Neuropeptide Y Suppresses Epileptiform Activity in Rat Hippocampus In Vitro

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INTRODUCTION

Several models have been developed to study the inappropriate, synchronous activity of neurons in epilepsy both in vivo and in vitro (McNamara 1994). The in vitro models have utilized several fundamentally different means, including a reduction of endogenous inhibition, an increase of excitation, or both, to produce synchronized electrical discharges resembling those recorded during seizures (ictal) or between seizures (interictal) in human epilepsy patients. Much of the synchrony observed in these models is thought to arise from collateral excitatory synaptic interactions within area CA3 of the hippocampus (Traub and Miles 1991; Wong and Traub 1983). We have therefore concentrated on this region.

We are interested in the biological role in the hippocampus of the endogenous peptide neuropeptide Y (NPY). Previously we showed that NPY selectively reduces feedforward synaptic excitation within the hippocampus (Colmers et al. 1987, 1988; Klapstein and Colmers 1993) involving a receptor subtype that has been identified as Y2 at CA3-CA1 pyramidal cell synapses (Colmers et al. 1991; Dumont et al. 1993). Activation of this receptor has no other demonstrable effects on these neurons (Bleakman et al. 1992; Colmers et al. 1987, 1988). We have hypothesized that NPY may normally play a role in the control of excitability within the hippocampus (Bleakman et al. 1993; Colmers and Bleakman 1994; Klapstein and Colmers 1993). In support of this hypothesis, it has recently been shown that NPY knockout mice exhibit spontaneous seizures or increased susceptibility to pentylentetrazole-induced seizures compared with wild-type or heterozygous littermates (Ericsson et al. 1996). Furthermore, hippocampal kindling induces the increased expression and release of hippocampal NPY (Bendotti et al. 1993; Schwarzzer et al. 1995; Vezzani et al. 1994) and increases the expression of NPY receptors in hippocampus (Roder et al. 1996). NPY has also been shown to be effective in the presence of γ-aminobutyric acid-A (GABA_A) receptor blockers in the rat hippocampus (Bijak and Smialowska 1995; Colmers et al. 1988).

A definitive test of the hypothesis awaits the availability of a Y2-selective antagonist. Instead, we have tested the corollary hypothesis that exogenously applied NPY will inhibit the epileptiform activity induced in three different in vitro hippocampal slice models of epilepsy. Two of these models involve alterations in the activity of endogenous transmitters. In the 0 Mg²⁺ bursting model (Anderson et al. 1986), Mg²⁺ is removed from the perfusate, relieving its voltage-dependent block of N-methyl-D-aspartate (NMDA) receptors and thereby potentiating the depolarizing effect of endogenous glutamate (Sah et al. 1989). In the picrotoxin bursting model, GABA_A receptors are blocked, thereby reducing the effect of endogenous GABA (Hablitz 1984).
Both of these treatments result in the development of spontaneous, synchronous population bursts (SBs) resembling interictal events in epilepsy. The third model is stimulus-induced bursting (STIB) (Stasheff et al. 1985), in which a series of high-frequency stimuli applied to the stratum radiatum of CA2/CA3 results in a widespread primary ictal afterdischarge (1stAD) in addition to delayed, spontaneous interictal bursts (SBs) and secondary ictal discharges (2ndADs) (Rafiq et al. 1993; Stasheff et al. 1985; Walther et al. 1986).

Together the three models produce a wide range of epileptiform activity in brain slices by fundamentally different mechanisms. They are therefore well suited as models with which to test the inhibitory effects of NPY.

Methods

Male Sprague-Dawley rats (15–40 days old) were decapitated according to guidelines of the University of Alberta Health Sciences Laboratory Animal Care Committee, and brains were removed into cold (4°C) artificial cerebrospinal fluid (ACSF) bubbled continuously with 95% O2-5% CO2 (carbogen). Hippocampi were removed with entorhinal cortex attached and sliced transversely (500–600 μm thick) with the use of a tissue chopper (Stoeplting). Slices were equilibrated at room temperature in carbonated ACSF for ≥1 h and then incubated, submerged at 34 ± 0.5°C (mean ± SE) in a perfusion chamber (flow rate 2.5–3.5 ml/min), for ≥15 min before the beginning of experiments.

0 Mg2+ and picrotoxin bursting

Composition of the ACSF used for dissection and storage of hippocampal slices (500 μm thick) and baseline control recordings was (in mM) 120 NaCl, 3.3 KCl, 1.2 MgSO4, 1.3 CaCl2, 1.23 NaH2PO4, 25 NaHCO3, and 10 glucose. Slices were transferred to the perfusion chamber, and extracellular glass recording electrodes (3–15 MΩ) filled with ACSF were placed in stratum pyramidale of area CA3. In some experiments, a bipolar stimulating electrode was placed on the mossy fiber tract in the dentate hilus to deliver single stimuli to optimize the placement of the recording electrode. Stimuli were not applied via this electrode during the remainder of the experiment. The perfusion medium was then changed to either 0 Mg2+ ACSF (ACSF with the MgSO4 omitted) or ACSF to which 100 μM picrotoxin and 1.7 mM KCl (total K+ = 5 mM) were added (picrotoxin ACSF), and the slice was monitored for development of SBs. Experiments were only initiated once SBs had reached a stable frequency. Concentrated stock solutions of NPY and other drugs were diluted in the appropriate ACSF immediately before bath application. Agonists were applied at concentrations near the median effective concentration for inhibition of the population excitatory postsynaptic potential in area CA1, i.e., 3 μM for the centrally truncated analogue 1–(6-aminohexanoic acid)–25–36 (ahlX25–36) NPY (Beck-Sicking et al. 1992) and 1 μM for all others (Klapstein and Colmers 1992; unpublished observations).

STIB

Methods were adapted from Stasheff et al. (1985, 1989). Briefly, hippocampal slices (600 μm thick) were prepared and maintained in ACSF containing (in mM) 120 NaCl, 3.3 KCl, 0.9 MgSO4, 1.1 MgCl2, 1.8 CaCl2, 1.23 NaH2PO4, 25 NaHCO3, and 10 glucose. ACSF used for perfusion was identical except for the omission of MgCl2 (final Mg2+ concentration = 0.9 mM). Stimuli were delivered through a bipolar tungsten electrode placed in the stratum radiatum of CA2/CA3a. Extracellular glass recording electrodes (3–15 MΩ) or intracellular patch pipette recording electrodes (3–15 MΩ) were placed in stratum pyramidale of CA3a, CA3b, or CA3c. In some extracellular experiments, single stimuli were delivered to facilitate placement of recording electrodes.

Extracellular electrodes were filled with ACSF. Electrodes used for whole cell slice-patch recordings were filled with a solution containing (in mM) 130 potassium gluconate, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, 11 ethylene glycol-bis(β-aminooxyethyl ether)-N,N,N’,N’-tetraacetic acid, 1 CaCl2, 2 MgATP, and 0.3 sodium glyoxaline-5’-triphosphate, pH buffered to 7.2 with KOH and adjusted to 270–280 mosM. Seal resistances were routinely >2 GΩ before breakin (cf. Hamill et al. 1981; Staley et al. 1992).

Stimulus trains were delivered every 4 or 5 min via the bipolar stimulating electrode and consisted of four stimuli (30 V, 100–200 μs) at 100 Hz repeated 15 times at 5 Hz (Larson et al. 1986). For some experiments, the number of repetitions was gradually reduced from 15 until the afterdischarge failed to occur, and then raised gradually until at least three consecutive typical afterdischarges occurred. Because of time constraints during whole cell recordings, this stimulus threshold determination was not conducted.

Electrophysiological signals were routinely filtered at 3 kHz. Experiments were recorded on chart paper (Gould RS3200) and plus code modulation (pcm) tape (Vetter 100) and analyzed offline. SB frequency and 1stAD duration were measured from chart recordings. Because there were no consistent changes in SB duration with NPY, we analyzed SBs from several experiments with the use of a coastline analysis routine. Individual SBs were digitized from tape with the use of Fetchex (Axon Instruments) and exported to Axum (Trimetrix). The coastline analysis program, written in Axum, measures the sum of the voltage differences between adjacent points on the digitized trace and provides an estimate of activity within a burst (Korn et al. 1987). To account for possible artifacts due to changes in electrical noise during experiments, coastlines were calculated for recording segments showing no burst activity (noise), which were of identical duration and immediately adjacent to those containing SBs (total). Statistics were calculated on the basis of the difference between the coastline of total and noise traces. Simultaneous dual patch-clamp recordings were captured from tape in Axotape (Axon Instruments) and plotted to show synchrony of events. Statistical comparisons were made with the use of Student’s t-test or paired t-test as appropriate.

Materials

dL–2-amino-5-phosphonovaleric acid (APV) and (+)-α-methyl-4-carboxyphenyl-glycine (MCPG) were obtained from Tocris Cookson (St. Louis). NPY was purchased from INRS Santé (Pointe-Claire, Canada). PY35–36 and NPY13–36 were gifts of Drs. A. Fournier and Y. Dumont, Douglas Hospital Research Centre (Verdun, Canada) or were purchased from Bachem California (Torrance, CA). [ahlX25–36] NPY was a gift of Dr. Annette Beck-Sicking, Swiss Federal Institute of Technology (Zurich, Switzerland); 6-nitro-7-sulphamoylbenzof(f)quinoline-2,3-dione (NBQX) was a gift of Dr. Lars Nordholm, Novo Nordisk Pharmaceuticals (Malmö, Denmark). CR231118 was a gift from Dr. Alex Daniels, Glaxo Wellcome (Research Triangle, NC). All other compounds were obtained from Sigma (St. Louis) or BDH (Toronto).

Results

Experiments consisted of 199 drug applications to a total of 96 hippocampal slice preparations. Whole cell recordings were made from 36 pyramidal cells and four interneurons.

0 Mg2+ and picrotoxin bursting

After a change of perfusate to 0 Mg2+ ACSF or picrotoxin ACSF, SBs appeared with latencies of 424 ± 72 s and...
294 ± 99 s, respectively. When recorded from the pyramidal cell body layer, SBs generally consisted of multiple, sharply negative population spikes superimposed on a slow, positive-going wave ~50–250 ms in duration (Fig. 1, insets). The shape of individual discharges was not remarkably different between the first and subsequent bursts; however, the frequency of these events generally tended to increase over a period of 0.5–1.5 h to rates between 6 and 36 per minute for 0 Mg$^{2+}$ and between 3 and 18 per minute for picrotoxin. Peptides were applied only after SB frequency appeared to be stable (i.e., not changing markedly over a period of 10–20 min). For statistical purposes, frequency was calculated by counting the number of SBs for a 60-s period under control conditions and again during the peak peptide effect. The interval was chosen because it is small relative to the duration of action of NPY, and especially of its shorter-acting analogues (this is necessary to accurately represent a “peak” effect), yet still large enough to have a high probability of giving a nonzero count under control conditions. Data were only included if the frequency of the events returned significantly toward control values on washout of the peptide.

0-Mg$^{2+}$-induced SBs. Bath application of NPY (1 μM) reduced the frequency of 0 Mg$^{2+}$-induced SBs recorded extracellularly in stratum pyramidale of area CA3 by 81.22 ± 4.7% (n = 14, P < 0.001, Fig. 1). PYY$_{3-36}$ (1 μM) and [ahx$^{5-24}$] NPY (3 μM), which are agonists with substantial selectivity for the Y$_2$ receptor relative to Y$_1$, inhibited the SB frequency by 63.7 ± 12.7% (n = 4, P < 0.01) and 43.0 ± 6.9% (n = 6, P < 0.001), respectively. The Y$_1$-preferring agonist Leu$^{31}$ Pro$^{34}$ NPY (1 μM) also inhibited burst frequency by 93.9 ± 3.7% (P < 0.001, n = 10), although this effect persisted in the presence of the Y$_2$-selective antagonist GR231118 (1 nM) (Daniels et al. 1995), where Leu$^{31}$ Pro$^{34}$ NPY (1 μM) still caused a 96.8 ± 3.3% reduction in SB frequency (P < 0.001, n = 4).

STIB

The STIB model produces several distinct electrographic events. Within 2 s of the end of a stimulus train applied to stratum radiatum of CA2/CA3 (see METHODS), large-amplitude, spontaneous 1<sup>st</sup>ADs were evident in both extracellular and whole cell patch recordings made from area CA3. These 1<sup>st</sup>ADs were generally clonic or tonic-clonic in nature and ranged from 5.2 to 58.4 s in duration.

In extracellular field recordings, bath application of NPY (1 μM) reduced the duration of the 1<sup>st</sup>ADs by 100 ± 14.8% (P < 0.001, n = 5, P < 0.001, n = 8). Within 2 s of the last NPY application, these 1<sup>st</sup>ADs stopped at lower frequency (5.5 ± 4.8% of control, P < 0.001, n = 5, P < 0.001, n = 8). ATIB was also evident during the application of NPY (1 μM) to CA2/CA3 slices prepared from the control conditions and then again during the peak peptide effect. The interval was chosen because it is small relative to the duration of action of NPY, and especially of its shorter- or duration, as was the case in the 0 Mg$^{2+}$-induced bursts. The Y$_2$-preferring agonists PYY$_{3-36}$ (1 μM) and [ahx$^{5-24}$] NPY (3 μM) inhibited the SB frequency by 100.0 ± 0.0% (P < 0.001, n = 6) and 81.9 ± 11.7% (P < 0.001, n = 7), respectively. The Y$_1$-preferring agonist Leu$^{31}$ Pro$^{34}$ NPY (1 μM) also inhibited burst frequency by 93.9 ± 3.7% (P < 0.001, n = 10), although this effect persisted in the presence of the Y$_2$-selective antagonist GR231118 (1 nM) (Daniels et al. 1995), where Leu$^{31}$ Pro$^{34}$ NPY (1 μM) still caused a 96.8 ± 3.3% reduction in SB frequency (P < 0.001, n = 4).

![Figure 1](http://jn.physiology.org/)

**FIG. 1.** Neuropeptide Y (NPY) reduces the frequency of spontaneous epileptiform bursts. AC-coupled chart records of extracellular field potentials from the pyramidal cell layer of area CA3 show spontaneous bursts (SBs) occurring in the presence of Mg$^{2+}$-free artificial cerebrospinal fluid (ACSF; 0 Mg$^{2+}$, top) and ACSF containing 100 μM picrotoxin (bottom). Insets: enlarged detail of individual bursts in the 0 Mg$^{2+}$ model under control conditions (left) and during NPY effect (right). Neither the shape nor the amplitude of these events is affected by application of 1 μM NPY.
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1.7 AD duration was also dramatically reduced by bath application of agonists that act at the Y₂ receptor, such as PYY (1 μM), which reduced 1.7 AD duration by 99.7 ± 0.3% (P < 0.001, n = 4). Similarly, 1.7 AD duration was inhibited by the Y₂-prefering agonists NPY₁₃₋₃₆ (3 μM, 100 ± 0.00%, P < 0.01, n = 6), [Ahx⁵₋²⁴] NPY (1 μM, 97.6 ± 2.1%, P < 0.001, n = 8), and PYY₁₃₋₃₆ (1 μM, 100.0 ± 0.0%, P < 0.01 n = 2). By contrast, the Y₁-prefering agonist Leu³¹Pro³⁴ NPY (1 μM) reduced 1.7 AD duration in only four of seven experiments, increasing the duration in the remaining three experiments. The average inhibitory effect was 13.1 ± 22.03% (P > 0.25, n = 7, Fig. 2A). The time course of action differed between agonist analogues, with the full peptides (NPY, PYY) having the longest duration of action and the centrally truncated analogue [Ahx⁵₋²⁴] NPY having both the fastest onset and recovery (Fig. 2B).

In extracellular field potential recordings in the STIB model, SBs resembling those seen in the 0 Mg²⁺ and picrotoxin models often began to occur spontaneously after several stimulus trains had been applied to a preparation. The frequency of these SBs was highly variable both within and between preparations, making statistical comparisons difficult. However, when SBs did occur, we consistently observed that their frequency was noticeably or entirely inhibited by bath application of NPY (1 μM, n = 6), PYY (1 μM, n = 2), NPY₁₃₋₃₆ (3 μM, n = 3), and [Ahx⁵₋²⁴] NPY (1 μM, n = 7). Interestingly, Leu³¹Pro³⁴ NPY, while having no effect on the 1.7 ADs, also inhibited the SBs (1 μM, n = 5, Fig. 2A).

Whole cell recordings. Whole cell patch-clamp recordings (see METHODS) were made from CA3 pyramidal neurons in slices prepared for the STIB model. Stimulus trains induced afterdischarges in individual pyramidal cells that resembled those observed in the extracellular field po-

FIG. 2. Effect of NPY receptor agonists on primary ictal afterdischarges (1.7 ADs) and SBs in the stimulus-train-induced bursting (STIB) model. A: extracellular recordings from a single preparation exhibit tonic-clonic (Control, top) or tonic (Recovery, top) 1.7 ADs immediately following stimulus trains (†) applied to stratum radiatum of area CA2/CA3. SBs (*) resembling those seen in 0 Mg²⁺ and picrotoxin models, occur between stimulus train applications and can be seen here preceding the stimulus trains. Bath application of the Y₂-prefering agonist [Ahx⁵₋²⁴] NPY (3 μM, top middle) eliminates the 1.7 AD and SBs. Leu³¹Pro³⁴ NPY (1 μM), a Y₁-prefering agonist (bottom middle), does not inhibit the 1.7 ADs but does inhibit SBs. Diagonal lines: interruptions in traces. B: time course of inhibition of the 1.7 AD by NPY, PYY₁₃₋₃₆, [Ahx⁵₋²⁴] NPY, and Leu³¹Pro³⁴ NPY. 1.7 AD duration was normalized to the duration of the AD immediately preceding agonist application, which is indicated by a bar. Points: means ± SE.
potential recordings described above. However, because of the difficulty of determining response thresholds while maintaining stable whole cell recordings, NPY's effects on afterdischarges were not systematically studied in whole cell patch-clamp recordings.

**SPONTANEOUS, RHYTHMIC SYNCHRONOUS EVENTS.** In whole cell recordings from CA3 pyramidal cells in slices that exhibited 1°ADs in the STIB model, spontaneous, rhythmic synchronous events (SRSEs) (Schwartzkroin and Haglund 1986; also referred to as synchronized synaptic potentials, Traub and Miles 1991) also usually appeared after several stimulus trains had been given and preceded the development of the SBs. The SRSEs appeared as outward currents when the membrane potential was held at about −55 mV in voltage clamp, reversed polarity between −63 and −68 mV, and were inhibited by the noncompetitive GABA, antagonist picrotoxin (100 μM, Fig. 3).

Once SRSEs occurred in a preparation, discontinuing the stimulus trains frequently permitted the appearance and development of inward currents superimposed on, and initially led by, the outward currents. These inward currents generally increased in amplitude and duration over time until they were no longer constrained by the voltage-clamp amplifier, and appeared to be unclamped SBs (Fig. 4), which could also be observed in extracellular field recordings from the same area (data not shown). In many preparations, these bursts would periodically increase in frequency to form clusters resembling the tonic phase of ictal discharges (2°ADs). After a period of relative quiescence following the spontaneous end of such a cluster, SRSEs reappeared and inward currents below action potential threshold, these events rhythmically depolarized the cell membrane, sometimes by 20−30 mV. As the hyperpolarizing current was reduced, each SRSE currents once again developed in their wake (Fig. 4A).

Simultaneous, whole cell patch-clamp recordings from pyramidal neurons in different regions of area CA3 (n = 4 pairs, Fig. 4) show that not only the 1°ADs but also the SRSEs and the spontaneous afterdischarge associated with them are very highly synchronized throughout that population of neurons.

The SRSEs recorded in CA3 pyramidal cells were not measurably affected in frequency or amplitude by bath application of 1 μM NPY (n = 7, Fig. 4, A and B). Because the SRSEs were initially hyperpolarizing, we tested the possible involvement of a hyperpolarization-activated cation current (Halliwell and Adams 1982; Maccaferri et al. 1993) in the coupling between the SRSEs and the SBs. Neither the development of the inward currents nor their coincidence with the SRSEs was prevented by bath application of 2 mM CsCl (n = 2, not shown). Likewise, no consistent change in SRSEs was caused by bath application of 50 μM APV (NMDA receptor antagonist, n = 4); however, they were completely and reversibly inhibited by the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptor antagonist NBQX (1 μM, n = 2, Fig. 5).

**INTERNEURONS.** To determine the origin of the SRSEs, whole cell patch-clamp recordings were made from CA3 interneurons (n = 4) identified by electrophysiological properties (Lacaille et al. 1989), location, and, in one cell, the inclusion of 0.2% Lucifer yellow in the pipette solution (not shown). In these cells, events occurred with the same time course as those observed in pyramidal cell recordings, including the appearance of 1°ADs, SRSEs, and paroxysmal depolarizations (Fig. 6). The most noticeable difference between these and pyramidal cell recordings was that in voltage-clamped interneurons, SRSEs were inward currents at holding potentials of −56 mV, with reversal potential estimated to be near −9 mV (Fig. 6, C−E). In current-clamp mode, when DC was passed to hyperpolarize the interneurons below action potential threshold, these events rhythmically depolarized the cell membrane, sometimes by 20−30 mV. As the hyperpolarizing current was reduced, each SRSE became capable of initiating an action potential (not shown).

SRSEs recorded in interneurons were not measurably affected by bath application of NPY (1 μM, n = 4), APV (50 μM, n = 2), or the metabotropic glutamate receptor antagonist MCPG (100 μM, n = 1) (McBain et al. 1994; Taylor et al. 1995). As in pyramidal cell recordings, however, these rhythmic events were completely and reversibly inhibited by bath application of 1 μM NBQX (n = 3, Fig. 6E).

**DISCUSSION**

In this study we used three different in vitro models of epileptiform activity to test whether NPY was capable of controlling the synchronous population discharges produced by different mechanisms. NPY, acting predominantly through Y2 receptors, was effective in reducing spontaneous and evoked excitability in all three models.

**0 Mg**

In the 0 Mg**2+** model, omission of Mg**2+** from the extracellular medium removes its physiological blockade of NMDA receptors, increasing the excitation mediated by glutamate (Sah et al. 1989). In the picrotoxin model, GABA_A receptors are blocked, reducing endogenous inhibition (Hablitz 1984). Both models exhibit spontaneous, synchronous bursts of neuronal activity that can readily be observed with extracellular recording electrodes placed in stratum pyramidale of area CA3. The complex waveform of these bursts is similar
FIG. 4. Simultaneous whole cell voltage-clamp recordings of 2 pyramidal neurons in CA3a/b and CA3b/c, illustrating STIB-related events throughout the CA3 region. A: continuous chart record of 2 neurons held at potentials indicated on top traces. Timing traces below recordings: chart speed (upward ticks, 1 s; downward ticks, 10 s). Application of a stimulus train (15 trains, see METHODS) resulted in a primary afterdischarge recorded in both neurons. Responses were synchronous. Between stimulus trains, SRSEs appeared. Voltage steps (potentials indicated near 2nd and 4th sets of traces) demonstrate that SRSE reversal potential was between $-53$ and $-63$ mV for the upper cell and between $-57$ and $-67$ mV for the lower cell. After the 4 afterdischarges shown, stimulus trains were discontinued and SBs were allowed to develop. Application of NPY abolished the occurrence of the SBs without affecting the frequency, appearance, or reversal potential of the outward currents. B: expanded view of recording segments obtained at time points indicated by asterisks in A. Note that the gradual development of inward currents superimposed on or closely following outward currents is abolished by the application of NPY.

in time course and shape to those observed to occur between seizures in human epilepsy patients and in animal models of epilepsy. These events have therefore been used as an in vitro model of interictal discharges (McNamara 1994). NPY reduced the frequency of these events without affecting their shape. Both $Y_2$- and, to a lesser extent, putative $Y_1$-receptor-
NPY INHIBITS EPILEPSY IN RAT HIPPOCAMPAL SLICES

receptor may be mediating the effects of Leu-Pro-NPY observed here.

All NPY agonists tested show greater inhibition of burst frequency in the picrotoxin model compared with the 0 Mg²⁺ model. This difference might be accounted for by the difference in burst frequencies seen in the two models. Because the measurement used involves counting the number of bursts in a 60-s interval, it is more likely that an incomplete inhibition of burst frequency will produce a burst-free 60-s interval in a preparation that had fewer bursts per minute under control conditions.

STIB model

AFTERDISCHARGES. The STIB model has been widely used as an acute in vitro model of electrographic seizure and epileptogenesis (Rafiq et al. 1993; Slater et al. 1985; Stasheff et al. 1985). A train or trains of high-frequency stimuli (such as those used in kindling studies), when applied to the stratum radiatum of area CA2/CA3, excites axons in that region, including the recurrent CA3 collateral projections that are thought to be important in the synchronization of activity seen in this region (MacVicar and Dudek 1980; Wong and Traub 1983). This model has already been shown to respond to anticonvulsants at concentrations close to those found clinically effective (Clark and Wilson 1995; Clark et al. 1992).

The 1st AD that immediately follows the stimulus trains is similar in appearance to the electrographic activity that occurs during seizures in human epilepsy patients and in vivo animal epilepsy models (McNamara 1994). The duration of the 1st AD is sensitive to inhibition by NPY and that

One possible explanation for this observation is the recently reported Y₅ receptor, which also has a high affinity for Leu-Pro-NPY (Gerald et al. 1996b). Some regions of the hippocampus, notably area CA3, the dentate hilus, and the dentate granule cell layer, demonstrate moderate but significant expression of Y₅ receptor mRNA (Gerald et al. 1996a,b). Given the affinity of Leu-Pro-NPY for this receptor subtype and the presence of mRNA for the receptor in CA3 and dentate gyrus, it is distinctly possible that this receptor may be mediating the effects of Leu-Pro-NPY observed here.

FIG. 4. (continued)

preferring agonists were also able to produce this effect; however, several lines of evidence suggest that the Y₁ receptor is not involved. GR231118, which is a potent and selective Y₁ receptor antagonist (Leban et al. 1995) in dissociated rat dentate granule cells (A. R. McQuiston and W. F. Colmers, unpublished data), but which does not inhibit Y₂-mediated responses at CA3-CA1 synapses (Colmers, unpublished observations), was unable to block the inhibition caused by the reportedly selective Y₁ receptor agonist Leu-Pro-NPY in our models. Leu-Pro-NPY has been shown to have a relative potency of <1:1000 compared with NPY in Y₂-receptor-containing tissues such as pig spleen (Krstenansky et al. 1990) and human SK-N-BE2 cells (Wahlestedt et al. 1990, 1992). Thus it is unlikely that Leu-Pro-NPY is acting via a Y₂ receptor in this preparation. As in dentate granule cells, NPY also causes an inhibition of N-type Ca²⁺ channels in acutely dissociated rat superior cervical ganglion cells. The agonist profile in these cells suggests that it does so via activation of receptors that are neither Y₁ nor Y₂ (Foucart et al. 1993). Although it is possible that a Y₃ receptor is mediating the burst frequency reduction caused by Leu-Pro-NPY, it must also be noted that no distinct Y₃-receptor-mediated (i.e., NPY-sensitive but PYY-insensitive) response has yet been observed in the rat hippocampal slice preparation.

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FIG. 5. Whole cell voltage-clamp recording of NPY-isolated rhythmic outward currents in a CA3b pyramidal cell. Expansions of points indicated by numbers under trace are shown at bottom. Holding potential: −55 mV. 1: outward currents isolated by 1 µM NPY. 2: i.e., 2-amino-5-phosphonovaleric acid (APV, 50 µM) caused a slight decrease in the duration and increase in the frequency of these events. 3: further application of 6-nitro-7-sulfamoylbenzo(1)quinazoline-2,3-dione (NBQX, 1 µM) caused a complete, reversible inhibition of the outward currents.
FIG. 6. Whole cell voltage- and current-clamp recordings from an interneuron in area CA3 in the STIB model. A: 1′AD following a stimulus train (bar) is similar in appearance to those recorded from pyramidal neurons. B: in voltage clamp, these rhythmic events appear as inward currents at potentials positive and negative to rest. C: plot of mean SRSE amplitude (from data illustrated in B) vs. holding potential. Reversal potential was calculated to be near −9 mV. D: paroxysmal depolarization shift recorded in current clamp. During the paroxysmal depolarization shift, the rhythmic events (†) reverse polarity, but their frequency does not seem to be altered. E: rhythmic excitatory events are insensitive to bath application of 1 μM NPY, 50 μM APV, or the metabotropic glutamate receptor antagonist (+)-α-methyl-4-carboxyphenyl-glycine (MCPG, 100 μM). NBQX (1 μM) completely inhibits the events.

this inhibition is mediated through the Y2 receptor, as evidenced by the fact that NPY, PYY, [αhxy3−28] NPY, and NPY13−36 are all able to drastically shorten or completely inhibit the AD, whereas the Y1- (and Y5-) receptor-prefering agonist Leu31-Pro34NPY is unable to do so.

SBS in STIB. Like the 1′ADs, the SBSs that occur in the STIB model are sensitive to Y2 receptor activation. As with 0 Mg2+ and picrotoxin-induced bursts, however, they are also somewhat inhibited by Leu31-Pro34NPY. This is, to our knowledge, the first means by which SBSs and the 1′ADs can be pharmacologically differentiated, which may prove useful in further studies of the pathways and mechanisms responsible for ictal and interictal behavior. A clue to how this occurs may be found in the origin of the different kinds of electrical events and the distribution of NPY receptor subtypes. In examining a 0 Mg2+ model of seizure, Lewis et al. (1990) reported that although electrographic discharges
the entorhinal cortex. Y1 receptors are particularly numerous capable of inhibiting synchronous excitatory discharges arising from the dentate gyrus, then progressed through CA3, CA1, and the entorhinal cortex. Y1 receptors are particularly numerous in the inner third of the molecular layer of the dentate gyrus; however, there is no evidence that they affect evoked post-synaptic potentials anywhere in the rat hippocampus (Colmers et al. 1991).

Activation of Y1 receptors causes a reduction in Ca2+ influx through N-type Ca2+ channels in the soma and dendrites of dentate granule cells (McQuiston et al. 1996). This action may reduce the Ca2+-dependent release of the inhibitory peptide dynorphin from the granule cells (Neumaier et al. 1988). Whether this is how Y1-preferring agonists inhibit SBs is unknown. It is also possible that the activation of Y1 receptors is sufficient to inhibit the occurrence of SBs but not to interrupt activity of the magnitude present during a stimulus train and subsequent afterdischarge. Alternatively, Leu3-Pro3-NPY, the Y1 agonist used in this study, may be acting at a receptor type other than Y1, as it seems to be in the picrotoxin bursting model. In any case, the differential distribution of the NPY receptor subtypes and their impact on the different forms of spontaneous electrical activity support the observations that the initiation of SBs and afterdischarges may involve different neuronal populations and projection pathways within the hippocampus.

Synaptically mediated events similar to those we describe here were reported to occur in epileptic human and normal monkey temporal lobe (Schwartzkroin and Haglund 1986). Whether or not they are associated with the pathology of epilepsy was unclear from that report, however, because the “normal” monkey temporal lobe reported was actually tissue contralateral to an epileptic focus and may therefore not have been entirely normal (Wyler et al. 1975).

Several factors may contribute to the development or occurrence of SRSEs in our preparations. For example, our experimental protocol differs from that of others in several ways. Because NPY’s actions in hippocampal slices are so prolonged (Colmers et al. 1988), they do not reverse within the lifetime of the slice if the peptide is applied for more than a few minutes. To accommodate a shorter agonist application period, stimulus trains were delivered at either 4- or 5-min intervals, rather than the 10-min intervals used by others (Raﬁq et al. 1993; Stasheff et al. 1985). Perhaps more importantly, the whole cell patch-clamp recordings used in this study allowed us to observe events that may be undetectable in extracellular field or intracellular sharp electrode recordings (Hamill et al. 1981; Staley et al. 1992).

In this study we observed that SRSEs are dependent on AMPA receptor activation. Greber et al. (1994) observed that NPY inhibits K+-stimulated glutamate release from hippocampal slices. Furthermore, 1 μM NPY has also been shown to inhibit glutamatergic transmission in CA3 by 45–55% (Klapstein and Colmers 1993), and yet the present data clearly show that NPY does not inhibit SRSEs in any measurable way. Therefore the glutamatergic drive resulting in SRSE expression must come from afferent inputs that are insensitive to NPY and that thus differ from those innervating pyramidal cells. The question remains as to where this afferent input originates. It may be relevant that the excitatory synaptic inputs to dentate granule cells in the rat are also insensitive to the actions of NPY (Klapstein and Colmers 1993). The present results clearly show that exogenous NPY is capable of inhibiting synchronous excitatory discharges arising in three mechanistically different models of epilepsy in the in vitro hippocampus. The question remains whether this is the biological role of the peptide. In addition to the present data, several observations support this concept. NPY is found in varicosities, thought to represent release sites, situated next to glutamatergic presynaptic terminals in the hippocampus (Milner and Veznedaroglu 1992). The peptide and its receptors are thus matched to the task of inhibiting glutamatergic transmission. Interneurons containing NPY receive collateral excitation from the axons of principal cells (La-caille et al. 1989), indicating that their activity would be regulated by that of the principal cells, and could provide feedback inhibition of local glutamatergic input in an activity-dependent manner. Finally, NPY knockout mice are prone to seizure (Erickson et al. 1996), suggesting that the absence of NPY is proconvulsant. It is probable that NPY subserves a more subtle physiological role in the hippocampus than the prevention of seizures. However, the present results support the concept that, within its biological role, endogenous NPY may well act to limit excitability within the hippocampus. On the basis of the present results, hippocampal NPY receptors may also be considered as potential targets for anticonvulsant therapy.

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