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Neurobiology of Disease

Differential Effect of Neuropeptides on Excitatory Synaptic Transmission in Human Epileptic Hippocampus

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Development of novel disease-modifying treatment strategies for neurological disorders, which at present have no cure, represents a major challenge for today’s neurology. Translation of findings from animal models to humans represents an unresolved gap in most of the preclinical studies. Gene therapy is an evolving innovative approach that may prove useful for clinical applications. In animal models of temporal lobe epilepsy (TLE), gene therapy treatments based on viral vectors encoding NPY or galanin have been shown to effectively suppress seizures. However, how this translates to human TLE remains unknown. A unique possibility to validate these animal studies is provided by a surgical therapeutic approach, whereby resected epileptic tissue from temporal lobes of pharmacoresistant patients are available for neurophysiological studies in vitro. To test whether NPY and galanin have antiepileptic actions in human epileptic tissue as well, we applied these neuropeptides directly to human hippocampal slices in vitro. NPY strongly decreased stimulation-induced EPSPs in dentate gyrus and CA1 (up to 30 and 55%, respectively) via Y2 receptors, while galanin had no significant effect. Receptor autoradiographic binding revealed the presence of both NPY and galanin receptors, while functional receptor binding was only detected for NPY, suggesting that galanin receptor signaling may be impaired. These results underline the importance of validating findings from animal studies in human brain tissue, and advocate for NPY as a more appropriate candidate than galanin for future gene therapy trials in pharmacoresistant TLE patients.

Key words: galanin; gene therapy; hippocampus; NPY; temporal lobe epilepsy

Introduction

One of the major challenges of translational research for brain diseases is how to validate in human specimens the therapeutic outcomes observed in animal models. To this goal, some cases of pharmacoresistant epilepsies, particularly temporal lobe epilepsy (TLE), where brain tissue-generating seizure activity is surgically resected and can be maintained alive as acute brain slices, provides a unique opportunity for in vitro validation of therapeutic compounds. Two such promising compounds are NPY and galanin, endogenous neuropeptides that exert strong seizure-suppressant effects in animal models (Vezzani et al., 1999; Mazzarati et al., 2001). These neuropeptides are currently considered putative candidates for gene therapy in epilepsy (Haberman et al., 2003; Richichi et al., 2004). Such novel treatment strategy for epilepsy addresses a strong unmet need as pharmacoresistant patients comprise 30–40% of all epilepsy cases (Duncan et al., 2006).

NPY is a 36 aa peptide found within subpopulations of GABAergic interneurons throughout the human and rodent brain, including hippocampus (de Quindt and Emson, 1986; Köhler et al., 1986; Morris, 1989; Furtinger et al., 2001). Galanin, consisting of 29 aa in rodents and 30 aa in humans, displays a more scattered distribution, but is present within neurons and
fibers in several brain regions of rodents and humans, including the hippocampus (Melander et al., 1986; Kordover et al., 1992; Yoshitake et al., 2004).

Several lines of evidence suggest that NPY and galanin are involved in controlling network excitability in the brain. Knockout animals for NPY and galanin are more prone than wild-type littermates to develop seizures (Erickson et al., 1996; Mazarati et al., 2000) and seizure-induced cell death following kainate treatment (Baraban et al., 1997; Mazarati et al., 2000), while animals overexpressing NPY (Vezzani et al., 2002) or galanin (Mazarati et al., 2000; Kokaia et al., 2001) are more resistant to seizures. Animals injected with adeno-associated viral vector encoding NPY or galanin into the hippocampus exhibit reduced seizure frequency and total time spent in seizures during status epilepticus induced by kainate administration (Lin et al., 2003; Richichi et al., 2004; Noë et al., 2008). These antiepileptic actions of NPY and galanin appear to be related to their ability to reduce presynaptic glutamate release via activation of Y2 (El Bahh et al., 2005) or GalR1 and GalR2 receptors (Zini et al., 1993; Mazarati et al., 2000, 2004), respectively.

For developing novel translational gene therapy strategies based on neuropeptides, it is important to determine whether NPY and galanin exhibit a seizure-suppressing effect in human epileptic tissue, as has been observed in rodents. Here we investigated the action of galanin and NPY on excitatory neurotransmission in human hippocampal slices derived from pharmacoresistant TLE patients. Our data demonstrate that NPY, but not galanin, suppresses excitatory synaptic transmission onto principal neurons in the human epileptic hippocampus, therefore, suggesting that NPY is a more appropriate choice for future gene therapy strategies in pharmacoresistant TLE patients.

Materials and Methods

Subjects and ethical permits. Only patients undergoing amygdala-hippocampectomy as a treatment for medically intractable TLE were included. Hippocampal tissue was resected en bloc while amygdala tissue was removed by suction (except in two cases). Patients of either sex were diagnosed before surgery based on seizure semiology, extracranial video EEG recording, neuropsychological testing, and structural MRI. In selected cases, intracranial EEG recording and functional imaging were part of the preoperative investigation. Patient details can be found in Table 1. Written informed consent was obtained from every patient before surgery. The study design was approved by the local Ethical Committee in Lund, Sweden (4212/2007) and Copenhagen, Denmark (H-2-2011-104), and performed in accordance with the Declaration of Helsinki.

Human tissue handling and slice preparation. In the surgery room and immediately after amygdala-hippocampectomy, the resected tissue was cut into coronal sections of approximately 5 mm thickness. These slices were quickly submerged into a transportation beaker containing ice-cold sucrose-based aCSF continuously oxygenated with carbogen (95% O2 and 5% CO2). This aCSF contained the following (in mM): 200 sucrose, 21 NaHCO3, 10 glucose, 3 KCl, 1.25 NaH2PO4, 1.6 CaCl2, 2 MgCl2, and 2 MgSO4, adjusted to 300–310 mOsm, 7.4 pH. Within 20–80 min, slices of 500 μm thickness were cut in sucrose-based aCSF at 4°C using a VT1200 vibratome (Leica Microsystems), and thereafter transferred to an incubation chamber containing the same solution held at 34°C. After 15–20 min, slices were transferred to another chamber containing slightly different aCSF and were allowed to fully recover for an additional 3 h. The latter aCSF solution was also used for slice perfusion during electrophysiological experiments and contained the following (in mM): 129 NaCl, 21 NaHCO3, 10 glucose, 3 KCl, 1.25 NaH2PO4, 1.6 CaCl2, 2 MgCl2, and 2 MgSO4, adjusted to 300–310 mOsm, 7.4 pH, and constantly oxygenated and maintained at 32–34°C.

Electrophysiology. Individual slices were placed in a submerged recording chamber and infrared differential interference contrast microscopy was used for visual identification of the neurons and approach of the recording pipette. For whole-cell patch-clamp recordings, glass pipettes were back-filled with a solution containing the following (in mM): 122.5 K-glutamate, 12.5 KCl, 10 KOH-HEPES, 0.2 KOH-EGTA, 2 Mg-ATP, 0.3 Na2GTP, and 8 NaCl, pH 7.2–7.4 (mOs 290–300) and had a tip resistance of 3–5 MΩ. The series resistance during whole-cell recordings was constantly monitored (15.4 ± 1.0 and 16.8 ± 1.1 MΩ; before and after drug application, respectively; n = 20) and if changed >20% over time, the recordings were excluded from the further analysis. Biocytin (5 mg/ml) was always included in the pipette solution for post hoc identification and morphological reconstruction of the recorded cells. Since induction of spontaneous seizure-like events in human slices is difficult when using submerged slice chambers, we focused on evaluating NPY effects on stimulation-evoked excitatory synaptic transmission.

Synaptic transmission was evoked in either dentate gyrus or CA1 by rectangular current pulses (0.1 ms duration) using a stimulation pipette filled with aCSF (0.5–1 MΩ tip resistance) positioned within the medial perforant path (MPP) or stratum radiatum (Schafer collaterals), respectively. In dentate gyrus, the field recording pipette was placed in the middle portion of the MPP, whereas whole-cell recordings of granule cells were obtained from stratum granulosum. In CA1, the field-recording pipette was positioned in the same subfield as the stimulation pipette. The stimulation current strength was adjusted to generate ~40–60% of maximal EPSC in whole-cell recordings or fEPSP in field recordings and was kept constant throughout baseline (at least 10 min), drug application (10 min), and washout period (30–60 min). Synthetic human NPY (1 μM; Schafer-N) or synthetic human galanin (0.5 or 1 μM; Schafer-N; 30 aa C terminal), stored in frozen aliquots, was dissolved in aCSF and applied to slices at a speed of 2–2.5 ml/min. The NPY Y2 receptor antagonist BIB0246 (Tocris Bioscience) was first dissolved in ethanol (25 mM) and subsequently added to the aCSF at a final concentration of 0.6 μM. Siliconized bottles, tubing, and recording chambers were used to minimize adhesion of neuropeptides to the walls. Interpulse

<table>
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<th>Gender</th>
<th>Age at surgery (years)</th>
<th>Age at epilepsy onset (years)</th>
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Table 1. Patient overview
Sprouting and severe degeneration of neurons in hippocampal tissue derived from patients with pharmacoresistant TLE. Example of hematoxylin (A–C) and MAP2 (D–F) staining seen in hippocampal tissue resected from an epileptic patient with severe sclerosis. While the overall architecture of the hippocampus is preserved, the pyramidal cell layer in CA1 is almost completely degenerated with scattered cells seen in surrounding layers (A, C, D, F). The dentate granule cell layer appears intact (A, B), but is accompanied by sprouting (D, E). Boxed areas are magnified. gcl, granule cell layer; ml, molecular layer; so, stratum oriens; pcl, principal cell layer; sr, stratum radiatum; sl-m, stratum lacunosum moleculare. Scale bars: A, D, 1 mm; B, C, E, F, 200 μm.

Figure 1.
before being exposed to [$^{35}$S]-sensitive Kodak BioMax MR film together for 5 min in ice-cold 50 mM Tris-HCl buffer, pH 7.4. Finally, sections were rinsed in ice-cold distilled H$_2$O and dried under a stream of cold air.

Excitatory neurotransmission in dentate granule cell synapses in sclerotic human hippocampal tissue is attenuated by NPY, but not galanin. A, Whole-cell patch-clamp recording of a dentate granule cell in hippocampal slice preparation derived from a TLE patient shows fast repetitive action potentials upon a 300 pA current ramp depolarization. Calibration: 20 mV and 200 ms. B, Spontaneous postsynaptic currents recorded in a dentate granule cell held at −70 mV. Boxed area is magnified on the right. Calibration: 10 pA, 200 and 20 ms, respectively. C, Post hoc visualization of recorded dentate granule cells revealed by immunohistochemistry. Alexa 488-conjugated streptavidin labels intracellular biocytin and shows apical dendrites extending into the molecular layers. Scale bar, 50 μm. D, Galanin application does not affect the amplitude of evoked EPSCs. Insert, Representative traces of evoked EPSCs during aCSF (black trace) and aCSF + galanin application (red trace). Calibration: 50 pA and 20 ms. E, PPR of EPSCs remains unaltered following galanin application. F, NPY application attenuates evoked EPSC amplitudes. Insert, Representative traces of evoked EPSCs during aCSF (black trace) and aCSF + NPY (blue trace) application. Calibration: 50 pA and 20 ms. G, NPY application increases the PPR, suggesting decreased release probability of glutamate. H, I, During 40 Hz stimulation, only NPY suppresses consecutive evoked EPSCs. EPSC amplitudes are normalized to baseline values for each condition. J, Examples of EPSC traces evoked by 40 Hz stimulation shown in aCSF (black), aCSF + galanin (red), and aCSF + NPY (blue), respectively. Calibration: 25 ms, 100 pA; *p < 0.05, **p < 0.01.

**Figure 2.** Excitatory neurotransmission in dentate granule cell synapses in sclerotic human hippocampal tissue is attenuated by NPY, but not galanin. A, Whole-cell patch-clamp recording of a dentate granule cell in hippocampal slice preparation derived from a TLE patient shows fast repetitive action potentials upon a 300 pA current ramp depolarization. Calibration: 20 mV and 200 ms. B, Spontaneous postsynaptic currents recorded in a dentate granule cell held at −70 mV. Boxed area is magnified on the right. Calibration: 10 pA, 200 and 20 ms, respectively. C, Post hoc visualization of recorded dentate granule cells revealed by immunohistochemistry. Alexa 488-conjugated streptavidin labels intracellular biocytin and shows apical dendrites extending into the molecular layers. Scale bar, 50 μm. D, Galanin application does not affect the amplitude of evoked EPSCs. Insert, Representative traces of evoked EPSCs during aCSF (black trace) and aCSF + galanin application (red trace). Calibration: 50 pA and 20 ms. E, PPR of EPSCs remains unaltered following galanin application. F, NPY application attenuates evoked EPSC amplitudes. Insert, Representative traces of evoked EPSCs during aCSF (black trace) and aCSF + NPY (blue trace) application. Calibration: 50 pA and 20 ms. G, NPY application increases the PPR, suggesting decreased release probability of glutamate. H, I, During 40 Hz stimulation, only NPY suppresses consecutive evoked EPSCs. EPSC amplitudes are normalized to baseline values for each condition. J, Examples of EPSC traces evoked by 40 Hz stimulation shown in aCSF (black), aCSF + galanin (red), and aCSF + NPY (blue), respectively. Calibration: 25 ms, 100 pA; *p < 0.05, **p < 0.01.

**Figure 2.**

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**Table 2.** Membrane properties of dentate granule cells recorded in slices from TLE patients

<table>
<thead>
<tr>
<th>Membrane property</th>
<th>Dentate granule cells (n = 9 cells from 9 patients)</th>
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<tbody>
<tr>
<td>Resting membrane potential (mV)</td>
<td>−71.38 ± 0.49</td>
</tr>
<tr>
<td>Input resistance (MΩ)</td>
<td>217.50 ± 9.78</td>
</tr>
<tr>
<td>Action potential threshold (mV)</td>
<td>−41.96 ± 0.66</td>
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<tr>
<td>Action potential amplitude (mV)</td>
<td>92.55 ± 0.58</td>
</tr>
<tr>
<td>Action potential duration (ms)</td>
<td>0.86 ± 0.01</td>
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tissue with severe sclerosis, a thin granule cell layer was normally observed in the dentate gyrus, and MAP2 immunohistochemical staining suggested substantial mossy fiber sprouting, particularly evident in the supragranular layers (Fig. 1D–F). Significant loss of pyramidal cells was found in CA2-CA3 areas and an almost complete degeneration of pyramidal cells was seen in the CA1 area of the hippocampus (Fig. 1C,F). In the CA1, remaining pyramidal cells did not form a distinct layer but were dispersed. In most cases, due to routine surgery procedures, the resected tissue did not contain the CA3 region of the hippocampus. Therefore, in all slices, field potentials were recorded in the dentate gyrus and CA1, while whole-cell patch-clamp recordings were performed only from dentate granule cells.

To validate the viability of the resected human tissue, we made one or several whole-cell patch-clamp recordings from dentate granule cells from a subset of the slices. These recordings could be
In our first series of experiments, we explored if galanin or NPY could suppress evoked synaptic transmission at MPP-dentate granule cell synapses. Whole-cell patch-clamp recordings of dentate granule cells revealed that average EPSC amplitudes and PPR were unaffected by galanin (amplitudes: in aCSF 215 ± 18 pA, in aCSF + galanin: 229 ± 35 pA, n = 11, nonsignificant, paired t test; PPR: in aCSF: 0.90 ± 0.08, in aCSF + galanin: 0.88 ± 0.08, n = 11, n-s, paired t test; Fig. 2D,E), whereas NPY exerted a strong effect (Fig. 2F,G), reducing EPSC amplitudes (in aCSF: 318 ± 46 pA, in aCSF + NPY: 143 ± 24 pA, n = 9, p < 0.01, paired t test) and increasing PPR (in aCSF: 0.75 ± 0.07, in aCSF + NPY: 0.87 ± 0.06, n = 9, p < 0.05, paired t test). These results with NPY are consistent with inhibition of presynaptic glutamate release shown previously in naive, nonepileptic rodents (Colmers et al., 1985, 1988; Qian et al., 1997). At high-frequency stimulations (40 Hz), none of the evoked EPSCs were affected by galanin (Fig. 2D,E). Blocking AMPA/kainate receptors with NBQX almost completely blocked AMPA component of EPSCs (Fig. 2D,F,G).}

**Effects of galanin and NPY in human dentate gyrus**

In our first series of experiments, we explored if galanin or NPY could suppress evoked synaptic transmission at MPP-dentate granule cell synapses. Whole-cell patch-clamp recordings of dentate granule cells revealed that average EPSC amplitudes and PPR were unaffected by galanin (amplitudes: in aCSF 215 ± 18 pA, in aCSF + galanin: 229 ± 35 pA, n = 11, nonsignificant, paired t test; PPR: in aCSF: 0.90 ± 0.08, in aCSF + galanin: 0.88 ± 0.08, n = 11, n-s, paired t test; Fig. 2D,E), whereas NPY exerted a strong effect (Fig. 2F,G), reducing EPSC amplitudes (in aCSF: 318 ± 46 pA, in aCSF + NPY: 143 ± 24 pA, n = 9, p < 0.01, paired t test) and increasing PPR (in aCSF: 0.75 ± 0.07, in aCSF + NPY: 0.87 ± 0.06, n = 9, p < 0.05, paired t test). These results with NPY are consistent with inhibition of presynaptic glutamate release shown previously in naive, nonepileptic rodents (Colmers et al., 1985, 1988; Qian et al., 1997). At high-frequency stimulations (40 Hz), none of the evoked EPSCs were affected by galanin (Fig. 2D,E). Blocking AMPA/kainate receptors with NBQX almost completely blocked AMPA component of EPSCs (Fig. 2D,F,G).
0.75 ± 0.04, in aCSF + galanin: 0.73 ± 0.04, n = 13, nonsignificant, paired t test; Fig. 3C). During 40 Hz stimulations, the effect of galanin was again subtle and only minor suppression of excitatory transmission was detected (Fig. 3D, only sixth pulse). NPY, on the other hand, caused a marked decrease in fEPSPs (in aCSF: 100 ± 15.1%, in aCSF + NPY: 69.2 ± 9.9%, n = 12, p < 0.01, paired t test; Fig. 3F), along with an increase in PPR (in aCSF: 0.71 ± 0.05, in aCSF + NPY: 0.83 ± 0.08, n = 12, p < 0.01, paired t test; Fig. 3G) and a profound reduction in excitatory transmission during the beginning of the 40 Hz stimulation train (Fig. 3H, first and second pulses). Application of NBQX almost completely blocked the fEPSPs (by 95.2 ± 1.2%, n = 9), confirming that MPP stimulation predominantly activated AMPA/kainate receptors in glutamatergic synapses on granule cells.

**Effects of galanin and NPY in human CA1**

To address whether the limited effect of galanin was specific to dentate gyrus or was more widespread in the hippocampus, similar experiments with field recordings were performed in the CA1 area. Electrical stimulation in stratum radiatum elicited fEPSPs of relatively short duration (Fig. 4A,B,F,G). The relatively short duration of fEPSPs was most likely from degeneration and the dispersion of the remaining CA1 pyramidal cells in the epileptic hippocampus. These fEPSPs were almost completely blocked following NBQX application (96.4 ± 0.8%, n = 12) without affecting the amplitude of the presynaptic fiber volley (Fig. 4B,G), confirming that these postsynaptic potentials were generated by AMPA/kainate receptor activation. Following galanin application, the initial slopes of the fEPSPs observed during single (in aCSF: 100 ± 21.0%, in aCSF + galanin: 92.2 ± 20.5%, n = 14, nonsignificant, paired t test; Fig. 4C) and 40 Hz stimulations (Fig. 4E) remained unchanged. This was also the case for PPR (in aCSF: 1.28 ± 0.20, in aCSF + galanin: 1.25 ± 0.17, n = 14, nonsignificant, paired t test; Fig. 4D). Similar results were found when measuring the amplitude (instead of initial slope) of the fEPSPs (data not shown). In contrast, the initial slopes of fEPSPs were strongly decreased following NPY application (in aCSF: 100 ± 25.8%, in aCSF + NPY: 46.1 ± 17.0%, n = 10, p < 0.01, paired t test; Fig. 4H), and this was accompanied by a significant increase in PPR (in aCSF: 0.91 ± 0.12, in aCSF + NPY: 1.33 ± 0.21, n = 10, p < 0.05, paired t test; Fig. 4I). During 40 Hz stimulations, only the first fEPSP was significantly suppressed, whereas consecutive responses displayed a trend of attenuation following NPY application (Fig. 4J). The inhibitory effect induced by NPY was also confirmed by measuring the amplitudes of the fEPSPs (data not shown). These data suggest that NPY can exert strong inhibitory action on presynaptic glutamate release in the CA1 area of the human epileptic hippocampus, whereas galanin has no effect on glutamatergic transmission.

Rodent data suggest that it is the Y2 receptor that mediates the inhibitory effects of NPY on hippocampal excitatory transmission (El Bahh et al., 2005). To evaluate whether the Y2 receptor also is important for this effect in human epileptic tissue, we repeated the paired-pulse fEPSP experiments in presence of the highly selective Y2 receptor antagonist BIIE0246 (Doods et al., 1999). Under these conditions, application of NPY to seven hippocampal slices from three TLE patients had no significant effect on fEPSP slopes (in aCSF + BIIE0246: 100 ± 40.1%, in aCSF + BIIE0246 + NPY: 119 ± 30.2%, paired t test) and PPR (in aCSF + BIIE0246: 1.47 ± 0.25, in aCSF + BIIE0246 + NPY: 1.39 ± 0.33, paired t test) in CA1 (Fig. 5A–C). Two additional field recordings were obtained from dentate gyrus and in both cases the effect of NPY was absent in the presence of BIIE0246 (Fig. 5D). These results clearly suggest that Y2 receptor activation is involved in mediating the inhibitory effect of NPY on presynaptic glutamate release in human CA1 and dentate gyrus.

**Receptor binding and functional binding in human tissue**

We speculated that lack of galanin effect in human epileptic tissue could be a consequence of compromised galanin receptor signaling. We found that galanin receptors were expressed within the hilus and molecular layer of the dentate gyrus and in strata lacunosum moleculare and radiatum of CA1, as indicated by specific binding of [125I]-galanin (Fig. 6A,C,M), but the functional receptor binding assay revealed impaired functionality of these receptors (Fig. 6G,I,N). These data suggest that, although galanin receptors are present in the human epileptic hippocampus, their ability for downstream signaling seems to be deficient. In con-
Figure 6. Galanin and NPY receptor binding and functional binding in the human epileptic hippocampus and amygdala. A, Galanin and B, NPY receptor binding in the human epileptic hippocampus. C, D, Nonspecific binding corresponding to A and B, respectively. E, Hematoxylin staining of an adjacent section showing the gross morphology of the layers analyzed. F, [125I]-galanin binding (top left), corresponding nonspecific binding (bottom left), [125I]-PYY binding (top right), and corresponding nonspecific binding (bottom right) in sections from the human amygdala. G, Galanin and NPY receptor functional binding. H, Basal and nonspecific (I) binding corresponding to G and K, respectively. J, Hematoxylin staining of an adjacent section showing the gross morphology of the layers analyzed. L, Galanin functional binding (top left), NPY functional binding (top right), and corresponding basal binding (bottom left) in sections from the human amygdala. M, Quantification of specific [125I]-galanin and [125I]-PYY receptor binding measured in hippocampal regions (n = 5) and amygdala (n = 2). N, Quantification of galanin and NPY receptor functional binding (i.e., peptide-stimulated binding minus basal binding) measured in hippocampal regions (n = 5) and amygdala (n = 2). Note almost complete absence of galanin receptor functional binding (M), despite specific [125I]-galanin binding found in M. Mol, stratum molecular; rad, stratum radiatum; lac-mol, stratum lacunosum moleculare. Scale bars: A–E, G–K, 3 mm; F, L, 4 mm.
Discussion
Here we demonstrate that resected hippocampus from TLE patients provides a unique possibility to validate, in diseased human brain tissue, the treatment outcomes obtained from animal models. Exogenously applied NPY effectively suppressed excitatory synaptic transmission, while galanin was ineffective. This approach is highly valuable to validate the most effective alternatives for future clinical gene therapy applications. Our data also indicate that downstream signaling of galanin receptors might be impaired in epileptic tissue, which may cause the ineffectiveness of galanin. These findings provide better understanding of the role of neuropeptides in icotogenesis and possibly epileptogenesis, and are important for developing neuropeptide-based gene therapy strategies for epilepsy and potentially other brain disorders associated with hyperexcitability (Rogawski, 2008; Kullmann, 2010; Santos et al., 2013).

The effect of exogenously applied NPY on human epileptic dentate gyrus was explored in one previous publication (Patyro et al., 1999). In this study, dentate granule cells were recorded with sharp pipettes, while the lateral perforant path (LPP; outer molecular layer) was electrically stimulated. In granule cells, LPP stimulation induced postsynaptic bursting activity, which was effectively attenuated by NPY application. The authors concluded that in human epileptic hippocampus NPY exerts a strong inhibitory effect on presynaptic glutamate release from LPP synapses. Our whole-cell patch-clamp and field recordings demonstrate that glutamate release is inhibited also from MPP synapses by NPY, although we cannot exclude that part of the observed effect may be related to EPSP(C)s generated by sprouted mossy fiber synapses activated by direct stimulation of granule cell dendrites. In addition, we show that glutamate release is also suppressed in Schaffer collateral–CA1 pyramidal cell synapses by NPY, suggesting its widespread inhibitory effect on excitatory transmission in the human epileptic hippocampus. Consistent with these findings, NPY receptors were expressed and functional in both the dentate gyrus and CA1 region as shown by conventional and functional binding assays. Moreover, we show that inhibitory effects of NPY in human hippocampal tissue are mediated by Y2 receptors, which have previously been found to be upregulated in TLE patients (Furtinger et al., 2001).

To our knowledge, possible effects of galanin on synaptic transmission in human brain have not been investigated previously. Our data suggest that galanin has limited action on excitatory synaptic transmission in the human epileptic hippocampus. Studies in animals show that both NPY (Qian et al., 1997; El Bahh et al., 2005) and galanin (Zini et al., 1993; Mazzari et al., 1998, 2000) can act as modulators of excitability by suppressing presynaptic glutamate release, particularly evident in the hippocampus. This is in accordance with several publications demonstrating inhibitory effect of NPY and galanin on seizures in various animal models of epilepsy (for review, see Woldbye and Kokaia, 2004; Lerner et al., 2008; Noé et al., 2009). Attenuation of epileptogenesis in a post status epilepticus model by viral vector-based overexpression of NPY in the hippocampus has also been demonstrated (Noé et al., 2008). Whether neuropeptides can exert similar effect in long-established chronic epileptic seizures is unknown. Our present data from human epileptic hippocampus suggest that only NPY, but not galanin, may be effective in suppressing such chronic seizures.

Why galanin fails to attenuate excitatory synaptic transmission in the human epileptic hippocampus is unclear. Our data show an almost complete absence of functional galanin binding in human epileptic hippocampus, thus indicating deficient galanin signaling. All previously observed seizure-suppressant effects of galanin have been reported in naïve, nonepileptic animals, and could therefore still be mediated by unaltered galanin receptor signaling. Alternatively, galanin effects could also be mediated by other mechanisms, partly by suppressing acetylcholine-mediated fEPSPs as shown in CA1 (Fisone et al., 1987; Yoshitake et al., 2011). Apart from presynaptic mechanisms, signaling through postsynaptic G-protein-regulated inwardly rectifying potassium (GIRK) channels that hyperpolarize neurons could also play a role (Smith et al., 1998).

One of the main translational implications of this study is that it suggests a tentative road map for future gene therapy trials in epi-
lepsy. In this regard, our data support the idea that viral vector-based gene therapy approaches to overexpress neuropeptides in the hippocampus should favor NPY rather than galanin as a candidate for therapeutic targeting, particularly if attenuation of glutamatergic excitatory synaptic transmission is an intended objective. In a more general perspective, the results of the present study imply three major points (Fig. 7). (1) Antiepileptic agents need to be tested in epileptic animals. Epileptic conditions may change the target, and the agent that is effective in naïve animals may lose its effectiveness in the epileptic brain. (2) The putative candidates need to be tested in human epileptic tissue. Indeed, refractory temporal lobe epilepsy cases provide a unique opportunity to test the novel antiepileptic agents in resected epileptic tissue from patients. This step may reveal the drugs that despite being effective in epileptic animals fail in the human epileptic brain. (3) The “GO/NOGO” decision for clinical trials needs to be based on the outcomes from both epileptic animals and, most importantly, on human epileptic tissue. Clinical trials are rather expensive to perform, and thorough preclinical examination involving human epileptic tissue may decrease the risks for negative outcomes.

References


