Y5 Receptors Mediate Neuropeptide Y Actions at Excitatory Synapses in Area CA3 of the Mouse Hippocampus

HUI GUO, PETER A. CASTRO, RICHARD D. PALMITER, and SCOTT C. BARABAN

INTRODUCTION

Brain peptides participate in our perceptions of pain, pleasure, and appetite (Bean et al. 1994). Over the last five years, evidence has accumulated that one such peptide, neuropeptide Y (NPY), acts as an endogenous anticonvulsant. First, brain NPY levels are increased following acute seizure activity (Marksteiner et al. 1990; Spiker et al. 1992; Stenfors et al. 1992; White and Gall 1987). Second, seizure activity up-regulates Y2/Y5 NPY receptor expression and down-regulates Y1 receptor (Y1R) expression in limbic structures (Kofler et al. 1997). Third, NPY inhibits epileptic discharge in a number of in vitro seizure preparations (Klapstein et al. 1999; Vezzani et al. 2000). Fourth, NPY knock out mice display mild seizure-like behaviors, are more susceptible to seizures induced by a GABA antagonist (pentyleneetrazol), and die in response to glutamate analogue kainic acid–evoked seizures (Baraban et al. 1997; Erickson et al. 1996).

Although these results provide strong support for the role of NPY as an endogenous anticonvulsant, precise receptor mechanisms responsible for these actions are not clear. In the hippocampus, NPY is prominently expressed by inhibitory GABAergic interneurons (Hendry et al. 1984) and is thought to inhibit calcium-mediated glutamate release (Greber et al. 1994). Exogenous NPY reduces field excitatory postsynaptic potentials (fEPSPs) elicited in CA1 or CA3 regions of a hippocampal slice (Klapstein and Colmers 1993). These effects can be mimicked by a Y2R-preferring agonist, NPY (Colmers et al. 1991). NPY or Y2R-preferring agonists, NPY (a Y2R-preferring agonist) reduces the frequency of spontaneous EPSCs but had no effect on sEPSC amplitude, rise time, or decay time. Furthermore, NPYergic modulation of spontaneous EPSCs in WT mice was mimicked by bath application of a novel YSR-selective peptide agonist ([cpp]hPP) but not the selective Y2R agonist (a [ahx5–24]NPY). In situ hybridization was used to confirm the presence of NPY, Y2, and Y5 mRNA in the hippocampus of WT mice and the absence of Y5R in knockout mice. These results suggest that the Y5 receptor subtype, previously believed to mediate food intake, plays a critical role in modulation of hippocampal excitatory transmission at the hilar-to-CA3 synapse in the mouse.


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unable to inhibit seizure activity in these studies indicating a requirement for Y5Rs but not Y2Rs. Furthermore, NPY application does not inhibit zero-magnesium–induced epileptiform activity in hippocampal slices from Y5R knockout (KO) mice but NPY, YNP$_{13-36}$, and hPP are equally effective in abolishing epileptiform activity in slices from wild-type (WT) mice (Marsh et al. 1999). Because high concentrations of NPY agonists (0.5–3 μM) are required to suppress seizure activity in vivo and inhibit excitability in hippocampal slices, it is possible that many NPY receptor subtypes are simultaneously activated in these studies. The lack of selective NPY receptor antagonists further complicates the pharmacological approach. Genetic inactivation of NPY receptors provides an alternative means of assessing the requirement of specific receptors in mediating NPY actions in the CNS. Here we present evidence, using KO mice and novel receptor-selective agonists, that NPYergic modulation of excitatory synaptic transmission onto CA3 pyramidal neurons requires a Y5R.

**METHODS**

**Animals**

Y5R KO mice on an inbred 129/Sv background were generated using homologous recombination techniques as described by Marsh et al. (1998) and maintained in a breeding colony at UCSF. WT 129/Sv mice were purchased from Jackson Laboratories. Young adult [postnatal day 15–20 (P15–P20)] mice were used for all experiments. All animal procedures complied with the National Institutes of Health Guidelines and were approved by the UCSF Committee on Animal Research.

**Slice preparation**

Hippocampal slices were prepared from young adult mice. The animals were decapitated under ketamine/xylazine anesthesia; their brains were rapidly removed in ice-cold sucrose artificial cerebrospinal fluid (ACSF) (Baraban et al. 1997) and bubbled continuously with 95% O$_2$–5% CO$_2$. Horizontal sections of the hippocampus (300 μm thick) were obtained using a Campden Instruments vibroslicer (Model NVSLM1). The resulting slices were transferred immediately to a gas-interface style (field recordings) recording chamber. Slices were then transferred to a gas-interface style (field recordings) recording chamber. Stimuli, and stimulation levels were then set at 1.5 times threshold for generation of a single population spike where they remained for the duration of the study. Whole cell recordings were made from CA3 pyramidal neurons, which were identified under an IR-DIC microscope (Olympus BX50WI). Patch pipettes (1–5 MΩ) filled with the following internal solution were used (in mM): 135 CsCl, 10 NaCl, 2 MgCl$_2$, 10 HEPES, 10 EGTA, 2 Na$_2$ATP, and 0.2 Na$_2$GTP (pH 7.2, 285–290 mOsm). Cells were held at −75 to −85 mV, and recordings were obtained with an Axopatch 1D amplifier, filtered at 1 kHz and digitized at 10 kHz (Axon Instruments 1320 A/D board running pClamp 8.0 software, Axon Instruments, Foster City, CA). Cells were allowed to stabilize for approximately 5 min after establishing the whole cell recording configuration. To obtain low-noise recordings, cell access resistance (range: 5–17 MΩ) was monitored frequently (but not compensated) by measuring the amplitude of the capacitative transient during a 10-mV depolarizing voltage step. Cells were not included for EPSC analysis if this value changed by >15% during the recording period. All cells had resting membrane potentials between −60 and −75 mV measured in current-clamp mode.

sEPSCs were recorded in the presence of 5–10 μM bicuculline to block the postsynaptic inhibitory currents caused by activation of GABA$_A$ receptors. Kynurenate application (100 mM) at the conclusion of each recording eliminated sEPSCs, indicating that these currents were glutamatergic. The amplitudes and frequencies of spontaneous synaptic events varied to some extent from cell to cell. TheMini Analysis Program (Synaptosoft, Leonia, NJ) was used to detect spontaneous or miniature EPSCs on the basis of amplitudes exceeding a threshold set just above the baseline noise of the recording, and kept constant throughout the analysis. All detected events were reexamined visually and either rejected or accepted. Events were included that had times-to-peak between 100 and 1,000 μs, decay times between 3 and 25 ms, and minimum peak amplitudes of 3 pA. For each cell we analyzed between 100 and 200 individual EPSC events. The program then measured amplitudes, frequencies, and decay time constants. For cumulative probability plots, statistical analysis for each neuron was performed using the Kolgomorov-Smirnov nonparametric test. Distributions were considered different in P < 0.01. All other data are presented as the means ± SE and were analyzed using a one-way ANOVA with Neuman-Keuls post hoc comparisons or Student’s t-tests. Unless otherwise indicated, significance was taken as P < 0.05.

**In situ hybridization**

Transcription of plasmids containing cDNAs of interest was performed with RNA polymerase (Roche, Basel) in the presence of digoxigenin-labeled uracil triphosphate (UTP) (Roche). Riboprobes included NPY, Y2 receptor, and Y5 receptor. Probes were hydrolyzed to 250 bp prior to use. Nonradioactive in situ hybridization of hippocampal tissue sections (P15–P16) was performed using a protocol obtained from S. Pleasure (University of California, San Francisco) (Kim et al. 2001).

**Drugs**

NPY (porcine), peptide YY (human), pancreatic polypeptide (human), NPY$_{13-36}$ (human), [d-Trp$^2$]NPY 1–36 (human), and [Leu$^3$Pro$^4$]NPY (porcine) were purchased from American Peptide Company (Sunnyvale, CA). [ahx$^5$]NPY and [cpp$^1$]NPY$_{19-23}$, A$^3$,Aib$^5$,Q$^9$] human pancreatic polypeptide were a generous gift of Dr. Annette Beck-Sickinger. Peptide stock solutions were prepared in distilled water and kept frozen at −20°C until immediately prior to use. 6-Cyano-7-nitroquinoline-2,3-dione (CNQX), di-2-amin-5-phosphonovaleric acid (d-APV), baclofen, and all other compounds were obtained from Sigma (St. Louis, MO).

**RESULTS**

**NPY effects on evoked activity**

NPY application reduces the fEPSP evoked by a stimulation electrode placed in either Schaffer collateral or mossy fiber pathways in rat hippocampal slices (Bleakman et al. 1992; Colmers et al. 1988; Klapstein and Colmers 1993). The inhibitory effect of NPY can be mimicked by NPY$_{13-36}$ or PYY$_{3-36}$.
suggesting the involvement of a Y2R subtype (Colmers et al. 1991; Klapstein and Colmers 1993; Qian et al. 1997). To determine whether similar actions are observed in mouse hippocampus, we examined the effect of peptide application in slices from WT mice. Experiments were performed in the hilar-CA3 region because high concentrations of NPY-immu-

FIG. 1. Neuropeptide Y (NPY) inhibits CA3 field excitatory postsynaptic potentials (fEPSPs). A: schematic of the hippocampal slice showing the location of stimulation and recording electrodes. B: hippocampal tissue section from a wild-type (WT) mouse stained with an antibody that recognizes NPY. C: representative fEPSP traces before (black) and 20 min after application of 1 μM NPY (red). Note the inhibition of fEPSP amplitude in a slice from a WT mouse (top set of traces) but the lack of effect in a slice from Y5 receptor knockout (Y5R KO) mice (bottom set of traces). Stimulation artifacts are clipped (*). D: plot of fEPSP amplitude vs. time for a typical NPY experiment; slice from WT mouse (●) and Y5R KO mouse (○). Traces in C were taken at the time indicated (arrow).

FIG. 2. NPYergic inhibition of CA3 fEPSPs requires a Y5R. A: representative fEPSP traces before (black) and 20 min after application of an NPY agonist (gray). Note the peptide-induced inhibition of fEPSP amplitude in slices from WT animals but the lack of effect in slices from Y5R KO mice. B: plot of fEPSP amplitudes following NPY agonist application for all slices from WT (black bars) and Y5R KO mice (gray bars). Data are presented as means ± SE. Each bar represents (7–10 slices). C: plot of the fEPSP amplitudes following drug application in slices from Y5R KO mice. Each drug was bath applied approximately 20 min after NPY agonist application.
noreactive neurons are located in this area of hippocampus (Gray and Morley 1986; Morris 1989) (Fig. 1, A and B). Bath application of 1 μM NPY significantly inhibited the amplitude of CA3 fEPSPs in hippocampal slices from WT mice (Fig. 1C). Comparable levels of fEPSP inhibition were observed with 0.25 and 0.5 μM NPY (n = 5). Inhibition of fEPSP amplitude was observed within 20 min of perfusion and was not readily reversible (Fig. 1D), as reported (Klapstein and Colmers 1993). Similar effects were observed with bath application of 1 μM peptide YY (PYY), an agonist at Y1, Y2, Y4, and Y5 receptor subtypes. Bath application of 1 μM NPY13–36, a Y2R-prefering agonist, in hippocampal slices from WT mice (Fig. 2A) mimicked the inhibition of fEPSP amplitude observed with the full agonists NPY and PYY (Fig. 2B). Application of 1 μM [Leu31Pro34]NPY, a Y1 receptor–preferring agonist, did not cause a significant change in the CA3 fEPSP in hippocampal slices from Y5R KO mice (Fig. 1, C and D). The peptide agonists PYY, NPY13–36, hPP, [β-Trp12]NPY and [Leu31Pro34]NPY were also unable to significantly alter CA3 fEPSPs in slices from Y5R KO mice (Fig. 2, A and B). One explanation for these findings would be a developmental alteration of the CA3 excitatory synapse induced by Y5R inactivation. Such an alteration could result in an inability to modulate synaptic transmission in these genetically altered mice. To test this possibility in Y5R KO mice, we applied drugs with demonstrated inhibitory actions at excitatory hippocampal synapses (Ault and Nadler 1983; Klapstein and Colmers 1992; Okada and Ozawa 1980). Following application of a peptide agonist (10- to 40-min exposure) and a 10- to 15-min washout/recovery period, drugs were applied. Bath application of 100 μM adenosine or 1 μM 2-chloroadenosine (A1 adenosine receptor agonist) significantly inhibited fEPSP amplitudes in hippocampal slices from Y5R KO mice (Fig. 2C). Similarly, 10 mM GABA or 10 μM baclofen (GABA_B receptor agonist) also inhibited mossy fiber fEPSPs recorded in slices from these animals (Fig. 2C). Drug effects were rapid (<15-min exposure) and reversible. Thus it is possible to modulate excitatory synaptic transmission in mice lacking the Y5R. Taken together, these results suggest that NPY inhibits excitatory transmission at CA3 synapses via activation of hippocampal Y5 receptors.

**NPY effects on spontaneous activity**

NPY application inhibits the frequency, but not the amplitude, of spontaneous EPSCs recorded from CA3 pyramidal...
neurons in rat hippocampal slices (McQuiston and Colmers 1996). The inhibitory effect of NPY can be mimicked by the Y2R-selective agonist [ahx\(^{5-24}\)]NPY. To determine whether similar actions are observed in mouse hippocampus, we examined the effect of peptide application in slices from WT mice. sEPSCs were isolated from inhibitory PSCs by the addition of 5–10 μM bicuculline to the bathing medium. sEPSCs reversed around 0 mV and were abolished by the addition of glutamate receptor antagonists (10 μM d-APV and 50 μM CNQX) to the bathing medium (Fig. 3, C and D). Bath application of 1 μM NPY significantly reduced sEPSC frequency (Figs. 3A and 5C). Similar effects were observed in other CA3 neurons with bath application of 1 μM PYY, 1 μM NPY\(^{13-36}\), 1 μM [d-Trp\(^{24}\)]NPY, or 1 μM hPP (Fig. 5, A and C). EPSC properties were not altered by application of a Y1R-selective agonist, 1 μM [Leu\(^{31}\)Pro\(^{34}\)]NPY (Fig. 5, C and E). Quantitative analysis of ≥100 EPSC events revealed that NPY agonists significantly reduced frequency but had no effect on EPSC amplitude (Fig. 5E), rise time, or decay time constant (Table 1). To further assess the effects of NPY in WT mice, we constructed cumulative probability plots for sEPSC interevent interval and amplitude. A representative analysis is shown in Fig. 3B. Note that NPY shifts the sEPSCs to larger interval ranges with no effect on amplitude.

To further examine NPYergic inhibition of excitatory synaptic transmission, we analyzed miniature EPSCs in the presence of 1 μM TTX (Fig. 4). TTX presumably eliminates EPSCs arising from presynaptic impulses. The cumulative interval and amplitude distributions of mEPSCs recorded in baseline and NPY were compared for CA3 pyramidal neurons in slices from WT mice (n = 4). Bath application of 1 μM NPY did not alter the frequency (Fig. 4B) or the amplitude (Fig. 4C) distribution of mEPSCs. The effects of NPY on group means for mEPSC intervals and amplitudes for all cells are shown in Fig. 4, D and E, respectively. Similar to findings in rat hippocampal slices (McQuiston and Colmers 1996), neither measure was significantly altered by NPY.

We next examined the effect of NPY agonists on sEPSCs recorded from CA3 pyramidal neurons in hippocampal slices from Y5R KO mice. Whole cell voltage-clamp recording experiments were conducted under the identical recording conditions used to study NPY effects in WT mice. Similar to our findings with CA3 field EPSPs, NPY and NPY receptor–preferring peptide agonists did not cause a significant change in the properties of sEPSCs recorded on

<table>
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<th>Peptide</th>
<th>Rise Time, ms</th>
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<tr>
<td></td>
<td>Control</td>
<td>Agonist</td>
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<tr>
<td>NPY</td>
<td>2.2 ± 0.05</td>
<td>2.0 ± 0.06</td>
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<tr>
<td>PYY</td>
<td>2.7 ± 0.07</td>
<td>2.4 ± 0.05</td>
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<td>13-36</td>
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<td>[d-Trp(^{24})]NPY</td>
<td>1.9 ± 0.10</td>
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<td>hPP</td>
<td>2.1 ± 0.03</td>
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<td>ahx</td>
<td>2.2 ± 0.07</td>
<td>2.3 ± 0.91</td>
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<td>[cpp(^{4})]PP</td>
<td>1.9 ± 0.11</td>
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Data are presented as means ± SE. Statistical comparisons were made using a Student’s t-test; P > 0.05 for all values. NPY, neuropeptide Y; EPSC, excitatory postsynaptic current; WT, wild-type.

CA3 pyramidal cells in these mice (Fig. 5B, 1–3; Table 1). To further illustrate a role for Y5 receptors in modulating hippocampal excitability at CA3 synapses, we tested two novel NPY receptor–selective peptide agonists, [ahx\(^{5-24}\)]NPY preferentially binds Y2 receptors, and [cpp\(^{4}\)]PP is selective for the Y5R subtype (Beck-Sickingler et al. 1992; Cabrele et al. 2000). In hippocampal slices from WT mice, bath application of 1 μM [ahx\(^{5-24}\)]NPY had no effect on sEPSCs recorded on CA3 pyramidal neurons (Fig. 6A). However, 1 μM [cpp\(^{4}\)]PP significantly reduced sEPSC frequency in a reversible manner (Fig. 6, B and C). A ramp command protocol was used to demonstrate that [cpp\(^{4}\)]PP had no effect on the membrane properties of CA3 pyramidal neurons (Fig. 6C). Because NPY agonists altered spontaneous EPSC frequency and not amplitude, did not alter CA3 membrane properties, and had no effect on miniature EPSCs, our results are consistent with the interpretation that NPY inhibits the presynaptic, Ca\(^{2+}\)-mediated release of glutamate onto CA3 neurons.

**NPY and NPY receptor expression**

Because genetic manipulation may alter neurodevelopment or cause compensation, we next examined NPY and NPY receptor expression patterns using in situ hybridization techniques. In hippocampal sections from control WT mice, NPY mRNA was found in interneurons in stratum oriens-alveus,

![FIG. 4. NPY does not affect mini EPSCs on CA3 pyramidal neurons. A: representative miniature EPSCs (mEPSCs) traces during baseline and approximately 5 min after perfusion with normal artificial cerebrospinal fluid containing 1 μM NPY. B: cumulative probability plot of the distribution of mEPSC intervals for a representative experiment; 1 μM NPY does not alter the distribution of mEPSC intervals. C: cumulative probability plot of the distribution of mEPSC amplitude for a representative experiment; 1 μM NPY does not alter the distribution of mEPSC amplitude. D: group mean mEPSC intervals (data from 4 neurons) were not significantly affected by NPY application compared with baseline. E: group mean mEPSC amplitudes (data from 4 neurons) were not significantly affected by NPY application compared with baseline. Data are presented as means ± SE.](http://jn.physiology.org/DownloadedFrom/10.1152/jn.00935.2001)
stratum lacunosum-moleculare, and the dentate hilus. A similar pattern of NPY expression was observed in hippocampal sections from age-matched Y5R KO mice. In both animals, NPY mRNA levels were highest in the dentate hilus (Fig. 7). In WT mice, moderate levels of Y2R and Y5R mRNA were found in neurons of CA1-CA3 and granule cells of the dentate gyrus (Fig. 7). Y2R mRNA expression patterns were comparable in hippocampal section from age-matched Y5R KO mice. Consistent with previous Northern blot analysis (Marsh et al. 1998), we did not observe Y5R mRNA in hippocampal sections from Y5R KO mice (Fig. 7). Thus the lack of NPYergic inhibition of excitatory synaptic transmission in Y5R KO mice

FIG. 5. NPY agonists inhibit sEPSC amplitude in slices from WT mice but not Y5R KO. A1: representative recordings of sEPSCs for CA3 pyramidal neurons in WT animals under baseline recording conditions (top, black traces) and 3 min after NPY agonist application (bottom, gray traces). B1: same for CA3 pyramidal neurons in Y5R KO mice. A2: group mean sEPSC frequencies for CA3 pyramidal neurons in WT mice. A3: group mean sEPSC amplitudes for CA3 pyramidal neurons in WT mice. B2 and B3: same for CA3 pyramidal neurons recorded in slices from Y5R KO mice. Data are presented as means ± SD. Each bar represents 5–15 cells. * Significance taken as $P < 0.05$ using a Student’s t-test.
Our data also indicate that commercially available NPY peptide receptor agonists (NPY13–36, hPP, and [d-Trp32]NPY) may not be adequate tools for assessing the role of specific NPY receptor subtypes in vitro.

One of the most thoroughly characterized CNS actions of NPY is its inhibition of excitatory hippocampal synaptic transmission (Bleckman et al. 1992; Colmers et al. 1987, 1988, 1991; Ho et al. 2000; Klapstein and Colmers 1993; McQuiston and Colmers 1996; Qian et al. 1997). NPY is believed to inhibit presynaptic glutamate release at hippocampal excitatory synapses (Colmers et al. 1991; Greber et al. 1994; Klapstein and Colmers 1993). Bath application of NPY at micromolar concentrations inhibits fEPSPs, extracellularly recorded population spikes, intracellularly recorded EPSPs, and sEPSCs at CA1 and CA3 synapses in both mouse and rat hippocampus. NPY-mediated inhibition of excitatory synaptic transmission was observed in slices pretreated with 4-aminopyridine (Klapstein and Colmers 1992), suggesting that presynaptic A-type potassium channels are not required. These actions were not associated with a change in the postsynaptic membrane properties of CA1 or CA3 pyramidal neurons (Ho et al. 2000; McQuiston and Colmers 1992) and were associated with an inhibition of presynaptic calcium monitored using a fluorescent Ca2+ indicator (Qian et al. 1997). To explore the receptor subtypes mediating the inhibitory actions of NPY, pharmacological studies have been performed with peptide fragments exhibiting a higher affinity for one receptor subtype versus another subtype. Using a strictly pharmacological approach, investigators have concluded that NPYergic inhibition of excitatory synaptic transmission in rat hippocampal slices is mediated by a Y2R (Colmers et al. 1991; McQuiston and Colmers 1996; Qian et al. 1997). The limitation of this approach is that peptide fragments bind NPY receptors with low nanomolar affinities and therefore can be classified as “receptor preferring” but are not strictly receptor selective. For example, NPY13–36, a widely used Y2R peptide fragment, exhibits the following receptor specificity: EC50 values for inhibition of forskolin-stimulated [cAMP] (in nM): 300 at Y1R, 2.2 at Y2R, >1,000 at Y4R, and 20 at Y5R (Gerald et al. 1996). Because peptides poorly penetrate tissue slices, in vitro studies are commonly performed at 1 μM peptide fragment concentrations, and it is likely that putative receptor-selective “agonists” activate multiple receptor subtypes (despite receptor selectivity in cell culture assays) in these studies. In light of this discussion, it should not be surprising that the NPYergic inhibition of fEPSPs and sEPSCs at CA3 synapses observed in hippocampal slices from WT mice could be mimicked by bath application of putative Y2R (1 μM NPY13–36) or Y5R (1 μM hPP or [d-Trp32]NPY) peptide agonists.

A surprising result was that no inhibition of CA3 fEPSP amplitude or CA3 sEPSC frequency was observed with application of NPY in hippocampal slices from Y5R KO mice, suggesting a critical role for the Y5 receptor subtype at this synapse. In our experiments, NPY’s inability to inhibit fEPSPs in Y5R KO mice was not associated with a general alteration of the excitatory CA3 synapse as application of adenosine or GABA potently inhibited fEPSP amplitude. The lack of effect of NPY (or peptide analogues) cannot be explained by a loss of Y2 receptors as in situ hybridization revealed comparable Y2R densities in hippocampal CA1 and CA3 regions of WT and Y5R KO mice. This is evident by the lack of effect of NPY (and a variety of NPY peptide fragments) on evoked or spontaneous excitatory synaptic transmission in hippocampal slices from mice lacking Y5 receptors.

**Discussion**

Our results demonstrate a strict requirement for Y5 receptors in mediating the modulatory actions of NPY at hilar-to-CA3 excitatory hippocampal synapses in the mouse. This is evident by the lack of effect of NPY (and a variety of NPY peptide agonists) on evoked or spontaneous excitatory synaptic transmission in hippocampal slices from mice lacking Y5 receptors. The inability to inhibit fEPSPs in Y5R KO mice cannot be explained by a loss or change in the expression of hippocampal Y2 receptors.
mRNA expression patterns between WT and Y5R KO mice. Note the absence of Y5R expression in Y5R KO mice. CA1, CA1 pyramidal; CA3, CA3 pyramidal; GC, dentate granule cells. Scale bar, 200 μM.

FIG. 7. NPY and NPY receptor expression in the mouse hippocampus. Nonradioactive in situ hybridization for probes as labeled in the figure (left). Note that NPY expression is most abundant in the dentate hilus. This is consistent with NPY immunostaining shown in Fig. 1. WT mice express both Y2 and Y5 receptors in GC, CA3, and CA1 cell body regions. NPY and Y2R expression patterns are similar for WT and Y5R KO mice. Note the absence of Y5R expression in Y5R KO mice. CA1, CA1 pyramidal; CA3, CA3 pyramidal; GC, dentate granule cells. Scale bar, 200 μM.

In summary, we have demonstrated that NPY inhibits fEPSPs and sEPSCs on CA3 pyramidal neurons in the mouse hippocampus and that these actions require Y5 receptors. Hippocampal NPY expression is dramatically up-regulated following a seizure (Marksteiner et al. 1990; Sperk et al. 1992; Stenfors et al. 1992; White and Gall 1987), and exogenous NPY application can exert powerful anticonvulsant actions (Bijak 1999; Klapstein and Colmers 1997; Marsh et al. 1999; Woldbye et al. 1997) that are likely to be mediated via NPYergic inhibition of excitatory synaptic transmission. Thus our findings may have important implications both for our understanding of brain peptide physiology and in the development of novel antiepileptic medications based on activation of hippocampal Y5 receptors.

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References


McQuiston AR and Colmers WF. Neuropeptide Y2 receptors inhibit the frequency of spontaneous but not miniature EPSCs in CA3 pyramidal cells of rat hippocampus. J Neurophysiol 76: 3159–3168, 1996.


