INTRODUCTION

Neuropeptide Y (NPY) is found within axons and somata in the hippocampus and many other regions of the brain (Chronwall et al. 1985). In the rat hippocampus, acting at a presynaptic site, NPY inhibits release of the excitatory transmitter glutamate and can thereby reduce hyperexcitability (Bleakman et al. 1992; Colmers et al. 1988; Klapstein and Colmers 1993) as has been demonstrated in acute models of seizurereactivity activity (Klapstein and Colmers 1997; Woldbye et al. 1997). Neurochemical studies suggest that there are changes in the expression of both NPY and its receptors in the hippocampus and dentate gyrus of epileptic humans and rodent models (de Lanerolle et al. 1992; Gruber et al. 1994; Marksteiner et al. 1990; Mathern et al. 1995; Schwarzer et al. 1998; Sperr et al. 1992). In most cases the levels of expression are increased, suggesting that NPY may serve as an endogenous mechanism to reduce seizure activity.

Electroencephalographic (EEG) and depth electrode studies have shown that temporal lobe epilepsy can have temporal neocortical or hippocampal onset (Spencer et al. 1993). In hippocampal slices from patients with temporal lobe epilepsy with cortical onset, perforant path stimulation generally leads to a single spike and a small excitatory postsynaptic potential (EPSP). In contrast, in slices from patients with hippocampal onset, the same stimulation leads to multiple spikes, and a larger and generally longer polysynaptic EPSP, indicative of dentate excitability (Williamson 1994). This chronic excitability may be due in part to enhanced N-methyl-D-aspartate (NMDA) receptor–mediated events and the presence of altered glutamatergic circuits. Therefore we tested the hypothesis that NPY would reduce the glutamatergic synaptic response evoked in hippocampal slices from epileptic patients with hippocampal onset. We compared the actions of NPY with another agonist that also acts at presynaptic receptors to reduce glutamate release, the metabotropic group 3 receptor agonist, L-2-amino-4-phosphonobutyric acid (L-AP4) (Gereau and Conn 1995; Macek et al. 1996). Although the importance of glutamatergic circuits has been well-documented in epileptic tissue, regulation of this activity by neuromodulators has received relatively little attention. The actions of NPY have not been studied in chronically epileptic tissue, or in human hippocampi.

METHODS

The tissue used for the physiological experiments was obtained from patients diagnosed with medically intractable temporal lobe epilepsy with hippocampal onset. This determination was based on intracranial EEG recordings and/or a presurgical evaluation suggestive of medial temporal lobe sclerosis (Spencer et al. 1993). Subsequent neuropathological examination showed cell loss, whereas structural lesions and/or tumors were not observed. The methods for preparation and maintenance of hippocampal slices from human tissue have been described previously (Williamson et al. 1995). These experiments were approved by the Yale University Human Investigation Committee, and informed consent was obtained.

Intracellular recordings from dentate granule cells were made with microelectrodes filled with 4 M K-acetate pulled on a Brown-Flaming electrode puller (Sutter Instruments). Perforant path fibers were activated by delivering electrical stimuli (0.1 Hz; 0.3 ms duration; 100–800 μA) to the outer molecular layer with bipolar stimulating electrodes. The stimulating current was maintained at a single intensity for any given cell. Drugs were dissolved in the bath medium immediately before use and were applied as microdrops using a picospritzer (General Valve). All compounds were obtained from Sigma. We examined neuronal input resistance, membrane potential, and maximal evoked response [number of action potentials, duration, and the area (measured as the time-voltage integral)] before and 15 s after the initiation of drug application. Data are presented as means ± SE, and statistical significance (P ≤ 0.05) was determined using a two-tailed paired Student’s t-test.

Histological sections (30–50 μm) were cut and then stained with rabbit NPY antiserum, a gift of Dr. T. Gorcs. The antiserum is specific for NPY, as described elsewhere (Csiffary et al. 1990). After overnight incubation in primary NPY antiserum, sections were washed, immersed in biotinylated goat anti-rabbit antiserum, washed, incubated in avidin-biotin-peroxidase complex (Vector Labs), and then stained with diaminobenzidine and hydrogen peroxide as previously described in greater detail (van den Pol 1997).
RESULTS

The data presented are from 32 neurons recorded from 22 patients. Intracellularly recorded cells exhibited a range of excitability that often appeared as a synaptically evoked burst of action potentials riding on complex EPSPs (Figs. 1 and 2). The amplitude and duration of these events were significantly ($P < 0.05$) and reversibly reduced by 2-amino-5-phosphonovaleric acid (APV; 200 μM), an NMDA receptor antagonist (Figs. 1A and 2C; $n = 13$ cells, 10 patients). Whether this excitability can also be attenuated by neuromodulators has not been extensively addressed in chronically epileptic tissue.

We examined the effects of L-AP4, a group 3 metabotropic glutamate receptor agonist, on evoked responses (Fig. 1B; $n = 8$ cells from 6 patients). L-AP4 (100 μM) significantly and reversibly decreased the number of action potentials by 29 ± 9.3% [mean ± SE, from mean 3.1 ± 1 (range, 1–10) to 2.1 ± 0.7 (range, 1–7) spikes; $P = 0.02$], the EPSP duration by 14.3 ± 3.2% [mean pre-L-AP4 82 ms duration (range 71–129 ms); $P = 0.006$] and the time-voltage integral of the synaptic response by 18.8 ± 2.8% [from mean 4,860 ± 730 mVs (range, 1,666–8,034 mVs) to 3,908 ± 565 mVs (range, 1,481–5,885); $P = 0.004$]. In contrast, L-AP4 did not alter the membrane potential or input resistance. These data suggest that mGluR activation can produce a modest decrease in glutamatergic synaptic actions in this tissue (Fig. 2C).

The effects of NPY (1 μM) on evoked responses were also examined ($n = 13$ cells from 5 patients). Although NPY produced no consistent change in either the membrane potential or input resistance, dramatic inhibitory effects were observed on the evoked excitatory responses (Fig. 2). Specifically, NPY reduced the number of action potentials evoked by a single stimulus by 53.4 ± 2.7% [mean pre-NPY 2.7 ± 0.6 (range, 0–6), post-NPY 0.9 ± 0.3 (range, 0–3) spikes; $P = 0.001$], the duration of the response by 18.1 ± 3.3% [mean pre-NPY control 135 ms duration; $P = 0.0002$], and the time-voltage integral by 52.0 ± 6.3% [mean pre-NPY mean 10,108 ± 1,432 (range, 5,323–25,003), post-NPY mean 4,615 ± 779 mVs (range, 1,688–10,424); $P = 0.0002$; Fig. 2C].

Additionally, NPY’s effect appeared to be greater in more excitable cells. Specifically, a positive correlation was found ($r = 0.56$) when the relative excitability of the neurons, as assessed by their control time-voltage integral, was plotted relative to the effect of NPY on their time-voltage integral (percent decrease). Furthermore, NPY had a greater effect in tissue in which an evoked inhibitory postsynaptic potential (IPSP) was not observed. In cells with a biphasic IPSP (4 of 13 cells), NPY produced a 34.5 ± 9.8% decrease in the time-voltage integral, whereas in the group of cells in which evoked IPSPs were not detected, NPY caused a more substantial (60.1 ± 6.3%) decrease (Fig. 2D; $P = 0.05$).

NMDA Receptor Antagonist Reduces Evoked Activity

**A** Patient 10409

Control  APV  Recovery

Class 3 mGluR Agonist Reduces Evoked Responses

**B1** Patient 20304

Control  L-AP4  Recovery

**B2** Patient 50512

Control  L-AP4  Recovery

FIG. 1. N-methyl-d-aspartate (NMDA) antagonist or metabotropic glutamate receptor agonist attenuates excitatory responses evoked in epileptic human tissue. A: NMDA antagonist 2-amino-5-phosphonovaleric acid (APV; 200 μM) reversibly reduced the duration and amplitude of a typical synaptically evoked burst. In both excitable, B1, and hyperexcitable cells, B2, the group 3 metabotropic glutamate receptor agonist L-2-amino-4-phosphonobutyric acid (L-AP4; 100 μM) reduced the duration of synaptically evoked bursts and spike number. Action potentials are purposefully truncated here and in Fig. 2.
Neuropeptide Y (NPY) inhibits perforant path–evoked responses in the epileptic human hippocampus. A1: NPY reduced a 3 spike burst to an attenuated excitatory postsynaptic potential (EPSP). Dashed line indicates baseline membrane potential and shows that an inhibitory postsynaptic potential (IPSP) was present in the cell before NPY application. A2: NPY significantly reduced the number of action potentials and the time-voltage integral of the evoked response in a hyperexcitable cell. B: effect of NPY on decreasing the amplitude and time-voltage integral of the evoked EPSP in this cell was long-lasting; in this example no recovery was found by 40 min after wash out of microdrop application of NPY. The data in A and B are from 3 different patients. In addition to reducing excitatory events, NPY also reduced the amplitude of the evoked IPSP (A1, A2, and B). Whereas this could be due to a direct NPY effect on GABAergic neurons, it could also be due to an indirect effect of reducing excitatory input to inhibitory cells as shown in the rat hippocampus (Bleakman et al. 1992; Klapstein and Colmers 1993). C: bar graph shows the relative decrease in the time-voltage integral (area) produced by APV (200 µM), L-AP4 (100 µM) and NPY (1 µM). NPY was the most potent of these compounds. n is the number of cells. D: inhibitory efficacy of NPY was greatest in cells lacking detectable IPSPs.
FIG. 3. NPY in axons and cells of epileptic human hippocampi. A: in this typical example of NPY immunostaining from an epileptic patient ($n = 4$) with hippocampal seizure onset, NPY immunoreactive axons are found in the inner (MLi) and outer molecular layer (MLo), granule cell layer, and in the hilus. B: Nomarski image of the same section. C: this micrograph is typical of an epileptic patient with cortical seizure onset. NPY immunoreactive fibers are preferentially in the outer molecular layer. D: Nomarski image of C. E: NPY immunoreactive neurons were more frequent in epileptic patients with cortical (E) compared with hippocampal (F) seizure onset, consistent with previous reports (Mathern et al. 1995).
Although recovery after L-AP4 and APV was generally complete, a comparable level of recovery was not noted after NPY wash out (Fig. 2B). This could be due to long-term actions of NPY on synaptic activity as previously reported in slice (Colmers et al. 1988) and culture (Obrietan and van den Pol 1996; van den Pol et al. 1996), but could in part also be due to the potentially sticky nature of this peptide in slices.

The electrophysiology experiments above were done on patients with hippocampal seizure onset. The pattern of NPY immunostaining in the dentate gyrus typical for this type of patient is shown in Fig. 3, A, B, and F. NPY immunoreactive fibers were found in the inner and outer molecular layer, the granule cell layer, and the hilus. Scattered NPY-positive neuron somata were found in the hilus. In contrast, immunostaining of the dentate gyrus from patients with cortical seizure onset, typically lacking signs of hippocampal sclerosis, showed a relative paucity of NPY fibers in the inner molecular layer and granule cell layer compared with the outer molecular layer in the same section (Fig. 3, C–E); NPY positive neurons were common in the hilus. These data are consistent with previous data suggesting a reorganization of the NPY fiber system in some patients with temporal lobe epilepsy (de Lanerolle et al. 1992; Mathern et al. 1995).

**Discussion**

NMDA receptor antagonists can reduce the excitability of responses evoked in granule cells from epileptic hippocampi (e.g., Fig. 1A of Williamson 1994). However, the role that neuromodulators may play in reducing this excitability has not been extensively examined, and the actions of NPY have not previously been reported in the human hippocampus. We report here that both L-AP4 and NPY can significantly reduce cellular excitability. Previous work in rodents has shown that NPY (Colmers et al. 1988; Klapstein and Colmers 1993) and the group 3 metabotropic glutamate receptor (Gereau and Conn 1995; Macek et al. 1996) act primarily on the presynaptic axon terminal to reduce glutamate release, although postsynaptic actions may also occur (McQuiston et al. 1995). Although we did not directly address the site of action here, our results showing a decrease in evoked excitatory responses without an apparent change in input resistance or membrane potential are consistent with the presynaptic receptor actions previously reported.

In this study we show that NPY can attenuate excitatory responses evoked by perforant path stimulation in dentate granule cells from epileptic humans. These data are in apparent contrast to parallel work on control rats where NPY had little effect on perforant path–evoked activity (Klapstein and Colmers 1993). Whereas this difference may be due to species-specific differences in receptor expression (Dumont et al. 1998), we cannot rule out the possibility that an NPY-mediated action on glutamate release at the recurrent collaterals of the granule cell mossy fibers might also be involved (Babb et al. 1991; Dudek et al. 1994; Houser et al. 1990; Patrylo and Dudek 1998; Sutula et al. 1989). Previous work in the rodent has shown that NPY can alter the release of glutamate from these fibers (Klapstein and Colmers 1993).

NPY has been shown to reduce excitatory activity in the rat hippocampus made acutely hyperexcitable by either reducing GABA inhibition or enhancing glutamate excitaiton (Klapstein and Colmers 1997; Woldbye et al. 1997). The relative efficacy (this study) and long-lasting effect of NPY (Colmers et al. 1988; van den Pol et al. 1996) in reducing excitatory activity in hyperexcitable neurons and in the epileptic human hippocampus, support the hypothesis that NPY may be particularly effective in reducing glutamate-mediated hyperactivity. The probable release pattern of NPY makes this peptide a prime candidate for an endogenous anticonvulsant. NPY is located within dense core vesicles (Pickel et al. 1995) that are believed to be released during high-frequency firing (Hökfelt 1991). Thus NPY may be released during a seizure and thereby limit its severity. Indeed, data from transgenic NPY knockout mice have shown that the threshold for seizure induction does not appear altered, yet the severity and lethality of induced seizures are significantly greater than in controls (Baraban et al. 1997). However, whether NPY plays a role in restricting seizure onset in chronically epileptic tissue is unknown, especially given the extensive changes in the NPY system seen in epileptic tissue.

Anatomic studies on the human epileptic hippocampus also suggest that NPY can reduce glutameric excitation. Although there appears to be a reduction in the number of NPY neurons in the hilus of temporal lobe epileptics with hippocampal seizure onset, there is an increase in the distribution of NPY immunoreactive axons in the inner molecular layer. In contrast, in control tissue and tissue from patients with a cortical seizure onset, the inner molecular layer shows relatively fewer axons (de Lanerolle et al. 1992; Mathern et al. 1995). This reorganization of NPY immunoreactive fibers in the patient population used in the present study (hippocampal seizure onset) occurs in the same area where glutamatergic circuits may be formed due to reorganization of the mossy fibers. NPY may therefore play a role in limiting activity at this recurrent excitatory synapse.

In conclusion, our data support the hypothesis that NPY can act as an endogenous neuromodulator that may limit hyperexcitability in the epileptic human dentate gyrus. We postulate that one neuronal mechanism that restricts the severity and propagation of seizures may be presynaptic inhibition of glutamate release, in part mediated by NPY.

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**References**


