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Up-Regulation of Neuropeptide Y-Y_2 Receptors in an Animal Model of Temporal Lobe Epilepsy

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ABSTRACT

Receptor autoradiography with the Y_2 receptor ligand ^{125}I-peptide $YY_{3–36}$ and *in situ* hybridization were applied to investigate changes in neuropeptide tyrosine-Y_2 receptor expression after kainic acid-induced recurrent seizures in the rat hippocampus. In the strata oriens and radiatum of CA1 to CA3, which are densely innervated by Y_2 receptor-bearing Schaffer collateral terminals, a transient 2-fold increase in Y_2 receptor affinity was observed after 4–12 hr, with a later slow decline. No change was seen in Y_2 mRNA expression in CA2/CA3 pyramidal cells, from which Schaffer collaterals originate. Conversely, in granule cells of the dentate gyrus, markedly elevated Y_2 mRNA concentrations were observed (by 740% in the dorsal hippocampus) 24–48 hr after kainate injection. At the same time, a marked and lasting (up to 6 months) increase in the number of Y_2 receptor sites (by 800%) was seen in the dentate hilus, which is innervated densely by mossy fibers. The early increase in Y_2 receptor affinity in Schaffer collaterals was accompanied by a 60% decrease in the EC_{50} of peptide $YY_{3–36}$ in inhibiting K^+-stimulated glutamate release in hippocampal slices from kainic acid-treated rats. Our data indicate transient up-regulation of presynaptic Y_2 receptors in Schaffer collaterals by a change in affinity and a permanent *de novo* synthesis of presynaptic Y_2 receptors in granule cells/mossy fibers. These changes may cause augmented presynaptic inhibition of glutamate release from different hippocampal sites and, in conjunction with increased concentrations of neuropeptide tyrosine in mossy fibers, may represent an endogenous reactive anticonvulsant mechanism.

Systemic injection of KA in the rat causes severe convulsions, seizure-induced brain damage, increased seizure susceptibility, and, after several weeks, spontaneous recurrent seizures (Ben-Ari, 1985; Sperk, 1994). These sequelae closely resemble those of human temporal lobe epilepsy. Therefore, KA-induced epilepsy has become a widely accepted animal model for this neurological disorder (Ben-Ari, 1985; Nadler, 1981). In this animal model, as in the human condition, the dentate gyrus of the hippocampus is presumed to be intimately involved in the generation of epileptic activity and exhibits characteristic neurochemical and histopathological changes (Sperk, 1994). Within granule cells of the dentate gyrus, immediate-early genes, neurotrophins, and various neuropeptides become strongly expressed during and after KA-induced seizures [for a review, see Sperk (1994)]. The expression of most of these molecules is transient. However, a persistent increase in the concentrations of NPY and its mRNA is seen in granule cell/mossy fibers (Bellmann *et al.*, 1991; Sperk *et al.*, 1992), and we recently proposed that this may represent a possible endogenous anticonvulsant mechanism (Greber *et al.*, 1994; Sperk *et al.*, 1992).

It is well accepted that in the hippocampus, NPY-Y_2 receptors are located mainly presynaptically on terminals of Schaffer collaterals (Dumont *et al.*, 1996; Haas *et al.*, 1987). They are prejunctionally innervated by NPY/GABA neurons (Haas *et al.*, 1987; Milner and Veznedaroglu, 1992) and mediate presynaptic inhibition of glutamate release from Schaffer collaterals (Colmers *et al.*, 1991; Greber *et al.*, 1994; Haas *et al.*, 1987). The same receptors seem to be minimally expressed on mossy fiber terminals (Dumont *et al.*, 1996; Röder *et al.*, 1996) .

We recently observed increased NPY receptor binding in various hippocampal subfields of rats subjected to KA-induced seizures (Röder *et al.*, 1996). To investigate these changes in NPY receptor binding in detail, we applied *in situ* hybridization to study Y_2 receptor mRNA expression and determined kinetic parameters (K_d and B_{max}) for the binding of ^{125}I-$PYY_{3–36}$, a specific Y_2 receptor agonist, in the terminal areas of Schaffer collaterals (strata oriens/radiatum of CA1) and mossy fibers (hilus of the dentate gyrus). Furthermore, we investigated the changes in Y_2 receptor-mediated presynaptic inhibition of glutamate release from hippocampal slices *ex vivo* obtained 4 hr after KA injection.

Experimental Procedures

Materials. All buffer substances and salts, glucose, paraformaldehyde, acetic anhydride, formamide, acetonitrile, Entellan, and the HPLC columns (LiChrospher 100RP-18, 5 μm, 125 $\times$ 3 mm, for

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ABBREVIATIONS: KA, kainic acid; GABA, γ-aminobutyric acid; SSC, standard saline citrate; NPY, neuropeptide tyrosine; PP, pancreatic polypeptide; PYY, peptide tyrosine-tyrosine; HPLC, high performance liquid chromatography.

determination of glutamate and LiChrospher 100RP-8, 5 μm, 250 × 4 mm, for separation of radioiodinated peptides) were purchased from Merck (Darmstadt, Germany). KA, the constituents of the hybridization buffer (Ficoll, polyvinylpyrrolidone, bovine serum albumin, salmon sperm DNA, yeast tRNA, dithiothreitol, dextran sulfate), and bacitracin were obtained from Sigma Chemical (St. Louis, MO). Bovine serum albumin, chloramine T, and cresyl violet originated from Serva (Heidelberg, Germany). Oligonucleotides were custom synthesized by Microsynth (Balgach, Switzerland). NPY, $NPY_{13–36}$, [D-Trp^{32}]NPY, PYY, $PYY_{3–36}$, [Pro^{34}]PYY, and PP (all analogs of the human sequence) were purchased from Neosystems (Strasbourg, France). BIBP 3226 [N2-(diphenylacetyl)-*N*-[(4-hydroxyphenyl)methyl]-D-argininamide] was a gift from Dr. A. Zimmer (Dr. Karl Thomae GmBH, Biberach, Germany). Terminal deoxynucleotidyltransferase was purchased from Boehringer-Mannheim Biochemicals (Mannheim, Germany). [^{35}S]α-Thio-dATP (1300 Ci/mmol; NEG 034H) and ^{125}I (2200 Ci/mmol; NEZ 033L) were obtained from DuPont-New England Nuclear (Boston, MA). The ^{125}I-microscales and βmax films for autoradiography were from Amersham (Buckinghamshire, UK). NTB-2 photoemulsion and photographic developer (D19) were from Kodak (Rochester, NY).

KA injection. Male Sprague-Dawley rats (250–350 g; Forschungsinstitut für Versuchstierzucht, Himberg, Austria) were housed at a constant temperature (23°) and relative humidity (60%) with a fixed 12-hr light/dark cycle and unlimited access to food and water. Procedures involving animals and their care were conducted in compliance with national laws and policies (EEC Council Directive 86/609, OJ L 358, 1, 12 December 1987; National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, NIH Publication No. 85–23, 1985). The experiments were performed with the consent of the Committee for Animal Protection of the Austrian Ministry of Science.

Rats were injected with 10 mg/kg KA intraperitoneally in buffered saline or with the corresponding amount of saline. As described in detail previously, KA initiated an acute behavioral syndrome that ranges from early staring, "wet dog shakes," and seizures from mild forehead nodding to severe limbic convulsions with rearing and foaming at the mouth (Sperk *et al.*, 1983). Rats were observed for ≥3 hr, and their behavior was rated as described previously (Sperk *et al.*, 1983). Animals exhibiting sustained limbic seizures (rating, 3–4; occurring in >80% of rats) were included in the studies. Depending on the experiment, rats were decapitated after different time intervals (4, 6, or 12 hr; 1, 2, or 7 days; and 1 and 6 months after KA injection). For *in situ* hybridization and receptor autoradiography, brains were rapidly removed from the skulls, divided by coronal cuts into three parts, immersed into cold isopentane (−40°C for 90 sec), and stored in sealed counting vials at −70°. For release studies, the hippocampi were dissected immediately on removal of the brains.

Brain sections. For *in situ* hybridization and Y_2 receptor autoradiography, 20-μm-thick coronal sections of the dorsal hippocampus and horizontal sections of the ventral hippocampus were cut and thaw-mounted onto gelatin-coated slides. The sections were kept desiccated at −30° until their use in the respective experiments.

***In situ* hybridization.** Two different synthetic oligonucleotide DNA probes were used (GAGTGAATGGCATCCAACCTCTGCTCACAGCGGAAGGCTGAGAGG and TGCTTGGAGATCTTGCTCTCCAGGTGGTAGACAATGCAACGATGTCGGTCC). The probes were complementary to different sequences of the rat Y_2 receptor mRNA (Dr. H. Herzog, personal communication, 1997) and highly homologous to the bases 1020–1064 and 451–501 of the recently published mouse cDNA, respectively (Nakamura *et al.*, 1996). Essentially, the same results were observed with both probes; data obtained with the later one are shown. The oligonucleotides were labeled with [^{35}S]α-thio-dATP (1300 Ci/mmol) via reaction with terminal deoxynucleotidyltransferase and precipitated with ethanol/sodium chloride. Matching sections from the same portion of the hippocampus of KA-treated rats and controls were assayed together. Frozen sections were rapidly immersed into 2% paraformaldehyde in phosphate-buffered saline (150 mM NaCl in 10 mM phosphate buffer, pH 7.2) for 10 min at room temperature, rinsed in phosphate-buffered saline, immersed in 0.25% acetic anhydride in 0.1 M triethylamine hydrochloride for 10 min, dehydrated by ethanol series, and delipidated with chloroform. They were hybridized at 42° for 18 hr with ≈50 fmol (1–1.5 × 10^6 cpm) labeled oligonucleotide probe in 50 μl of hybridization buffer consisting of 50% formamide, 5× SSC (1× SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.2), 500 μg/ml salmon sperm DNA, 250 μg/ml yeast tRNA, 1× Denhardt's solution (0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 10% dextran sulfate, and 20 mM dithiothreitol. At the end of the incubation, the slides were briefly rinsed twice in 1× SSC/50% formamide. They were washed four times in 50% formamide in 2× SSC (42° for 15 min), rinsed in 1× SSC, and dipped briefly in water. The sections were then dipped in 70% ethanol, dried, and exposed to βmax films for 2 weeks. Subsequently, the slides were dipped in Kodak NTB-2 photosensitive emulsion (diluted 1:1 with distilled water), air-dried, and exposed for 6 weeks. The βmax films and dipped slides were developed with Kodak D19 developer. Sections were counter-stained superficially with cresyl violet, dehydrated, cleared in xylol, and coverslipped with Entellan. The corresponding radiolabeled sense DNA was used to exclude nonspecific hybridization of the probe. Sections prehybridized for 2 hr with an excess (1 nmol) of unlabeled probe were included as additional controls in some experiments.

Preparation of ^{125}I-$PYY_{3–36}$. $PYY_{3–36}$ was radiolabeled according to the procedure of Hunter and Greenwood (1962) as described previously in greater detail for NPY (Bellmann *et al.*, 1991; Dumont *et al.*, 1995). Briefly, 10 μg of $PYY_{3–36}$ was allowed to react with 1 mCi of ^{125}I (2200 Ci/mmol), 30 μg of chloramine T in 10 μl of H_2O, and 60 μl of 0.5 M phosphate buffer, pH 7.0, at room temperature for 45 sec. The reaction was stopped by the addition of 100 μl of 10% bovine serum albumin. The resulting mixture was separated on a C8 reverse-phase column (LiChrospher 100RP-8, 5 μm, 250 × 4 mm) by HPLC, which was eluted with 0.1 M tetraethyl-formiate buffer, pH 2.5, and acetonitrile (application of a linear gradient from 25% to 40% during 55 min) at a flow rate of 1.0 ml/min. Two major radioactive peptide peaks were recovered at 30% and 32% acetonitrile; they exhibited similar binding properties, and aliquots of the first peak were used. Because the terminal tyrosine becomes preferentially iodinated and migrates early on HPLC (Sheikh *et al.*, 1989), the peak may contain monoiodinated [Tyr^{36}]^{125}I-PYY.

^{125}I-$PYY_{3–36}$ receptor autoradiography. Receptor binding was performed as described by Dumont *et al.* (1996). Slides containing either one brain section (time course study) or three sections placed on the same slide (kinetic analysis and displacement studies) were thawed and preincubated for 30 min at room temperature in 200 ml of Krebs-Henseleit-Tris buffer (118 mM NaCl, 4.8 mM KCl, 1.3 mM $MgSO_4$, 1.2 mM $CaCl_2$, 50 mM glucose, 15 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 10 mM Tris, pH 7.3). The incubation was performed in Coplin jars containing 20 ml of the same buffer supplemented with 0.1% bovine serum albumin, 0.05% bacitracin, and ^{125}I-$PYY_{3–36}$. Time course studies were performed at 25 pM ^{125}I-$PYY_{3–36}$. Kinetic analysis of the receptor binding was performed in sections of the dorsal hippocampus of controls and 6 or 48 hr after KA injection. ^{125}I-$PYY_{3–36}$ was used at concentrations of 2.5, 5, 10, 15, and 25 pM. Displacement studies were performed at 25 pM ^{125}I-$PYY_{3–36}$. Thirty or 100 nM concentration of PYY, $PYY_{3–36}$, $NPY_{13–36}$, [D-Trp^{32}]NPY, [Pro^{34}]PYY, or PP or 5 or 15 μM concentration of the Y_1 antagonist BIBP 3226 was included in the incubation medium. Incubations were performed at room temperature for 2 hr. Unspecific binding was determined in the presence of 1 μM NPY; it was uniformly distributed throughout the section and was <5% (e.g., in Schaffer collaterals). The sections were dipped twice and then washed for 30 sec in ice-cold Krebs-Henseleit-Tris buffer, dipped in deionized water, and rapidly dried under a stream of cold air. They were exposed to βmax films for 10 days together with ^{125}I-microscales.

Quantification of *in situ* hybridization and receptor autoradiography and statistics. The autoradiograms were developed,

digitized through the Appligene Image System (Illkirch, France), and analyzed using the public domain NIH Image program (written by Wayne Rasband at the National Institutes of Health and available from the Internet by anonymous FTP from zippy.nimh.nih.gov). For quantification of mRNA signals, absorbance was quantified in the strata granulosum and pyramidale. For each rat, values obtained for both sides were averaged. Data are given as mean values obtained from 3–6 animals per time interval and 12 age-matched control animals. For binding experiments, absorbance was measured in the strata oriens and radiatum of CA1 and in the hilus of the dentate gyrus. Absolute values were calculated by using a dose-response curve of the absorbance obtained through concomitant autoradiography of ^{125}I-microscales and expressed as fmol/mg of wet tissue weight. Specific binding was calculated by subtracting the unspecific binding, as determined in the presence of 1 μM NPY, from total binding. For the time course study, mean values obtained from pairs of hippocampi were averaged for each time interval after KA injection (3–6 rats/interval) and from 12 age-matched control animals killed concomitantly with the experimental animals. For displacement studies, mean values determined in three to six sections per animal were averaged. These values were used for calculating the mean values for 5 rats. Data are expressed as percentage of ^{125}I-PYY$_{3–36}$ binding in the presence of the respective unlabeled peptide analog of control binding obtained in the absence of cold ligands using sections from the same rats. Statistical analysis was done for time course and displacement studies by analysis of variance and the multiple-comparison Dunnett *posteriori* test.

Kinetic evaluation was performed separately for each animal using Scatchard blot analysis for the strata oriens and radiatum CA1 and the dentate hilus. For each concentration of ^{125}I-PYY$_{3–36}$, binding was determined in nine coronal sections of the dorsal hippocampus per animal (three sections per slide). Values of individual sections were obtained by calculating the mean values of each hemisphere. Mean unspecific binding for each ligand concentration was assessed in 35 sections and deducted. K_d and B_{max} values were determined individually for each animal using regression analysis. Data were averaged, and a two-tailed Student's t test was used for statistical comparison.

Cell counts were performed to evaluate relative cell loss in interneurons of the hilus of the dentate gyrus, CA1, CA3a, and CA3c pyramidal neurons. Counts were performed in 20-μm Nissl-stained sections of the dorsal hippocampus in areas of 25,000 μm^2 (CA1 and CA3a) and 100,000 μm^2 (hilus and CA3c), respectively.

Superfusion of hippocampal slices. Superfusion experiments were performed as described previously (Greber *et al.*, 1994). Control and KA-treated rats (4 hr after treatment) were killed by decapitation. Their brains were rapidly removed and transferred into ice-cold Krebs-Henseleit-Tris buffer (118 mM NaCl, 4.8 mM KCl, 1.3 mM $MgSO_4$, 1.2 mM $CaCl_2$, 15 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 10 mM Tris, 10 mM glucose, adjusted to pH 7.3 by gassing with 95% O_2/5% CO_2). Hippocampi were dissected keeping brains immersed in chilled buffer. Slices (300 μm) of the septal extension of the hippocampus were obtained using a McIllwain tissue chopper and placed in superfusion chambers at 34° (Greber *et al.*, 1994). They were superfused at a flow rate of 1.0 ml/min with the above buffer and continuously gassed with 95% O_2/5% CO_2. Glutamate release was provoked by increasing the K^+ concentration to 45 mM for 90 sec while the Na^+ concentration was correspondingly reduced. Slices were stimulated twice (after 50 and 100 min, respectively). PYY$_{3–36}$ was included at concentrations of 0, 1, 3, 10, 30, or 100 nM together with 0.01% bovine serum albumin in the superfusion medium 8 min before and during the second K^+ pulse. Fractions of 1.0 ml were collected, lyophilized, and used for determination of glutamate. Brain slices were finally sonicated in 1.0 ml of 0.1 M $HClO_4$. This homogenate was centrifuged and the supernatants were diluted 1:80 with 0.4 M $NaBO_4$ buffer, pH 8.4, for determination of tissue glutamate. Pellets were solubilized in 100 μl of 0.5 M NaOH (20 min, 90°) and assayed for protein content (Lowry *et al.*, 1951) .

Determination of glutamate by HPLC. Glutamate was determined by HPLC and fluorimetric detection after precolumn derivatization with *o*-phtaldialdehyde as described in detail previously (Greber *et al.*, 1994). Basal release rates were defined by the mean glutamate concentrations of two fractions before and two fractions after stimulation. Potassium stimulated glutamate release was calculated by the glutamate concentration in the two fractions obtained during stimulation with high K^+ and subtraction of basal release. The ratio of potassium stimulated glutamate release during the second and the first stimuli (S_2/S_1) was calculated. Dose-response curves and EC_{50} values were calculated by sigmoidal analysis using the computer program Origin 4.1.

Results

Behavioral and neuropathological changes. On injection of KA, rats exposed a typical limbic seizure syndrome, characterized by early staring, "wet dog shakes," and seizures ranging from mild forehead nodding to severe limbic convulsions with rearing and foaming at the mouth, as described in more detail previously (Sperk, 1994; Sperk *et al.*, 1983). Maximal seizure activity was observed in >80% of the rats between 1 and 2 hr after KA injection and declined thereafter. Physically, the rats recovered entirely after ≈1 week. Rats exhibiting the full seizure syndrome were included in the study. These rats exposed typical neurodegenerative changes in the hippocampal formation (Beu-Ari, 1985; Sperk, 1994). Two days after injection of KA, neurodegeneration was evident by loss of interneurons of the dentate hilus (by 68 ± 9.9%; mean ± standard error) and pyramidal cells in CA1 (by 38 ± 12.5%), CA3a (by 53 ± 8.8%), and CA3c (by 75 ± 9.9%). At the early intervals (4–6 hr after KA injection), neuronal damage was not detected.

***In situ* hybridization of Y_2 receptor mRNA.** In control rats, Y_2 receptor mRNA was expressed preferentially in the pyramidal cell layers of CA3 and CA2 of the dorsal and ventral hippocampus (Figs. 1, 2, and 3). Although the transcript was essentially absent in the CA1 pyramidal neurons and in granule cells of the dorsal hippocampus, it was observed in the respective neurons of the ventral hippocampus, indicating a septotemporal gradient of Y_2 mRNA expression. After KA injection, high concentrations of Y_2 receptor mRNA were found in granule cells of both extensions of the hippocampus (Figs. 1 and 2). Expression of the receptor mRNA was maximally increased after 24 hr in the dorsal (by ≈740%; Fig. 3) and after 48 hr in the ventral hippocampus (by ≈190%). It declined slowly thereafter but remained significantly elevated even 30 days after injection of the convulsant (Fig. 3). In the ventral hippocampus, the increase in Y_2 receptor mRNA in granule cells was preceded by an initial decrease (by 73% of control) in message 4–6 hr after KA injection (Figs. 1 and 2). In CA1, slight and transient expression of Y_2 receptor mRNA was detected after 24–48 hr (Fig. 1). No Y_2 receptor mRNA was detected in interneurons of the hippocampus.

Characterization of Y_2 receptor binding by displacement studies. Displacement studies using various peptide analogs at concentrations of 30 and 100 nM are summarized in Table 1. Although PYY, NPY$_{13–36}$, and PYY$_{3–36}$ potently inhibited ^{125}I-PYY$_{3–36}$ binding at a concentration of 30 nM, moderate (by 40–45%) inhibition was seen with [Pro^{34}]PYY and PP only at 100 nM. [D-Trp^{32}]NPY and the nonpeptidergic

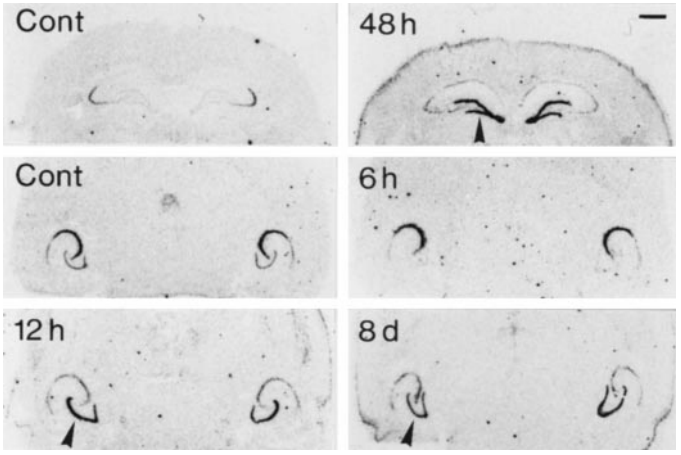

Fig. 1. Film autoradiograms of *in situ* hybridization of Y_2 receptor mRNA in the dorsal (*top*) and ventral hippocampus (*center* and *bottom*) are shown for controls (*Cont*) and at various intervals after KA injection. In controls, Y_2 receptor mRNA is highest in CA2/CA3. In the ventral hippocampus, the transcript is also detected in CA1 and the stratum granulosum. *Arrowheads*, marked increase in Y_2 receptor mRNA in the granule cell layer after 12 and 48 hr (*h*) in the ventral and dorsal hippocampus, respectively, which is still present after 8 days (*d*) (shown for the ventral hippocampus). *Scale bar*, 2 mm.

Y_1 receptor antagonist BIBP 3226 (at concentrations of 5 and 15 μM) did not significantly inhibit ^{125}I-PYY$_{3-36}$ binding.

Time course of changes in Y_2 receptor binding. In controls, Y_2 receptor binding was observed at high concentrations in the strata oriens and radiatum of CA1 to CA3 (Fig. 4). Considerably lower concentrations were observed in all other areas of the hippocampus, including the hilus and the molecular layer of the dentate gyrus. Only a thin band in the supergranular zone of the inner molecular layer of the dentate gyrus was labeled.

In agreement with our previous experiments using full-length ^{125}I-PYY (Roden *et al*., 1996), a rapid and pronounced increase in binding of the Y_2 receptor specific agonist ^{125}I-PYY$_{3-36}$ was observed in the strata oriens and radiatum of CA1 to CA3 (Figs. 3 and 4 for stratum oriens in the dorsal hippocampus). In this area, binding was maximally elevated 4–12 hr after KA injection (by 80–100%; Fig. 3) and decreased (by 50%) below control levels after 1 month. In the hilus of the dentate gyrus, ^{125}I-PYY$_{3-36}$ binding increased more slowly but dramatically. It was maximally elevated after 24–48 hr, as shown in Fig. 3 for the dorsal hippocampus. Although essentially absent in the hilus of controls, the extent of ^{125}I-PYY$_{3-36}$ binding was then comparable to that in the strata oriens and radiatum (Figs. 3 and 4). At later intervals, receptor binding decreased also in the hilus but remained elevated 3-fold even 6 months after KA injection (Fig. 3). After 8–30 days, binding in the stratum lucidum (presumably located on mossy fiber terminals) remained increased despite its decline in the adjacent stratum radiatum. Although in the molecular layer of the dentate gyrus of controls Y2 receptor binding was restricted to the inner molecular layer, moderate binding was detected throughout the molecular layer 1–2 days after KA injection (Fig. 4).

Kinetic analysis of Y_2 receptor binding. The distinctly different expression of Y_2 receptor mRNA in granule cells versus CA3 pyramidal neurons and the different time courses of changes in Y_2 receptor binding in the hilus compared with the strata radiatum and oriens suggest different mechanisms of Y_2 receptor up-regulation in granule cells/mossy fibers and in CA3 pyramidal neurons/Schaffer collaterals. To investigate this in more detail, we conducted kinetic analysis of ^{125}I-PYY$_{3-36}$ receptor autoradiography 6 hr after KA injection in the strata oriens and radiatum of CA1 (representing terminal areas of Schaffer collaterals) and after 48 hr in the hilus of the dentate gyrus (a terminal area of mossy fibers that is not overlapping with Schaffer collaterals). Six

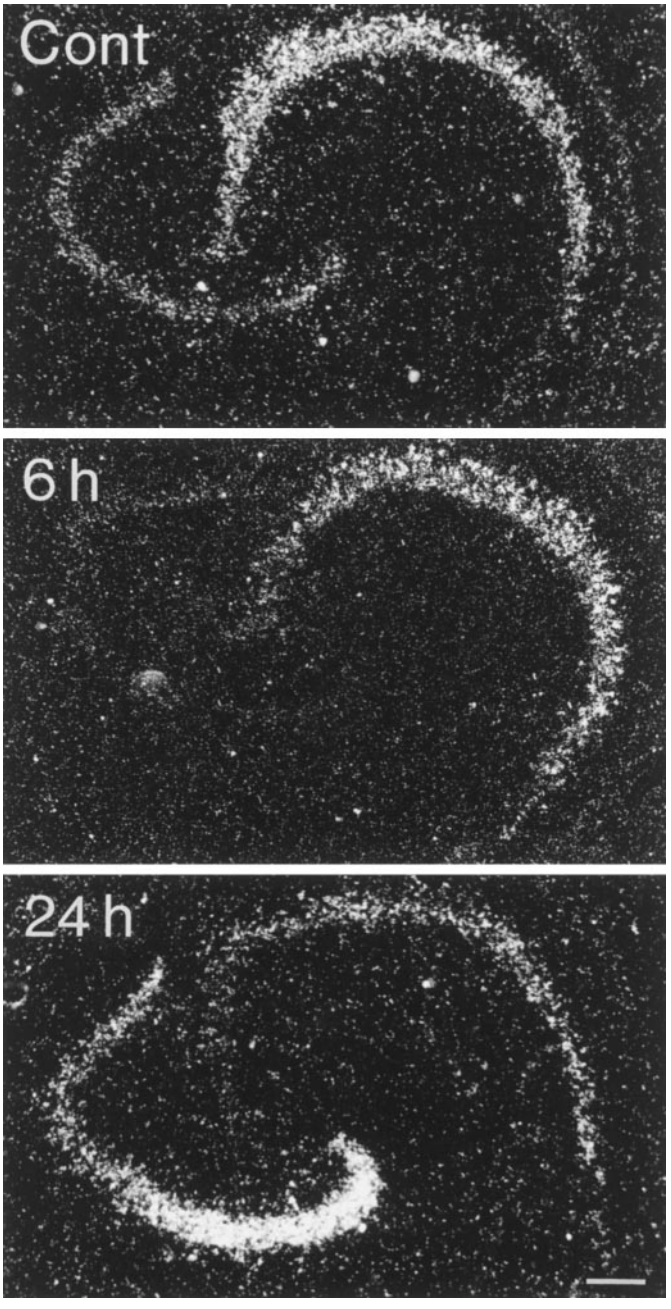

Fig. 2. High-magnification, dark-field photographs of photoemulsion-dipped sections of the ventral hippocampus after *in situ* hybridization of Y_2 receptor mRNA. In controls (*Cont*), the highest mRNA concentrations are present in the pyramidal cell layer of CA2/CA3. Lower concentrations are found in CA1 and the granule cell layer. At 6 hr (*h*) after KA-induced seizures, a reduction in Y_2 receptor mRNA is seen in the granule cell layer. At later intervals (24 hr), a marked increase in mRNA is seen in the same cells. At this interval, there is more message present in the supra-pyramidal than in the infrapyramidal blade of the stratum granulosum. *Scale bar*, 20 μm.

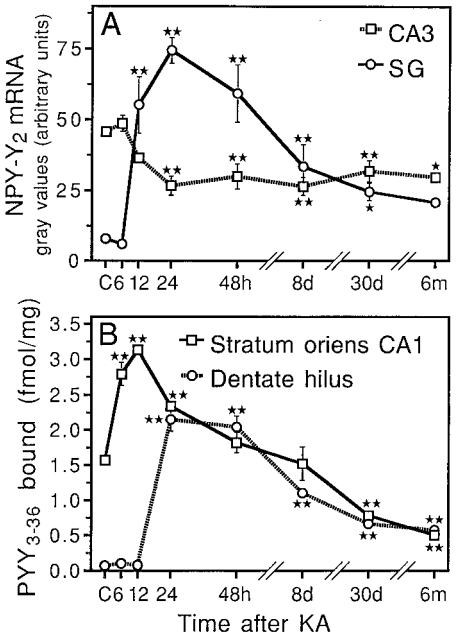

Fig. 3. Time course of Y_2 receptor mRNA expression and ^{125}I-$PYY_{3–36}$ receptor binding after KA-induced seizures. A, Changes in Y_2 receptor mRNA expression for granule and CA3 pyramidal cells of the dorsal hippocampus. Film autoradiograms were evaluated from three to six experimental animals per time interval and 12 age-matched control animals. Note the strong increase in Y_2 receptor mRNA in the stratum granulosum after 12–24 hr (*h*), which persisted (although less prominently) up to 6 months (*m*). Mostly due to seizure-related neurodegeneration, concentrations of Y_2 mRNA become decreased in CA3 pyramidal neurons at later intervals. A similar time course of changes was observed in the ventral hippocampus (data not shown). B, ^{125}I-$PYY_{3–36}$ receptor binding for the stratum oriens of CA1 and in the hilus of the dentate gyrus. Note the fast but transient increase in Y_2 receptor binding in stratum oriens (presumably located prejunctionally on Schaffer collaterals). In the dentate hilus, ^{125}I-$PYY_{3–36}$ binding presumably is located on mossy fibers (see Discussion); it increases with some delay and remains elevated up to 6 months. *In situ* hybridization and ^{125}I-$PYY_{3–36}$ receptor binding were performed in sections of the same animals (three to six experimental animals per interval and 12 age-matched control animals). $^{**}p < 0.01$, $^{*}p < 0.05$, compared with control animals by analysis of variance and a Dunnett *posteriori* test.

TABLE 1

Displacement of [^{125}I]$PYY_{3–36}$ binding by various NPY receptor ligands

Peptide analogs to human sequences were used. Values represent the mean ± standard error for five animals (determined in three to six different sections) and are shown as percentage of binding obtained in the presence versus that in the absence of the respective peptide analog. Data are shown for binding in the stratum oriens of control rats. Essentially the same values were obtained in the stratum oriens 4 hr and in the dentate hilus 1 day after KA injection.

Receptor ligand	[^{125}I]$PYY_{3–36}$ binding	
	30 nM	100 nM
	% of control	
PYY	20 ± 8.1[a]	<5[a]
$PYY_{3–36}$	17 ± 4.0[a]	5 ± 2.8[a]
$NPY_{13–36}$	27 ± 8.3[a]	<5[a]
PP	88 ± 7.3	61 ± 8.5[a]
[D-Trp^{32}]NPY	108 ± 14.8	76 ± 6.6
[Pro^{34}]NPY	104 ± 6.4	56 ± 9.6[a]
BIBP 3226	103 ± 13.1	85 ± 8.8

[a] $p < 0.01$ versus control animals by analysis of variance with a Dunnett *posteriori* test.

hours after KA injection, a 2-fold increase in the affinity of ^{125}I-$PYY_{3–36}$ binding without a significant change in the number of receptor sites was observed in CA1 (Fig. 5A; Table 2). The change in K_d was transient and returned to control

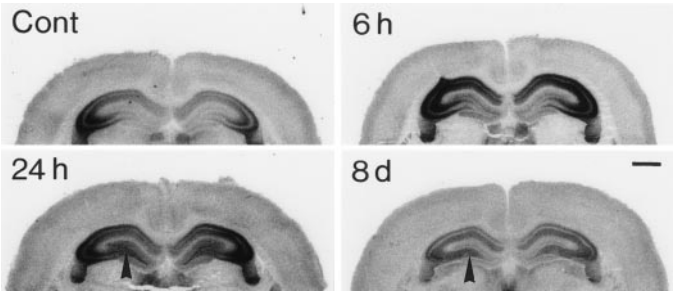

Fig. 4. Y_2 receptor autoradiography was performed using 25 pM ^{125}I-$PYY_{3–36}$ as radioligand. Receptor binding was enhanced in the strata radiatum and oriens of CA1 to CA3 6–24 hr after KA injection. *Arrowheads*, lasting increase in binding in the hilus of the dentate gyrus, as shown for 24 hr (*h*) and 8 days (*d*) after KA injection. *Cont*, controls. *Scale bar*, 2 mm.

levels after 2 days (data not shown). In contrast, in the hilus of the dentate gyrus, a marked (9-fold) increase in the number of Y_2 receptor sites was detected 2 days after KA injection (Fig. 5B; Table 2). Binding affinity was unchanged in this area.

Altered Y_2 receptor-mediated inhibition of glutamate release. This experiment was designed to investigate whether the increased Y_2 receptor affinity may result in increased efficacy of a Y_2 receptor agonist to inhibit glutamate release from Schaffer collaterals. The experiments were done in slices of the dorsal hippocampus from rats 4 hr after KA injection where they contained essentially no detectable Y_2 receptor mRNA or Y_2 receptor binding in the granule cell/mossy fiber system (Figs. 3 and 4). Thus, modulation of

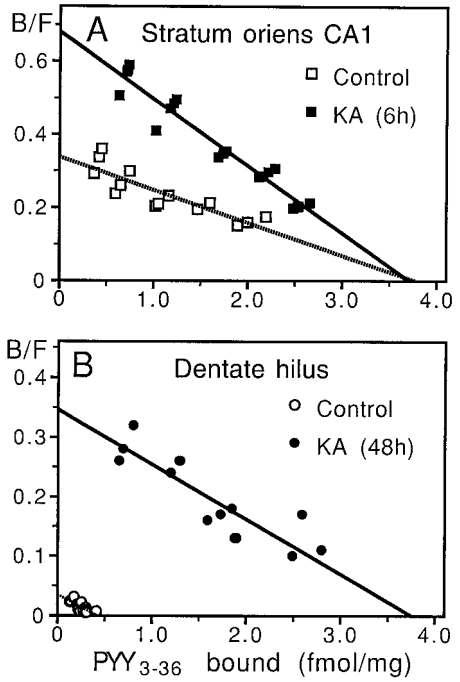

Fig. 5. Kinetic analysis of receptor autoradiography of ^{125}I-$PYY_{3–36}$ binding to Y_2 receptors. Binding was evaluated at various concentrations of the radioligand (A) in the stratum oriens of CA1 (representing binding in Schaffer collaterals) 6 hr (*h*) after KA injection (identical results were obtained for the stratum radiatum) and (B) in the hilus of the dentate gyrus (representative for presumable binding to mossy fibers) after 48 hr and the respective control rats. For details, see Experimental Procedures; for statistics, see Table 2.

TABLE 2

Kinetic parameters of $[^{125}I]PYY_{3–36}$ binding after KA-induced seizures

Kinetic analysis was calculated from autoradiograms of sections of the dorsal hippocampus incubated at five different concentrations of $[^{125}I]PYY_{3–36}$ (see Experimental Procedures). Data are expressed as mean ± standard error. The number of animals is given.

	K_d		B_{max}	
	pM	*n*	*fmol/mg*	*n*
Stratum oriens				
Control	10.7 ± 0.92	3	3.6 ± 0.22	3
6 hr after KA	5.4 ± 0.48[a]	4	3.7 ± 0.13	4
Dentate gyrus				
Control	10.0 ± 0.92	3	0.4 ± 0.03	3
48 hr after KA	10.0 ± 0.54	3	3.6 ± 0.24[a]	3

[a] $p < 0.01$ versus control animals by two-tailed Student's *t* test.

K^+-stimulated glutamate release was primarily mediated through Y_2 receptors on Schaffer collaterals.

The rate of K^+-stimulated glutamate release in the absence of $PYY_{3–36}$ was 550 ± 47 (35 experimental points) and 1060 ± 71 (37 experimental points) pmol/mg of protein/90-sec stimulus (mean ± standard error) in control and KA-treated rats, respectively. As shown in Fig. 6, the Y_2 receptor agonist $PYY_{3–36}$, in a sigmoidal dose-response relationship, reduced K^+-stimulated glutamate release by up to 47% in both control and KA-injected rats. In slices from KA-treated rats, $PYY_{3–36}$ inhibited K^+-stimulated glutamate release at significantly lower concentrations than in slices of control rats (Fig. 6). EC_{50} values for $PYY_{3–36}$-mediated inhibition of glutamate release were 10.5 ± 0.61 nM (35 experimental points) and 4.2 ± 0.13 nM (37 experimental points) (mean ± standard error) in control and KA-treated rats, respectively.

Discussion

In accordance with our previous study (Röder *et al.*, 1996), the current results indicate a marked increase in Y_2 receptor binding in the hippocampus of rats after KA induced limbic seizures. Our data demonstrate that these changes are mediated by two regionally different mechanisms: (1) a fast increase in affinity of Y_2 receptors in the strata oriens and radiatum (presumably located there on Schaffer collaterals) and (2) *de novo* synthesis of Y_2 receptors in granule cells/mossy fibers.

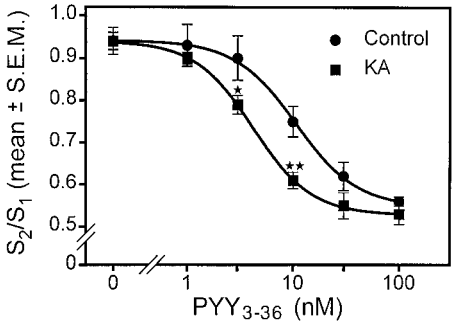

Fig. 6. Dose-response curves for inhibition of K^+-stimulated glutamate release by the Y_2 receptor agonist $PYY_{3–36}$ in slices of the *dorsal* hippocampus obtained 4 hr after KA injection and from control animals. EC_{50} values were 10.5 ± 0.61 and 4.2 ± 0.13 nM in control and KA-treated rats, respectively. At this interval, in the dorsal hippocampus Y_2 receptors are not expressed in granule cells/mossy fibers; data therefore indicate a change in receptor affinity in Schaffer collaterals. Values are means of 5–10 independent release experiments per concentration (35 versus 37 experimental points obtained in slices of KA-treated rats and control animals, respectively). **, $p < 0.001$; *, $p < 0.01$ compared with control animals.

Specificity of ^{125}I-$PYY_{3–36}$ as Y_2 receptor agonist. PP and [Pro^{34}]PYY, which are thought to be potent agonists at Y_4 and Y_5 sites in transfected cells (but do not bind significantly to Y_2 receptors), only weakly inhibited ^{125}I-$PYY_{3–36}$ binding (Bard *et al.*, 1995; Blomqvist and Herzog, 1997; Gerald *et al.*, 1996; Hu *et al.*, 1994). In addition, the selective Y_5 receptor ligand [D-Trp^{32}]NPY did not inhibit this binding. On the other hand, the potent Y_2 agonist $NPY_{13–36}$, which exerts only moderate or no activity at Y_4 and Y_5 sites, was similarly active in displacing ^{125}I-$PYY_{3–36}$ binding as PYY or $PYY_{3–36}$. The moderate inhibition by the Y_1, Y_4, and Y_5 agonist [Pro^{34}]PYY and the lack of an effect of the nonpeptidergic, Y_1-selective receptor antagonist BIBP 3226 support the lack of binding of the radioligand to Y_1 receptors. Prominent displacement by PYY excludes binding to Y_3 receptors. There is no evidence for the presence of Y_6 receptors in the hippocampus (originally also referred to as Y_5 receptors; Weinberg *et al.*, 1996). Taken together, these studies indicate that ^{125}I-$PYY_{3–36}$ under our conditions may bind mainly to Y_2 receptors. Furthermore, the affinities of $PYY_{3–36}$ to Y_5 and Y_4 receptors are ≈7-fold and >1000-fold lower than for Y_2 receptors, respectively (Bard *et al.*, 1995; Blomqvist and Herzog, 1997; Gerald *et al.*, 1996), and the distribution of Y_2 receptors is in excellent agreement with the *in situ* hybridization data using a Y_2-specific probe.

Increased affinity of Y_2 receptors in Schaffer collaterals. The dense binding sites for ^{125}I-$PYY_{3–36}$ within the strata oriens and radiatum could be located either on dendrites of pyramidal neurons or terminals of Schaffer collaterals arising from CA3. There is, however, considerable evidence for prejunctional Y_2 receptors located on Schaffer collaterals. Milner and Veznedaroglu (1992) demonstrated NPY-containing terminals near axon terminals in the stratum oriens of CA1 and CA3. Electrophysiological evidence for a presynaptic localization of Y_2 receptors on terminals of Schaffer collaterals and their presynaptic inhibitory action on glutamate release was provided previously (Colmers *et al.*, 1985, 1991; Haas *et al.*, 1987), and Y_2 receptors have been found especially enriched in the strata oriens and radiatum of CA1 to CA3 (Dumont *et al.*, 1996) (Figs. 3 and 4). Further support for a presynaptic localization of Y_2 receptors on Schaffer collaterals comes from the *in situ* hybridization data. The highest concentrations of Y_2 receptor mRNA are contained in CA3 and CA2 neurons. The fact that minor amounts of Y_2 mRNA are also expressed in CA1 neurons of the ventral hippocampus and, 48 hr after KA injection in the dorsal hippocampus indicates either that some Y_2 receptors may be also present on dendrites of CA1 pyramidal neurons or, more likely, that they become targeted to terminals of CA1-subicular projection neurons.

KA seizures induce a rapid increase in Y_2 receptor binding (after 4–12 hr) without a concomitant increase in concentrations of the respective mRNA in pyramidal cells. These observations argue against enhanced synthesis of Y_2 receptors in Schaffer collaterals as a cause for the increased receptor binding. Accordingly, kinetic analysis of ^{125}I-$PYY_{3–36}$ receptor binding in this area revealed an increase in receptor affinity without a significant change in the number of binding sites. This change in affinity likely may be caused by post-translational modification. Indeed a cysteine, as a pos-

sible site for palmitoylation, and several serine residues, which might be involved in regulatory phosphorylation, are present in the receptor molecule (Rose *et al.*, 1995).

Increased affinity of Y_2 receptors may facilitate presynaptic inhibition of glutamate release from Schaffer collaterals. The decreased EC_{50} values found for $PYY_{3–36}$ in presynaptic inhibition of glutamate release are in good agreement with the increased affinity of Y_2 receptors in the binding studies and thus indicates a mechanism pertinent to the pathophysiology of KA-induced epilepsy. In control animals and 4 hr after KA injection, almost no Y_2 receptors are present on mossy fibers, and only a minor portion of Y_2 binding is present in the dentate molecular layer. Therefore, glutamate released from mossy fibers may largely contribute to the portion of glutamate release not responding to the Y_2 receptor agonist.

It is interesting to note that in slices from KA-treated rats, there was an almost 2-fold increase in basal K^+-stimulated glutamate release. This certainly is an indicator for the markedly enhanced excitatory transmission in these rats. Despite this, similar inhibition of glutamate release, by ≈50%, was seen in control and KA-treated rats at maximal concentrations (100 nM) of the Y_2 receptor agonist.

Elevated Y_2 receptor synthesis in granule cells/mossy fibers. In the stratum lucidum, Y_2 receptor binding to mossy fibers overlaps with the strong labeled area of Schaffer collaterals. Binding to mossy fibers was therefore investigated in the hilus of the dorsal dentate gyrus, in which essentially no Y_2 receptors are present in controls. Thus, the concomitant pronounced increases in Y_2 receptor mRNA concentration in granule cells and of the number of Y_2 receptor sites in the hilus of the dentate gyrus are clear indicators of newly synthesized receptors. Essentially no Y_2 receptor mRNA was observed in interneurons of the dentate gyrus of control and KA-treated rats, excluding an other possible localization of Y_2 receptor binding in the hilus. Y_2 receptor mRNA occurs rather early (already after 12 hr) and throughout the granule cell layer. It is not restricted to newly formed granule cells, which are found primarily at the inner surface of the granule cell layer and can be labeled after 3–13 days by incorporation of deoxyuridine (Parent *et al.*, 1997) .

In the ventral aspect of the hippocampus (in which in control animals, measurable concentrations of Y_2 receptor mRNA are present), the increase of Y_2 receptor transcript is preceded by an early (4–6 hr after KA injection) decrease in message. This indicates high lability and fast turnover of the transcript. As observed for the Ammon's horn, in the dentate hilus, the increase in Y_2 receptor binding becomes attenuated after some time. Concentrations of Y_2 receptor mRNA in granule cells and Y_2 receptor binding in the dentate hilus remain significantly elevated 1–6 months after the KA-induced seizures, indicating permanent expression of these receptors in epileptic rats.

To which mechanisms in epileptogenesis do the changes in Y_2 receptors relate? Changes in Y_2 receptor binding and mRNA expression could be either initiated by the strong and repeated neuronal stimulation during or after acute KA-induced seizures or may be related to adaptive changes in the course of epileptogenesis (Sperk, 1994). Similar to the augmented expression of NPY in mossy fibers (Marksteiner *et al.*, 1990), we are proposing extensive stimulation during epileptic seizures as a primary mechanism. Thus, increases in Y_2 binding in Schaffer collaterals are already maximal before extended neurodegeneration or subsequent plastic changes of mossy fibers are detected. Furthermore, we recently observed similar expression of Y_2 receptor mRNA and binding in granule cells/mossy fibers of electrically kindled rats that are devoid of neuronal damage (Gobbi *et al.*, in press).

It is interesting to note that in our current work (using KA-treated rats), there even was a tendency for a negative correlation of Y_2 binding in the hilus of the dentate gyrus and neuronal damage occurring at the same time in CA3c neurons (preliminary data, not shown), indicating a possible protective effect in rats with increased Y_2 receptor expression in granule cells/mossy fibers.

On the other hand, it must be noted that the late decreases in Y_2 receptor binding in the strata oriens and radiatum may be due to neurodegenerative processes. As shown in our previous experiments (Röder *et al.*, 1996), in animals treated with thiopental early after seizure induction to prevent neurodegeneration, similar but more persistent increases in NPY receptor binding can be seen.

NPY may mediate an endogenous anticonvulsive action through Y_2 receptors. It is well established that the hippocampal formation plays a crucial role in the generation and propagation of seizures in temporal lobe epilepsy (Nadler, 1981; Sommer, 1880). In the hippocampus of control rats, NPY is contained within numerous GABAergic interneurons (Köhler *et al.*, 1986). Some of these interneurons are terminating prejunctionally on Schaffer collaterals (Milner and Veznedaroglu, 1992). In CA3 and CA1, NPY inhibits glutamate release from these fibers through Y_2 receptors (Colmers *et al.*, 1991). During the acute seizures, reaching their maximal extent ≈90 min after KA injection, a rapid decrease in NPY levels indicates pronounced release of the peptide (Bellmann *et al.*, 1991). In conjunction with the rapid increase in Y_2 receptor affinity, this may represent an important mechanism for suppressing excessive glutamate release from Schaffer collaterals and counteracting the spread of excitation through the hippocampal circuits. The possible relevance of this mechanism is underscored by our current work on glutamate release *ex vivo*. Furthermore, NPY infused into the brain has a potent anticonvulsive action (Smialowska *et al.*, 1996; Woldbye *et al.*, 1996), and NPY-deficient mice exhibit an increased seizure susceptibility (Erickson *et al.*, 1996) .

Mossy fibers exert an important role in passing the afferent excitatory information of the perforant path to CA3 pyramidal neurons. Because granule cells are resistant to seizure-induced brain damage, they are prone to chronically perpetuate excitation in the epileptic animals. Thus, expression of NPY and its presynaptic Y_2 receptors in mossy fibers may result in presynaptic inhibition of glutamate release; this may aid in attenuating the excitatory/excitotoxic action of glutamate on CA3 pyramidal neurons. Considering the highly effective action of NPY on glutamate release *ex vivo*, this may be a rather important mechanism in the epileptic animal.

In contrast to NPY released from GABA/NPY interneurons and acting presynaptically on Schaffer collaterals, NPY released from mossy fibers acts at its own nerve terminals. Thus, its rate of release and its final action are controlled by the activity of mossy fibers themselves. Within mossy fiber

terminals, the peptide presumably is stored in large dense core vesicles. Although under physiological conditions or low frequency stimulation, primarily the small synaptic vesicles (containing glutamate but no peptides) become exocytosed, NPY can be released from large, dense core vesicles at high frequency stimulation (for a review, see Hökfelt, 1991). This indicates that the peptide will be primarily liberated during episodes of acute seizures and may not interfere significantly with the physiological transmission of the hippocampus during seizure-free intervals. Expression of NPY and its receptors in mossy fibers therefore may represent a potent endogenous anticonvulsive mechanism that may be programmed to become particularly activated during acute seizure episodes.

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References

Bard JA, Walker MW, Branchek TA, and Weinshank RL (1995) Cloning and functional expression of a human Y_4 subtype receptor for pancreatic polypeptide, neuropeptide Y, and peptide YY. *J Biol Chem* **270**:26762–26765.

Bellmann R, Widmann R, Olenik C, Meyer DK, Maas D, Marksteiner J, and Sperk G (1991) Enhanced rate of expression and biosynthesis of neuropeptide Y after kainic acid induced seizures. *J Neurochem* **56**:525–530.

Ben-Ari Y (1985) Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience* **14**:375–403.

Blomqvist AG and Herzog H (1997) Y-receptor subtypes: how many more? *Trends Neurosci* **20**:294–298.

Colmers WF, Klapstein GJ, Fournier A, St-Pierre S, and Treherne KA (1991) Presynaptic inhibition by neuropeptide Y in rat hippocampal slice *in vitro* is mediated by a Y_2 receptor. *Br J Pharmacol* **102**:41–44.

Colmers WF, Lukowiak K, and Pittmann QJ (1985) Neuropeptide Y reduces orthodromically evoked population spike in rat hippocampal CA1 by a possibly presynaptic mechanism. *Brain Res* **346**:404–408.

Dumont Y, Fournier A, St-Pierre S, and Quirion R (1995) Characterization of neuropeptide Y binding sites in rat brain membrane preparations using [^{125}I][Leu^{31},Pro^{34}]PYY and [^{125}I]PYY$_{3–36}$ as selective Y_1 and Y_2 radioligands. *J Pharmacol Exp Ther* **272**:673–680.

Dumont Y, Fournier A, St-Pierre S, and Quirion R (1996) Autoradiographic distribution of [^{125}I][Leu^{31},Pro^{34}]PYY and [^{125}I]PYY$_{3–36}$ binding sites in the rat brain evaluated with two newly developed Y_1 and Y_2 receptor radioligands. *Synapse* **22**:139–158.

Erickson JC, Clegg KE, and Palmiter RD (1996) Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. *Nature (Lond)* **381**:415–418.

Gerald C, Walker MW, Criscione L, Gustafson EL, Batzl-Hartmann C, Smith KE, Vaysse P, Durkin MM, Laz TM, Linemeyer DL, Schaffhauser AO, Witebread S, Hofbauer KG, Taber RI, Branchek TA, and Weinshank RL (1996) A receptor subtype involved in neuropeptide-Y-induced food intake. *Nature (Lond)* **283**:168–171.

Gobbi M, Gariboldi M, Piwko C, Hoyer D, Sperk G, and Vezzani A (1998) Distinct changes in the binding of neuropeptide Y to Y_1 and Y_2 receptors and their mRNA expression in the hippocampus during kindling epileptogenesis. *J Neurochem*, in press.

Greber S, Schwarzer C, and Sperk G (1994) Neuropeptide Y inhibits potassium-stimulated glutamate release through Y_2 receptors in rat hippocampal slices in vitro. *Br J Pharmacol* **113**:737–740.

Haas HL, Herman A, Greene RW, and Chan-Palay V (1987) Action and location of neuropeptide tyrosine (Y) on hippocampal neurons of the rat in slice preparations. *J Comp Neurol* **257**:208–215.

Hökfelt T (1991) Neuropeptides in perspective: the last ten years. *Neuron* **7**:867.

Hu Y, Bloomquist BT, Cornfield LJ, DeCarr LB, Flores-Riveros JR, Friedman L, Jiang P, Lewis-Higgins L, Sadlowski Y, Schaefer J, Velasquez N, and McCaleb ML (1994) Identification of a novel hypothalamic neuropeptide Y receptor associated with feeding behavior. *J Biol Chem* **271**:26325–26319.

Hunter WM and Greenwood FC (1962) Preparation of iodine-^{131}I labelled growth hormone of high specific activity. *Nature (Lond)* **194**:495–496.

Köhler C, Eriksson L, Davies S, and Chan-Palay V (1986) Neuropeptide Y innervation of the hippocampal region in the rat and monkey brain. *J Comp Neurol* **244:**384–400.

Lowry OH, Rosebough NJ, Farr AL, and Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**:265–275.

Marksteiner J, Prommegger R, and Sperk G (1990) Effect of anticonvulsant treatment on kainic acid induced increases in peptide levels. *Eur J Pharmacol* **181**: 241–246.

Milner TA and Veznedaroglu E (1992) Ultrastructural localization of neuropeptide Y-like immunoreactivity in the rat hippocampal formation. *Hippocampus* **2**:107–126.

Nadler JV (1981) Kainic acid as a tool for the study of temporal lobe epilepsy. *Life Sci* **29**:2031–2042.

Nakamura M, Aoki Y, and Hirano D (1996) Cloning and functional expression of a cDNA encoding a mouse type 2 neuropeptide Y receptor. *Biochim Biophys Acta* **1284**:134–137.

Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, and Lowenstein DH (1997) Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *J Neurosci* **17**:3727–3738.

Röder C, Schwarzer C, Vezzani A, Gobbi M, Mennini T, and Sperk G (1996) Autoradiographic analysis of neuropeptide Y receptor binding sites in the rat hippocampus after kainic acid-induced limbic seizures. *Neuroscience* **70**:47–55.

Rose PM, Fernandes F, Lynch JS, Frazier ST, Fisher SM, Kodukula K, Kienzle B, and Seethala R (1995) Cloning and functional expression of a cDNA encoding a human type 2 neuropeptide Y receptor. *J Biol Chem* **270**:22661–22664.

Sheikh SP, O'Hare MT, Torton O, and Schwartz TW (1989) Binding of monoiodinated neuropeptide Y to hippocampal membranes and human neuroblastoma cell lines. *J Biol Chem* **254**:6648–6654.

Smialowska M, Bijak M, Sopala M, and Tokarski K (1996) Inhibitory effect of NPY on the picrotoxin-induced activity in the hippocampus: a behavioral and electrophysiological study. *Neuropeptides* **30**:7–12.

Sommer W (1880) Erkrankung des Ammonshorn als aetiologisches Moment der Epilepsie. *Arch Psychiatr Nervenkrankh* **10**:631–675.

Sperk G (1994) Kainic acid seizures in the rat. *Prog Neurobiol* **42**:1–32.

Sperk G, Lassmann H, Baran H, Kish SJ, Seitelberger F, and Hornykiewicz O (1983) Kainic acid induced seizures: neurochemical and histopathological changes. *Neuroscience* **10**:1301–1315.

Sperk G, Marksteiner J, Gruber B, Bellmann R, Mahata M, and Ortler M (1992) Functional changes in neuropeptide Y and somatostatin containing neurons induced by limbic seizures in the rat. *Neuroscience* **50**:831–846.

Weinberg DH, Sirinathsinghji DJS, Tan CP, Shiao L-L, Morin N, Rigby MR, Heavens RH, Rapoport DR, Bayne ML, Cascieri MA, Strader CD, Linemeyer DL, and MacNeil DJ (1996) Cloning and expression of a novel neuropeptide Y receptor. *J Biol Chem* **271**:16435–16438.

Woldbye DPD, Madsen TM, Larsen PJ, Mikkelsen JD, and Bolwing TG (1996) Neuropeptide Y inhibits hippocampal seizures and wet dog shakes. *Brain Res* **737**:162–168.

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