.S. Interactions of angiotensin ii and gaba-ergic agents at the threshold of convulsive reactions. *Acta Physiol. Pharmacol. Bulg.,* **1986,** *12*(2), 14-20.

[245] Tchekalarova, J.; Georgiev, V. Angiotensin peptides modulatory system: How is it implicated in the control of seizure susceptibility? *Life Sci.,* **2005,** *76*(9), 955-970.

[246] De Bundel, D.; Smolders, I.; Vanderheyden, P.; Michotte, Y. Ang ii and ang iv: Unraveling the mechanism of action on synaptic plasticity, memory, and epilepsy. *CNS Neurosci. Ther.,* **2008,** *14*(4), 315-339.

[247] Stragier, B.; Clinckers, R.; Meurs, A.; De Bundel, D.; Sarre, S.; Ebinger, G.; Michotte, Y.; Smolders, I. Involvement of the somatostatin-2 receptor in the anti-convulsant effect of angiotensin iv against pilocarpine-induced limbic seizures in rats. *J. Neurochem.,* **2006,** *98*(4), 1100-1113.

[248] Pechlivanova, D.M.; Stoynev, A.G.; Tchekalarova, J.D. The effects of chronic losartan pretreatment on restraint stress-induced changes in motor activity, nociception and pentylenetetrazol generalized seizures in rats. *Folia Med. (Plovdiv),* **2011,** *53*(2), 69-73.

[249] Lukawski, K.; Janowska, A.; Jakubus, T.; Czuczwar, S.J. Interactions between angiotensin at receptor antagonists and second-generation antiepileptic drugs in the test of maximal electroshock. *Fundam. Clin. Pharmacol.,* **2013,** doi: 10.1111/fcp.12023. [Epub ahead of print]

[250] Lukawski, K.; Janowska, A.; Jakubus, T.; Tochman-Gawda, A.; Czuczwar, S.J. Angiotensin at1 receptor antagonists enhance the anticonvulsant action of valproate in the mouse model of maximal electroshock. *Eur. J. Pharmacol.,* **2010,** *640*(1-3), 172-177.

[251] Sarro, G.D.; Paola, E.D.; Gratteri, S.; Gareri, P.; Rispoli, V.; Siniscalchi, A.; Tripepi, G.; Gallelli, L.; Citraro, R.; Russo, E. Fosinopril and zofenopril, two angiotensin-converting enzyme (ace) inhibitors, potentiate the anticonvulsant activity of antiepileptic drugs against audiogenic seizures in dba/2 mice. *Pharmacol. Res.,* **2012,** *65*(3), 285-296.

[252] Hadjiivanova, C.H.; Georgiev, V. *In vitro* effect of angiotensin ii on gaba release in rat hippocampus. *Neuropeptides,* **1998,** *32*(5), 431-434.

[253] Tchekalarova, J.; Kambourova, T.; Georgiev, V. Effects of angiotensin iii and angiotensin iv on pentylenetetrazol seizure susceptibility (threshold and kindling): Interaction with adenosine a(1) receptors. *Brain Res. Bull.,* **2001,** *56*(2), 87-91.

[254] Kovacs, Z.; Dobolyi, A.; Juhasz, G.; Kekesi, K.A. Nucleoside map of the human central nervous system. *Neurochem. Res.,* **2010,** *35*(3), 452-464.

[255] Kovacs, Z.; Juhasz, G.; Palkovits, M.; Dobolyi, A.; Kekesi, K.A. Area, age and gender dependence of the nucleoside system in the brain: A review of current literature. *Curr. Top. Med. Chem.,* **2011,** *11*(8), 1012-1033.

[256] Vanderheyden, P.M. From angiotensin iv binding site to at4 receptor. *Mol. Cell. Endocrinol.,* **2009,** *302*(2), 159-166.

[257] Chai, S.Y.; Fernando, R.; Peck, G.; Ye, S.Y.; Mendelsohn, F.A.; Jenkins, T.A.; Albiston, A.L. The angiotensin iv/at4 receptor. *Cell. Mol. Life Sci.,* **2004,** *61*(21), 2728-2737.

[258] Benoist, C.C.; Wright, J.W.; Zhu, M.; Appleyard, S.M.; Wayman, G.A.; Harding, J.W. Facilitation of hippocampal synaptogenesis and spatial memory by c-terminal truncated nle1-angiotensin iv analogs. *J. Pharmacol. Exp. Ther.,* **2011,** *339*(1), 35-44.

[259] McCoy, A.T.; Benoist, C.C.; Wright, J.W.; Kawas, L.H.; Bule-Ghogare, J.M.; Zhu, M.; Appleyard, S.M.; Wayman, G.A.; Harding, J.W. Evaluation of metabolically stabilized angiotensin iv analogs as procognitive/antidementia agents. *J. Pharmacol. Exp. Ther.,* **2013,** *344*(1), 141-154.

[260] Grossman, A.; Costa, A. The regulation of hypothalamic crh: Impact of *in vitro* studies on the central control of the stress response. *Funct. Neurol.,* **1993,** *8*(5), 325-334.

[261] Gallagher, J.P.; Orozco-Cabal, L.F.; Liu, J.; Shinnick-Gallagher, P. Synaptic physiology of central crh system. *Eur. J. Pharmacol.,* **2008,** *583*(2-3), 215-225.

[262] Pilcher, W.H.; Joseph, S.A. Co-localization of crf-ir perikarya and acth-ir fibers in rat brain. *Brain Res.,* **1984,** *299*(1), 91-102.

[263] Pranzatelli, M.R. On the molecular mechanism of adrenocorticotrophic hormone in the cns: Neurotransmitters and receptors. *Exp. Neurol.,* **1994,** *125*(1), 142-161.

[264] Yan, X.X.; Toth, Z.; Schultz, L.; Ribak, C.E.; Baram, T.Z. Corticotropin-releasing hormone (crh)-containing neurons in the immature rat hippocampal formation: Light and electron microscopic features and colocalization with glutamate decarboxylase and parvalbumin. *Hippocampus,* **1998,** *8*(3), 231-243.

[265] Baram, T.Z.; Yi, S.; Avishai-Eliner, S.; Schultz, L. Development neurobiology of the stress response: Multilevel regulation of corticotropin-releasing hormone function. *Ann. N. Y. Acad. Sci.,* **1997,** *814*, 252-265.

[266] Wu, J.; Ma, D.L.; Ling, E.A.; Tang, F.R. Corticotropin releasing factor (crf) in the hippocampus of the mouse pilocarpine model of status epilepticus. *Neurosci. Lett.,* **2012,** *512*(2), 83-88.

[267] Smith, M.A.; Weiss, S.R.; Abedin, T.; Kim, H.; Post, R.M.; Gold, P.W. Effects of amygdala kindling and electroconvulsive seizures on the expression of corticotropin-releasing hormone in the rat brain. *Mol. Cell. Neurosci.,* **1991,** *2*(2), 103-116.

[268] Smith, M.A.; Weiss, S.R.; Berry, R.L.; Zhang, L.X.; Clark, M.; Massenburg, G.; Post, R.M. Amygdala-kindled seizures increase the expression of corticotropin-releasing factor (crf) and crf-binding protein in gabaergic interneurons of the dentate hilus. *Brain Res.,* **1997,** *745*(1-2), 248-256.

[269] Piekut, D.T.; Phipps, B. Corticotropin-releasing factor--immunolabeled fibers in brain regions with localized kainate neurotoxicity. *Acta Neuropathol.,* **1999,** *98*(6), 622-628.

[270] Foradori, C.D.; Lund, T.D.; Nagahara, A.H.; Koenig, J.I.; Handa, R.J. Corticotropin-releasing hormone heterogeneous nuclear rna (hnrna) and immunoreactivity are induced in extrahypothalamic brain sites by kainic-acid-induced seizures and are modulated by estrogen. *Brain Res.,* **2007,** *1164*, 44-54.

[271] Piekut, D.T.; Phipps, B. Increased corticotropin-releasing factor immunoreactivity in select brain sites following kainate elicited seizures. *Brain Res.,* **1998,** *781*(1-2), 100-113.

[272] Hatalski, C.G.; Brunson, K.L.; Tantayanubutr, B.; Chen, Y.; Baram, T.Z. Neuronal activity and stress differentially regulate hippocampal and hypothalamic corticotropin-releasing hormone expression in the immature rat. *Neuroscience,* **2000,** *101*(3), 571-580.

[273] Baram, T.Z.; Schultz, L. Corticotropin-releasing hormone is a rapid and potent convulsant in the infant rat. *Brain Res. Dev. Brain Res.,* **1991,** *61*(1), 97-101.

[274] Brunson, K.L.; Schultz, L.; Baram, T.Z. The *in vivo* proconvulsant effects of corticotropin releasing hormone in the developing rat are independent of ionotropic glutamate receptor activation. *Brain Res. Dev. Brain Res.,* **1998,** *111*(1), 119-128.

[275] Ribak, C.E.; Baram, T.Z. Selective death of hippocampal ca3 pyramidal cells with mossy fiber afferents after crh-induced status epilepticus in infant rats. *Brain Res. Dev. Brain Res.,* **1996,** *91*(2), 245-251.

[276] Hollrigel, G.S.; Chen, K.; Baram, T.Z.; Soltesz, I. The pro-convulsant actions of corticotropin-releasing hormone in the hippocampus of infant rats. *Neuroscience,* **1998,** *84*(1), 71-79.

[277] Baram, T.Z.; Hirsch, E.; Snead, O.C., 3rd; Schultz, L. Corticotropin-releasing hormone-induced seizures in infant rats originate in the amygdala. *Ann. Neurol.,* **1992,** *31*(5), 488-494.

[278] Pascual, J.; Heinrichs, S.C. Olfactory neophobia and seizure susceptibility phenotypes in an animal model of epilepsy are normalized by impairment of brain corticotropin releasing factor. *Epilepsia,* **2007,** *48*(4), 827-833.

[279] Baram, T.Z.; Koutsoukos, Y.; Schultz, L.; Rivier, J. The effect of 'astressin', a novel antagonist of corticotropin releasing hormone (crh), on crh-induced seizures in the infant rat: Comparison with two other antagonists. *Mol. Psychiatry,* **1996,** *1*(3), 223-226.

[280] Maecker, H.; Desai, A.; Dash, R.; Rivier, J.; Vale, W.; Sapolsky, R. Astressin, a novel and potent crf antagonist, is neuroprotective in the hippocampus when administered after a seizure. *Brain Res.,* **1997,** *744*(1), 166-170.

[281] Combi, R.; Dalpra, L.; Ferini-Strambi, L.; Tenchini, M.L. Frontal lobe epilepsy and mutations of the corticotropin-releasing hormone gene. *Ann. Neurol.,* **2005,** *58*(6), 899-904.

[282] Sansoni, V.; Forcella, M.; Mozzi, A.; Fusi, P.; Ambrosini, R.; Ferini-Strambi, L.; Combi, R. Functional characterization of a crh missense mutation identified in an adnfle family. *PLoS One,* **2013,** *8*(4), e61306.

[283] Lerman, P.; Kivity, S. The efficacy of corticotropin in primary infantile spasms. *J. Pediatr.,* **1982,** *101*(2), 294-296.

[284] Hrachovy, R.A.; Frost, J.D., Jr.; Glaze, D.G. High-dose, long-duration versus low-dose, short-duration corticotropin therapy for infantile spasms. *J. Pediatr.,* **1994,** *124*(5 Pt 1), 803-806.

[285] Dooley, J.M.; Camfield, P.R.; Goulden, K.J.; Macken, S.R. Low-dose alternate-day corticotropin therapy in the treatment of childhood seizures. *Am. J. Dis. Child.,* **1989,** *143*(11), 1263-1265.

[286] Baram, T.Z.; Mitchell, W.G.; Tournay, A.; Snead, O.C.; Hanson, R.A.; Horton, E.J. High-dose corticotropin (acth) versus prednisone for infantile spasms: A prospective, randomized, blinded study. *Pediatrics,* **1996,** *97*(3), 375-379.

[287] Brunson, K.L.; Khan, N.; Eghbal-Ahmadi, M.; Baram, T.Z. Corticotropin (acth) acts directly on amygdala neurons to down-regulate corticotropin-releasing hormone gene expression. *Ann. Neurol.,* **2001,** *49*(3), 304-312.

[288] Wang, W.; Murphy, B.; Dow, K.E.; David Andrew, R.; Fraser, D.D. Systemic adrenocorticotropic hormone administration down-regulates the expression of corticotropin-releasing hormone (crh) and crh-binding protein in infant rat hippocampus. *Pediatr. Res.,* **2004,** *55*(4), 604-610.

[289] Baram, T.Z.; Schultz, L. Acth does not control neonatal seizures induced by administration of exogenous corticotropin-releasing hormone. *Epilepsia,* **1995,** *36*(2), 174-178.

[290] Avishai-Eliner, S.; Yi, S.J.; Baram, T.Z. Developmental profile of messenger rna for the corticotropin-releasing hormone receptor in the rat limbic system. *Brain Res. Dev. Brain Res.,* **1996,** *91*(2), 159-163.

[291] Brunson, K.L.; Eghbal-Ahmadi, M.; Baram, T.Z. How do the many etiologies of west syndrome lead to excitability and seizures? The corticotropin releasing hormone excess hypothesis. *Brain Dev.,* **2001,** *23*(7), 533-538.

[292] Holmes, M.C.; Catt, K.J.; Aguilera, G. Involvement of vasopressin in the down-regulation of pituitary corticotropin-releasing factor receptors after adrenalectomy. *Endocrinology,* **1987,** *121*(6), 2093-2098.

[293] Grammatopoulos, D.K. Insights into mechanisms of corticotropin-releasing hormone receptor signal transduction. *Br. J. Pharmacol.,* **2012,** *166*(1), 85-97.

[294] Wang, W.; Dow, K.E.; Fraser, D.D. Elevated corticotropin releasing hormone/corticotropin releasing hormone-r1 expression in postmortem brain obtained from children with generalized epilepsy. *Ann. Neurol.,* **2001,** *50*(3), 404-409.

[295] Baram, T.Z.; Chalmers, D.T.; Chen, C.; Koutsoukos, Y.; De Souza, E.B. The crf1 receptor mediates the excitatory actions of corticotropin releasing factor (crf) in the developing rat brain: *In vivo* evidence using a novel, selective, non-peptide crf receptor antagonist. *Brain Res.,* **1997,** *770*(1-2), 89-95.

[296] Bobele, G.B.; Ward, K.E.; Bodensteiner, J.B. Hypertrophic cardiomyopathy during corticotropin therapy for infantile spasms. A clinical and echocardiographic study. *Am. J. Dis. Child.,* **1993,** *147*(2), 223-225.

[297] Zorrilla, E.P.; Koob, G.F. Progress in corticotropin-releasing factor-1 antagonist development. *Drug Discov. Today,* **2010,** *15*(9-10), 371-383.

[298] Liang, L.; Angleson, J.K.; Dores, R.M. Using the human melanocortin-2 receptor as a model for analyzing hormone/receptor interactions between a mammalian mc2 receptor and acth(1-24). *Gen. Comp. Endocrinol.,* **2013,** *181*, 203-210.

[299] Jaworska-Feil, L.; Turchan, J.; Przewlocka, B.; Budziszewska, B.; Leskiewicz, M.; Lason, W. Effects of pilocarpine- and kainate-induced seizures on thyrotropin-releasing hormone biosynthesis and receptors in the rat brain. *J. Neural. Transm.,* **1999,** *106*(5-6), 395-407.

[300] Knoblach, S.M.; Kubek, M.J. Thyrotropin-releasing hormone release is enhanced in hippocampal slices after electroconvulsive shock. *J. Neurochem.,* **1994,** *62*(1), 119-125.

[301] Deng, P.Y.; Porter, J.E.; Shin, H.S.; Lei, S. Thyrotropin-releasing hormone increases gaba release in rat hippocampus. *J. Physiol.,* **2006,** *577*(Pt 2), 497-511.

[302] Nie, Y.; Schoepp, D.D.; Klaunig, J.E.; Yard, M.; Lahiri, D.K.; Kubek, M.J. Thyrotropin-releasing hormone (protirelin) inhibits potassium-stimulated glutamate and aspartate release from hippocampal slices *in vitro*. *Brain Res.,* **2005,** *1054*(1), 45-54.

[303] Yatsugi, S.; Yamamoto, M. Anticonvulsive properties of ym-14673, a new trh analogue, in amygdaloid-kindled rats. *Pharmacol. Biochem. Behav.,* **1991,** *38*(3), 669-672.

[304] Osonoe, K.; Osonoe, M.; Ariga, K.; Mori, N. The effect of thyrotropin-releasing hormone (trh) on limbic status epilepticus in rats. *Epilepsy Res.,* **1994,** *18*(3), 217-225.

[305] Ujihara, H.; Xie, R.M.; Sasa, M.; Ishihara, K.; Fujita, Y.; Yoshimura, M.; Kishimoto, T.; Serikawa, T.; Yamada, J.; Takaori, S. Inhibition by thyrotropin-releasing hormone of epileptic seizures in spontaneously epileptic rats. *Eur. J. Pharmacol.,* **1991,** *196*(1), 15-19.

[306] Przewlocka, B.; Labuz, D.; Mika, J.; Lipkowski, A.; van Luijtelaar, G.; Coenen, A.; Lason, W. Protective effects of trh and its analogues in chemical and genetic models of seizures. *Pol. J. Pharmacol.,* **1997,** *49*(5), 373-378.

[307] Takeuchi, Y.; Takano, T.; Abe, J.; Takikita, S.; Ohno, M. Thyrotropin-releasing hormone: Role in the treatment of west syndrome and related epileptic encephalopathies. *Brain Dev.,* **2001,** *23*(7), 662-667.

[308] Tanaka, C.; Maegaki, Y.; Koeda, T.; Ohta, S.; Takeshita, K. Successful treatment of progressive myoclonus epilepsy with trh. *Pediatr. Neurol.,* **1998,** *18*(5), 442-444.

[309] Matsumoto, A.; Kumagai, T.; Takeuchi, T.; Miyazaki, S.; Watanabe, K. Clinical effects of thyrotropin-releasing hormone for severe epilepsy in childhood: A comparative study with acth therapy. *Epilepsia,* **1987,** *28*(1), 49-55.

[310] Kubek, M.J.; Liang, D.; Byrd, K.E.; Domb, A.J. Prolonged seizure suppression by a single implantable polymeric-trh microdisk preparation. *Brain Res.,* **1998,** *809*(2), 189-197.

[311] Veronesi, M.C.; Kubek, D.J.; Kubek, M.J. Intranasal delivery of a thyrotropin-releasing hormone analog attenuates seizures in the amygdala-kindled rat. *Epilepsia,* **2007,** *48*(12), 2280-2286.

[312] Kubek, M.J.; Domb, A.J.; Veronesi, M.C. Attenuation of kindled seizures by intranasal delivery of neuropeptide-loaded nanoparticles. *Neurotherapeutics,* **2009,** *6*(2), 359-371.

[313] Heuer, H.; Schafer, M.K.; O'Donnell, D.; Walker, P.; Bauer, K. Expression of thyrotropin-releasing hormone receptor 2 (trh-r2) in the central nervous system of rats. *J. Comp. Neurol.,* **2000,** *428*(2), 319-336.

[314] Gary, K.A.; Sevarino, K.A.; Yarbrough, G.G.; Prange, A.J., Jr.; Winokur, A. The thyrotropin-releasing hormone (trh) hypothesis of homeostatic regulation: Implications for trh-based therapeutics. *J. Pharmacol. Exp. Ther.,* **2003,** *305*(2), 410-416.

[315] Inanaga, K.; Kumashiro, H.; Fukuyama, Y.; Ohtahara, S.; Shirouzu, M. Clinical study of oral administration of dn-1417, a trh analog, in patients with intractable epilepsy. *Epilepsia,* **1989,** *30*(4), 438-445.

[316] Renming, X.; Ishihara, K.; Sasa, M.; Ujihara, H.; Momiyama, T.; Fujita, Y.; Todo, N.; Serikawa, T.; Yamada, J.; Takaori, S. Antiepileptic effects of cnk-602a, a novel thyrotropin-releasing hormone analog, on absence-like and tonic seizures of spontaneously epileptic rats. *Eur. J. Pharmacol.,* **1992,** *223*(2-3), 185-192.

[317] Momiyama, T.; Ishihara, K.; Kimura, K.; Todo, N.; Fujita, Y.; Serikawa, T.; Sasa, M. Long-term antiepileptic effects of chronic intake of cnk-602a, a thyrotropin-releasing hormone analogue, on spontaneously epileptic rats. *Epilepsia,* **1996,** *37*(4), 328-331.

[318] Rajput, S.K.; Singh, J.N.; Ingole, S.; Jain, G.; Kaur, N.; Monga, V.; Meena, C.L.; Jain, R.; Sharma, S.S. Neuropharmacological profile of l-pglu-(1-benzyl)-l-his-l-pronh2, a newer thyrotropin-releasing hormone analog: Effects on seizure models, sodium current, cerebral blood flow and behavioral parameters. *Epilepsy Res.,* **2009,** *87*(2-3), 223-233.

[319] Rajput, S.K.; Krishnamoorthy, S.; Pawar, C.; Kaur, N.; Monga, V.; Meena, C.L.; Jain, R.; Sharma, S.S. Antiepileptic potential and behavioral profile of l-pglu-(2-propyl)-l-his-l-pronh2, a newer thyrotropin-releasing hormone analog. *Epilepsy Behav.,* **2009,** *14*(1), 48-53.

[320] Sah, N.; Rajput, S.K.; Singh, J.N.; Meena, C.L.; Jain, R.; Sikdar, S.K.; Sharma, S.S. L-pglu-(2-propyl)-l-his-l-pronh(2) attenuates 4-aminopyridine-induced epileptiform activity and sodium current: A possible action of new thyrotropin-releasing hormone analog for its anticonvulsant potential. *Neuroscience,* **2011,** *199*, 74-85.

[321] Lee, H.J.; Macbeth, A.H.; Pagani, J.H.; Young, W.S., 3rd Oxytocin: The great facilitator of life. *Prog. Neurobiol.,* **2009,** *88*(2), 127-151.

[322] Caffe, A.R.; Van Ryen, P.C.; Van der Woude, T.P.; Van Leeuwen, F.W. Vasopressin and oxytocin systems in the brain and upper spinal cord of macaca fascicularis. *J. Comp. Neurol.,* **1989,** *287*(3), 302-325.

[323] Knobloch, H.S.; Charlet, A.; Hoffmann, L.C.; Eliava, M.; Khrulev, S.; Cetin, A.H.; Osten, P.; Schwarz, M.K.; Seeburg, P.H.; Stoop, R.; Grinevich, V. Evoked axonal oxytocin release in the central amygdala attenuates fear response. *Neuron,* **2012,** *73*(3), 553-566.

[324] Zhang, L.; Hernandez, V.S. Synaptic innervation to rat hippocampus by vasopressin-immuno-positive fibres from the hypothalamic supraoptic and paraventricular nuclei. *Neuroscience,* **2013,** *228*, 139-162.

[325] Sun, Q.; Pretel, S.; Applegate, C.D.; Piekut, D.T. Oxytocin and vasopressin mrna expression in rat hypothalamus following kainic acid-induced seizures. *Neuroscience,* **1996,** *71*(2), 543-554.

[326] Loyens, E.; Vermoesen, K.; Schallier, A.; Michotte, Y.; Smolders, I. Proconvulsive effects of oxytocin in the generalized pentylenetetrazol mouse model are mediated by vasopressin 1a receptors. *Brain Res.,* **2012,** *1436*, 43-50.

[327] Erbas, O.; Yilmaz, M.; Korkmaz, H.A.; Bora, S.; Evren, V.; Peker, G. Oxytocin inhibits pentylentetrazol-induced seizures in the rat. *Peptides,* **2013,** *40*, 141-144.

[328] Zaninetti, M.; Raggenbass, M. Oxytocin receptor agonists enhance inhibitory synaptic transmission in the rat hippocampus by activating interneurons in stratum pyramidale. *Eur. J. Neurosci.,* **2000,** *12*(11), 3975-3984.

[329] Raggenbass, M.; Wuarin, J.P.; Gahwiler, B.H.; Dreifuss, J.J. Opposing effects of oxytocin and of a mu-receptor agonistic opioid peptide on the same class of non-pyramidal neurones in rat hippocampus. *Brain Res.,* **1985,** *344*(2), 392-396.

[330] Croiset, G.; De Wied, D. Proconvulsive effect of vasopressin; mediation by a putative v2 receptor subtype in the central nervous system. *Brain Res.,* **1997,** *759*(1), 18-23.

[331] Brinton, R.E.; McEwen, B.S. Vasopressin neuromodulation in the hippocampus. *J. Neurosci.,* **1989,** *9*(3), 752-759.

[332] Muhlethaler, M.; Dreifuss, J.J.; Gahwiler, B.H. Vasopressin excites hippocampal neurones. *Nature,* **1982,** *296*(5859), 749-751.

[333] Chapman, C.D.; Frey, W.H., 2nd; Craft, S.; Danielyan, L.; Hallschmid, M.; Schioth, H.B.; Benedict, C. Intranasal treatment of central nervous system dysfunction in humans. *Pharm. Res.,* **2012**. [Epub ahead of print]

[334] Neumann, I.D.; Maloumby, R.; Beiderbeck, D.I.; Lukas, M.; Landgraf, R. Increased brain and plasma oxytocin after nasal and peripheral administration in rats and mice. *Psychoneuroendocrinology,* **2013,** doi: 10.1016/j.psyneuen.2013.03.003. [Epub ahead of print]

[335] Zhu, J.; Jiang, Y.; Xu, G.; Liu, X. Intranasal administration: A potential solution for cross-bbb delivering neurotrophic factors. *Histol. Histopathol.,* **2012,** *27*(5), 537-548.

[336] Stoop, R. Neuromodulation by oxytocin and vasopressin. *Neuron,* **2012,** *76*(1), 142-159.

[337] Young, W.S.; Li, J.; Wersinger, S.R.; Palkovits, M. The vasopressin 1b receptor is prominent in the hippocampal area ca2 where it is unaffected by restraint stress or adrenalectomy. *Neuroscience,* **2006,** *143*(4), 1031-1039.

[338] Paban, V.; Alescio-Lautier, B.; Devigne, C.; Soumireu-Mourat, B. The behavioral effect of vasopressin in the ventral hippocampus is antagonized by an oxytocin receptor antagonist. *Eur. J. Pharmacol.,* **1998,** *361*(2-3), 165-173.

[339] Manning, M.; Misicka, A.; Olma, A.; Bankowski, K.; Stoev, S.; Chini, B.; Durroux, T.; Mouillac, B.; Corbani, M.; Guillon, G. Oxytocin and vasopressin agonists and antagonists as research tools and potential therapeutics. *J. Neuroendocrinol.,* **2012,** *24*(4), 609-628.

[340] Hicks, C.; Jorgensen, W.; Brown, C.; Fardell, J.; Koehbach, J.; Gruber, C.W.; Kassiou, M.; Hunt, G.E.; McGregor, I.S. The nonpeptide oxytocin receptor agonist way 267,464: Receptor-binding profile, prosocial effects and distribution of c-fos

expression in adolescent rats. *J. Neuroendocrinol.,* **2012,** *24*(7), 1012-1029.

[341] Decaux, G.; Soupart, A.; Vassart, G. Non-peptide arginine-vasopressin antagonists: The vaptans. *Lancet,* **2008,** *371*(9624), 1624-1632.

[342] Pitt, G.R.; Batt, A.R.; Haigh, R.M.; Penson, A.M.; Robson, P.A.; Rooker, D.P.; Tartar, A.L.; Trim, J.E.; Yea, C.M.; Roe, M.B. Non-peptide oxytocin agonists. *Bioorg. Med. Chem. Lett.,* **2004,** *14*(17), 4585-4589.

[343] Wasterlain, C.G.; Mazarati, A.M.; Naylor, D.; Niquet, J.; Liu, H.; Suchomelova, L.; Baldwin, R.; Katsumori, H.; Shirasaka, Y.; Shin, D.; Sankar, R. Short-term plasticity of hippocampal neuropeptides and neuronal circuitry in experimental status epilepticus. *Epilepsia,* **2002,** *43 Suppl 5*, 20-29.

[344] Robertson, C.R.; Flynn, S.P.; White, H.S.; Bulaj, G. Anticonvulsant neuropeptides as drug leads for neurological diseases. *Nat. Prod. Rep.,* **2011,** *28*(4), 741-762.

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