Neuropeptide Y₁ Receptors Inhibit N-Type Calcium Currents and Reduce Transient Calcium Increases in Rat Dentate Granule Cells

A. Rory McQuiston,¹ Jeffrey J. Petrozzino,² John A. Connor,² and William F. Colmers¹

¹Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7, and ²Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110-1199

Neuropeptide Y (NPY) is far more abundant in the dentate gyrus than elsewhere in the hippocampal formation, but it does not alter the synaptic excitation of dentate granule cells (DGCs) as it does for pyramidal cells in areas CA1 and CA3. NPY inhibited depolarization-induced increases in intracellular Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$) in DGCs in hippocampal slices, without altering the resting $[\text{Ca}^{2+}]_i$. NPY inhibited Ca^{2+} currents (I_{Ca}) via a Y_1 receptor in 84% of acutely isolated DGCs and via a Y_2 receptor in 31% of the NPY-responsive cells tested. I_{Ca} inhibition was completely occluded by ω -conotoxin-GVIA but not by nimodipine. The inhibition of I_{Ca} was accompanied by a change in the time course of I_{Ca} activation in only 27% of NPY-responsive cells. Only 23% of DGCs responded to NPY when Ba^{2+}

was substituted for extracellular Ca^{2+} and when $[\text{Ca}^{2+}]_i$ was strongly buffered.

Therefore, NPY inhibits an N-type I_{Ca} in DGCs, mainly via Y_1 receptors. Furthermore, it seems that more than one mechanism, one of which may be sensitive to $[\text{Ca}^{2+}]_i$, may couple NPY receptors to the Ca^{2+} channels in DGCs. Because the release of dynorphin from DGCs depends in part on N-type currents, NPY receptors are poised to regulate the release of opioid peptides from DGC somata and dendrites.

Key words: neuropeptide Y; Y_1 receptor; voltage-dependent calcium current; dentate gyrus granule cell; acutely dissociated neurons; intracellular calcium imaging

Neuropeptide Y (NPY) and its receptors are found in large quantities in widely distributed regions of the vertebrate peripheral nervous system (PNS) and CNS (Dumont et al., 1990, 1992, 1993; Hendry, 1993; Sundler et al., 1993; Wahlestedt and Reis, 1993). NPY has a substantial number of documented actions on physiology and/or behavior in the mammalian PNS and CNS (Colmers and Bleakman, 1994; Wahlestedt and Reis, 1993). The cellular mechanisms of the actions of NPY, however, especially in the CNS, are not well understood.

In the mammalian CNS, very high concentrations of NPY and its receptors are found in the hippocampus (Dumont et al., 1992, 1993; Hendry, 1993). NPY binding is found in high density in the stratum radiatum and oriens of hippocampal areas CA1 and CA3, where NPY potently and selectively inhibits (glutamatergic) synaptic excitation of CA1 (Colmers et al., 1987, 1988; Haas et al., 1987) and CA3 pyramidal neurons (McQuiston and Colmers, 1992; Klapstein and Colmers, 1993) via a receptor of the Y_2 subtype (Colmers and Bleakman, 1994). In the hippocampal formation of the rat, NPY is most concentrated in the dentate gyrus (Hendry, 1993), and substantial specific binding for NPY is present in the molecular layer of the dentate gyrus (Dumont et al., 1990, 1993), where afferent inputs from the entorhinal cortex form synapses with the dendrites of dentate

granule cells (DGCs) (Laatch and Cowan, 1967; Hjorth-Simonsen and Jeune, 1972). Receptor binding studies indicate that the NPY receptor in the dentate gyrus is mostly Y_1 , in contrast to the predominantly Y_2 receptors of areas CA1 and CA3 (Dumont et al., 1990, 1992, 1993; Aicher et al., 1991; Wahlestedt and Reis, 1993). Despite this, however, no effect of NPY has been observed on synaptic inputs to DGCs or on the passive electrical properties of the DGCs (Klapstein and Colmers, 1993), with the exception of a single report of a direct excitatory effect (Brooks et al., 1987). Therefore, a function for NPY and its receptors in the dentate gyrus remains elusive.

NPY inhibits N-type voltage-dependent calcium channels (VDCCs) of vertebrate PNS neurons such as sympathetic C cells, rat myenteric plexus neurons, and rat sensory neurons (for review, see Bleakman et al., 1993; Colmers and Bleakman, 1994). NPY also inhibits both N- and L-type VDCCs of frog melanotrophs (Valentijn et al., 1994). In most cases, NPY inhibits the N-type VDCCs by activating a Y_2 receptor. By contrast, in some nodose ganglion neurons of the rat, NPY potentiates the same N-type VDCC via a Y_1 receptor that it inhibits via a Y_2 receptor (Wiley et al., 1993). NPY also augments VDCCs in rat vascular smooth muscle (Xiong et al., 1993). Furthermore, NPY often is coupled to intracellular calcium mobilization in neurons and other cell types (Michel, 1991; Dumont et al., 1992; Wahlestedt and Reis, 1993).

Here we investigated the possibility that NPY acts either to alter intracellular calcium homeostasis [possibly by elevating intracellular Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$)] or to modulate VDCCs of rat DGCs. We have found that NPY inhibits N-type VDCCs and reduces activity-dependent Ca^{2+} increases in rat DGCs, predominantly by activating a Y_1 receptor.

MATERIALS AND METHODS

Calcium imaging experiments. Experiments were performed on DGCs in 450 μm transverse slices of 21- to 30-d-old male Sprague-Dawley rats.

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Correspondence should be addressed to Dr. William F. Colmers, Department of Pharmacology, University of Alberta, 9-36 MSB, Edmonton, Alberta, Canada T6G 2H7.

Dr. McQuiston's present address: Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA 94305.

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Rats were anesthetized with halothane and decapitated. Brains were removed rapidly and placed in ice-cold saline (4°C). The hippocampus was then dissected from the brain and cut on a Vibratome. Slices were placed on a polypropylene mesh, submerged, and continuously perfused with saline (23°C) containing (in mM): 124 NaCl, 2 KCl, 1.3 MgSO₄, 1.25 KH₂PO₄, 18 NaHCO₃, 2.5 CaCl₂, and 10 glucose equilibrated with 95% O₂/5% CO₂, pH 7.4. Recordings from DGCs were obtained in the whole-cell patch-clamp configuration (Blanton et al., 1989) using patch electrodes (5–9 MΩ) filled with 200 μM Fura-2 (K⁺ salt; Molecular Probes, Eugene, OR) dissolved in the intracellular solution consisting of (in mM): 135 K-gluconate, 2 Mg-ATP, 0.3 Na-GTP, and 10 HEPES, pH 7.2. Neurons were held at -60 mV in the continuous voltage-clamp mode using an Axoclamp 2A amplifier (Axon Instruments, Burlingame, CA), and 500 msec voltage steps to 0 mV were made periodically to stimulate voltage-dependent Ca²⁺ influx.

Imaging experiments were performed from the top surface of the slice using an upright microscope (Zeiss Axioscope) and a 40× water immersion objective (Zeiss, Thornwood, NY). Digital images were taken with a cooled charge-coupled device camera system (Photometrics, Tucson, AZ) in the frame-transfer mode to acquire image pairs at 360 and 380 nm excitation wavelengths. Ca²⁺ levels were calculated from background-corrected images using the ratio method (Grynkiewicz et al., 1985; Tsien, 1989). Calibration of the indicator was carried out under *in vitro* conditions using general procedures described previously (Connor, 1986). For these measurements, a light path of 20 μm was achieved by confining the indicator-saline mixtures in a 20 × 50 μm² capillary (Vitro Dynamics, Rockaway, NJ).

Dissociated cell preparation. Single DGC neurons were isolated from 21- to 30-d-old male Sprague-Dawley rats by a procedure described previously (Kay, 1989; White et al., 1993). Rats were anesthetized with halothane and decapitated. Brains were rapidly removed and placed in ice-cold (4°C) bicarbonate-based artificial CSF (aCSF) saturated with 95% O₂/5% CO₂. The brains were hemisected sagittally with a razor blade and cut on a Vibratome into 500 μm transverse slices containing the hippocampal formation. The dentate gyrus was microdissected from these slices and placed in a spinner flask (Belco) containing warm (30°C) dissociation solution saturated with 100% O₂. Slices were allowed to rest for ~15 min and then treated with 0.2 mg/ml proteinase K (Sigma, St. Louis, MO) in the dissociation solution. After 5 min, the solution was exchanged for another solution containing 1 mg/ml trypsin type XI (Sigma) and treated for an additional 30 min. After trypsin treatment, the slices were removed, placed in enzyme-free dissociation solution, and allowed to return to room temperature. The slices rested for 1–2 hr, and then two slices were triturated in dissociation solution (to which 0.5 mg/ml bovine serum albumin was added) through two fire-polished Pasteur pipettes with tip diameters of ~500 and 200 μm, respectively. Cells were placed in a recording chamber and allowed to settle onto poly-L-lysine-coated glass coverslips for 10–15 min before they were washed with a HEPES-based aCSF in a recording chamber placed on the stage of an inverted microscope (Nikon Diaphot, Nikon, Mississauga, Ontario, Canada).

Calcium current recording. Currents were recorded from DGC neurons at room temperature (~22°C) using the whole-cell patch-clamp configuration (Hamill et al., 1981) with an Axopatch 1D amplifier and analyzed with pClamp software (Axon Instruments). Patch electrodes (3–5 MΩ) made from borosilicate glass (World Precision Instruments, Sarasota, FL) were not fire-polished or insulated for the measurement of the relatively slow Ca²⁺ currents we recorded. Whole-cell recording was obtained in the HEPES-buffered aCSF, which was then promptly switched to the calcium current solution. Series resistance and membrane capacitance were electronically compensated by 80–90% via the amplifier circuitry and were continuously monitored throughout the experiment. Currents were leak-subtracted on-line using a P/N protocol and filtered at 1 kHz. I_{Ca} was elicited at 20 sec intervals with 40 msec voltage steps to +5 mV from a holding potential of -60 mV or with voltage ramps swept from -80 to +70 mV over 100 msec. Drugs were applied by bath superfusion in the calcium (or barium) current solution.

Significant differences in mean values were tested using the Wilcoxon signed rank test or the Kruskal-Wallis test. Values were considered significantly different for $p < 0.05$. Data are presented as mean ± SEM.

Solutions and materials. The bicarbonate-buffered aCSF used for the dissection consisted of (in mM): 126 NaCl, 2.5 KCl, 6 MgCl₂, 2 CaCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃, 10 glucose, and 1 kynurenic acid. The dissociation solution consisted of (in mM): 115 NaCl, 5 KCl, 20 PIPES, 1 CaCl₂, 4 MgCl₂, 25 glucose, 1 kynurenic acid, and 1 sodium pyruvate. The HEPES-buffered solution used to wash the isolated cells consisted of (in

mM): 135 NaCl, 2.5 KCl, 2 MgCl₂, 2 CaCl₂, 20 HEPES, 20 glucose, and 1 Na-pyruvate. The calcium current solution consisted of (in mM): 100 NaCl, 2.5 KCl, 2 MgCl₂, 5 CaCl₂, 20 HEPES, 3 CsCl, 30 tetraethylammonium chloride (TEA-Cl), 5 4-aminopyridine, 10 glucose, and 1 Na-pyruvate. All extracellular solutions had a pH of 7.4 and an osmolarity of 315–320 mOsm. The intracellular electrode solution consisted of (in mM): 100 CsCl, 30 TEA-Cl, 10 HEPES, 4 Mg-ATP, 0.3 Na₃-GTP, 0.1 bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetra-acetic acid tetrapotassium salt (BAPTAK₄), 14 ditris phosphocreatine, and 50 U/ml creatine phosphokinase, pH 7.2 (with CsOH); the solution had an osmolarity of 280–290 mOsm. For the barium current experiments, the calcium of the extracellular calcium current solution was replaced by equimolar barium, and the BAPTAK₄ of the intracellular electrode solution was replaced by (BAPTA)-free acid (20 mM). The CsCl concentration in the pipette was reduced to compensate for the increased BAPTA concentration.

NPY was obtained from Richelieu Biotechnology (Quebec, Canada); [Leu³¹Pro³⁴]NPY, NPY_{13–36}, and peptide YY (PYY) were obtained from Bachem (Torrance, CA); ω-conotoxin GVIA (ω-CTX GVIA) was obtained from Peninsula Laboratories (Belmont, CA); nimodipine was a generous gift from Miles (West Haven, CT); baclofen (Lioresal) was a gift of Ciba-Geigy (Basel, Switzerland) and creatine phosphokinase was obtained from Boehringer Mannheim (Laval, Quebec, Canada). All other compounds were obtained from Sigma.

RESULTS

NPY inhibits depolarization-induced increases in [Ca²⁺]_i of DGCs

We first sought to determine whether NPY affected either resting [Ca²⁺]_i or Ca²⁺ influx in DGCs. For this, we made spatially resolved measurements of [Ca²⁺]_i in DGCs loaded with Fura-2 (Grynkiewicz et al., 1985) (see Materials and Methods) via a whole-cell patch electrode that also served to measure voltage and injected current. An experiment showing the effects of NPY is illustrated in Figure 1. The resting [Ca²⁺]_i was not altered by the bath application of 1 μM NPY ($n = 6$). To investigate a possible NPY modulation of Ca²⁺ influx, we depolarized the soma from -60 mV to 0 mV for 500 msec via the patch pipette. No attempts were made to isolate calcium currents (I_{Ca}), and the depolarization produced Na⁺- and Ca²⁺-dependent action potentials, which we did not attempt to control. NPY (1 μM) significantly decreased the depolarization-induced increase in [Ca²⁺]_i compared with that in controls (Fig. 1C,D). Washout of NPY partially reversed the decrease (Fig. 1E). The data from five experiments are summarized in the histogram (Fig. 1F). NPY (1 μM) decreased the depolarization-induced increase in [Ca²⁺]_i to $66.6 \pm 2.3\%$ of the control increase in the soma (Fig. 1F) and to $55.8 \pm 5.9\%$ of the control increase in the dendrite (Fig. 1F) ($p < 0.05$, Wilcoxon signed rank test). The larger decrease of [Ca²⁺]_i in the dendrites compared with the soma was not significantly different ($p > 0.05$, Wilcoxon signed rank test).

The most likely means by which NPY could inhibit this depolarization-induced increase in [Ca²⁺]_i are by (1) inhibiting VDCCs directly or (2) reducing the excitability of the DGC, such as by increasing K⁺ channel activity or decreasing Na⁺ channel activity and thereby the influx of Ca²⁺. We first tested the hypothesis that NPY inhibited VDCCs of acutely dissociated neurons.

NPY inhibits voltage-dependent I_{Ca} s of acutely dissociated DGCs

We isolated DGCs acutely from rat hippocampal slices and isolated I_{Ca} (as described in Materials and Methods). DGCs were distinguished from other neuronal types by their small round cell bodies (Mody et al., 1989). Larger DGCs were selected because they usually had a larger I_{Ca} , which also ran down more slowly and recovered more completely from NPY inhibition. DGCs with larger dendrites were also selected because NPY-binding sites are concentrated in

Figure 1. NPY inhibits depolarization-induced increases in $[Ca^{2+}]_i$ in DGCs from hippocampal slices. Image of a DGC in a hippocampal slice of the rat. *A*, Fluorescence image with the cell body of the neuron at the top and a bifurcating dendrite underneath. The $[Ca^{2+}]_i$ coding for the false color image is on the far right of the figure. The cell was voltage-clamped at -60 mV and periodically depolarized to 0 mV for 500 msec. *B*, Ratiometric image of the $[Ca^{2+}]_i$ of the cell at rest. *C*, The control increase in $[Ca^{2+}]_i$ in response to a depolarizing step. *D*, The presence of NPY ($1 \mu M$) inhibits the increase in $[Ca^{2+}]_i$ after a depolarizing step. *E*, The increase in $[Ca^{2+}]_i$ after a depolarizing step recovers toward control levels after washing out NPY. *F*, Histogram summarizing results of similar experiments ($n = 5$). NPY ($1 \mu M$) significantly ($p < 0.05$, Wilcoxon signed rank test) inhibited depolarization-induced increases in $[Ca^{2+}]_i$ of Dendrites in the molecular layer and Soma of DGCs of rat hippocampal slices.

their dendrites (Dumont et al., 1990, 1993; Aicher et al., 1991). Unless otherwise indicated, I_{Ca} s from DGCs were recorded with 5 mM Ca^{2+} in the extracellular solution as the charge carrier and with a pipette solution having low Ca^{2+} buffering (0.1 mM BAPTA with no added Ca^{2+} ; see Materials and Methods). I_{Ca} s of these DGCs were typically between 200 and 1000 pA in peak amplitude. The maximum peak amplitudes were elicited with voltage steps from -60 mV to between $+5$ and $+10$ mV.

The I_{Ca} of 42 of 50 acutely dissociated DGCs was inhibited by bath application of $1 \mu M$ NPY (Fig. 2). Not all cells recovered fully from NPY inhibition, and recovery in many cells took several minutes longer than was required for the complete exchange of the solution in the recording chamber. Some cells showed no apparent recovery within the recording period. Recovery of I_{Ca} from NPY inhibition was also obscured by the rapid rate of I_{Ca} rundown, despite the presence in the pipette of an ATP-regenerating solution (see Materials and Methods). Repeated administration of NPY often resulted in a loss of the ability of NPY to inhibit I_{Ca} (see below).

NPY produced two types of inhibition of I_{Ca} in DGCs. First, in a great majority of DGCs ($33/45$ cells), NPY caused a reversible inhibition of I_{Ca} without an alteration in the time course of I_{Ca} activation (Fig. 2*A*). The second type of I_{Ca} inhibition by NPY in DGCs was accompanied by a slowing of the activation time course of I_{Ca} (Fig. 2*B*). This second type of inhibition was seen less frequently ($12/45$ cells); however, it was often more robust and reversible than the other (Figs. 2*B*, 3*A,C,D*). Peak I_{Ca} in DGCs that showed a clear change in I_{Ca} time course after NPY application was inhibited by $38.8 \pm 5.4\%$ ($12/45$ DGCs), as opposed to

the $22.3 \pm 0.9\%$ ($33/45$ DGCs; $p < 0.001$, Kruskal-Wallis test) inhibition of peak I_{Ca} observed in DGCs with no accompanying change in I_{Ca} activation time course.

NPY inhibits I_{Ca} of DGCs predominantly by activating a Y_1 receptor

At present, NPY seems to have three receptor subtypes, termed Y_1 , Y_2 , and Y_3 (Grundemar et al., 1993; Wahlestedt and

Figure 2. NPY inhibits I_{Ca} of acutely dissociated DGCs. NPY inhibits the I_{Ca} of two different DGCs. The I_{Ca} was elicited by a 40 msec voltage step to $+5$ mV from a holding potential of -60 mV. Voltage steps were elicited at 20 sec intervals. *A*, NPY ($1 \mu M$) inhibits the I_{Ca} of this DGC without markedly altering the time course of activation of the current. *B*, In a different DGC, NPY ($1 \mu M$) inhibits I_{Ca} and slows the time course of activation of the current.

Figure 3. Selective NPY agonists inhibit I_{Ca} of acutely dissociated DGCs. *A*, Time plot of peak I_{Ca} from a DGC elicited at 20 sec intervals by a 40 msec voltage step to +5 mV from a holding potential of -60 mV. The peak current elicited was plotted against the time elapsed from the beginning of the experiment. Drugs were applied during the times indicated by the bars at the top of the plot. NPY, [Leu³¹Pro³⁴]NPY, and PYY inhibited the I_{Ca} but NPY₁₃₋₃₆ did not. All agonists were applied at 1 μ M. *B*, The I_{Ca} elicited at the times indicated by the corresponding letters in *A*. Control traces were taken immediately before the respective peptide was applied. Time and current scales are as indicated. *C*, Time plot of I_{Ca} as in *A*. I_{Ca} in this cell was inhibited by all the agonists tested (at 1 μ M), including the Y_2 -selective agonist NPY₁₃₋₃₆. *D*, Mean $\pm$ SEM percent reduction of I_{Ca} of all cells responding to 1 μ M of the indicated agonist (Y_1 = [Leu³¹Pro³⁴]NPY; Y_2 = NPY₁₃₋₃₆). The reductions of I_{Ca} by the individual agonists are not significantly different ($p > 0.30$, Kruskal-Wallis test). All NPY-responsive cells also responded to [Leu³¹Pro³⁴]NPY and PYY when tested. Only 5 of 16 NPY-responsive cells also responded to NPY₁₃₋₃₆.

Reis, 1993). We tested the receptor profile for NPY using [Leu³¹Pro³⁴]NPY (Y_1 - and Y_3 -selective), NPY₁₃₋₃₆ (Y_2 - and somewhat Y_3 -selective), and PYY (Y_1 - and Y_2 -selective but not Y_3 -selective), in addition to the nonselective agonist NPY itself.

Figure 3*A* illustrates the time plot of an experiment in which NPY and the receptor-selective agonists were applied to the same cell. NPY, [Leu³¹Pro³⁴]NPY, and PYY significantly inhibited the peak I_{Ca} , whereas NPY₁₃₋₃₆ did not (Fig. 3*A,B*). In this case, because NPY₁₃₋₃₆ can activate both Y_2 and Y_3 receptors (but was inactive here) and PYY was active, NPY must inhibit the I_{Ca} of this DGC via a Y_1 receptor. The current traces show that in this cell, NPY, [Leu³¹Pro³⁴]NPY, and PYY slow the activation time course of the I_{Ca} (Fig. 3*B*).

Although most DGCs seem to express only Y_1 receptors (Fig. 3), other DGCs express more than one NPY receptor subtype (Fig. 3*C*). In the DGC illustrated in Figure 3*C*, NPY and all three selective agonists inhibited the I_{Ca} . This experiment shows that more than one NPY receptor subtype must be expressed by this DGC, although this protocol did not distinguish whether Y_2 and/or Y_3 receptors were present on this cell.

Agonist studies on I_{Ca} inhibition are summarized in Figure 3*D*. I_{Ca} was inhibited by between 21.8 and 30.2% by individual agonists; however, there was no significant difference between the

four different agonists ($p > 0.30$, Kruskal-Wallis test; Fig. 3*D*). The effects of NPY₁₃₋₃₆ were similar in magnitude to the other agonists, although it was effective in only 31% (5/16 cells) of DGCs that responded to NPY. By contrast, [Leu³¹Pro³⁴]NPY ($n = 19$) and PYY ($n = 13$) inhibited the I_{Ca} in all of the NPY-responsive DGCs on which they were tested.

NPY does not modulate the voltage-dependence of I_{Ca} activation

The I_{Ca} of these small cells often ran down rapidly, making the construction of a current-voltage relationship of the I_{Ca} from a family of voltage steps under several conditions very difficult. To circumvent this problem, we ramped the voltage of the DGC membrane (V_m) from -80 to +70 mV over 100 msec (Fig. 4). The I_{Ca} of this DGC was inhibited by NPY with no change in the V_m range of I_{Ca} activation or the V_m where I_{Ca} reached a maximum (Fig. 4). This DGC was insensitive to NPY₁₃₋₃₆ (Fig. 4). NPY ($n = 7$), [Leu³¹Pro³⁴]NPY ($n = 4$), and PYY ($n = 3$) inhibited I_{Ca} elicited by voltage ramps in other DGCs without shifting the voltage range of activation or the V_m at which I_{Ca} peaked. We did not see an inhibition of voltage-ramped I_{Ca} by NPY₁₃₋₃₆ ($n = 2$). The I_{Ca} of one voltage-ramped DGC did not respond to NPY.

Figure 4. NPY inhibition of I_{Ca} does not shift the current-voltage relationship of I_{Ca} . NPY but not NPY_{13-36} inhibited I_{Ca} elicited in a DGC by a 100 msec voltage ramp from -80 to $+70$ mV. NPY did not shift the voltage range of I_{Ca} activation or the voltage at which I_{Ca} reached its maximum.

NPY inhibits an N-type I_{Ca}

We next sought to determine the type of I_{Ca} that NPY affected in DGCs. We rarely observed low voltage-activated I_{Ca} in our DGC preparations and, therefore, we used pharmacological inhibitors of high voltage-activated currents to determine the type or types of I_{Ca} inhibited by NPY in DGCs. We applied either NPY or $[Leu^{31}Pro^{34}]NPY$ first in the absence and then in the presence of the L-type I_{Ca} inhibitor nimodipine ($1-5 \mu M$) or the N-type I_{Ca} inhibitor ω -CTX GVIA ($3-5 \mu M$). The peak I_{Ca} was inhibited by $44 \pm 6\%$ ($n = 9$) by ω -CTX GVIA and by $23 \pm 6\%$ ($n = 7$) by nimodipine. The time plot of Figure 5A demonstrates that ω -CTX GVIA completely occluded the $[Leu^{31}Pro^{34}]NPY$ inhibition of I_{Ca} in this DGC. The same observation was made in seven other cells inhibited by NPY and in one other cell inhibited by $[Leu^{31}Pro^{34}]NPY$. The data are summarized in Figure 6. Figure 5B illustrates that ω -CTX GVIA completely occludes the inhibition of I_{Ca} by NPY ($n = 7$). By contrast, nimodipine did not significantly reduce the inhibition of I_{Ca} by NPY (Fig. 5B; $n = 7$).

Two factors complicated this observation. First, we observed that the magnitude of I_{Ca} suppression by NPY diminishes by $32 \pm 10\%$ ($n = 8$) with the second application of NPY to the same neuron (Fig. 5C). Because we always first tested the effect of NPY alone, the response ran down, resulting in a systematic underestimate of the inhibition of I_{Ca} by NPY in nimodipine. We therefore corrected the observed value of I_{Ca} inhibition by NPY in nimodipine for the rundown of the NPY response (Fig. 5D, *Corrected*). Second, if NPY does not affect L-type VDCCs, then it should inhibit a greater percentage of the I_{Ca} that remains after nimodipine treatment. We therefore predicted the effect of NPY on the nimodipine-insensitive I_{Ca} on the basis of the first effect of NPY and the amount of current inhibited by nimodipine (Fig. 5D, *Predicted*). There was no significant difference between the two (Wilcoxon signed rank test, $p > 0.73$), suggesting that there is indeed no effect of nimodipine on the I_{Ca} reduced by NPY receptors in these neurons.

Inhibition of I_{Ca} by NPY is Ca^{2+} -sensitive

In experiments on dissociated cells recorded under conditions that favored a moderate resting $[Ca^{2+}]_i$ (Ca^{2+} as the charge

carrier and minimal buffering of $[Ca^{2+}]_i$), 42 of 50 DGCs responded to NPY (Fig. 6). By contrast, under experimental conditions favoring low $[Ca^{2+}]_i$ (Ba^{2+} as the charge carrier and strong buffering of $[Ca^{2+}]_i$), NPY inhibited the I_{Ca} of only 23% of DGCs ($n = 121$; Fig. 6). In the same cells, the I_{Ba} was reversibly inhibited by at least 15% in 22 of 27 cells tested with the application of $10 \mu M$ baclofen (not shown), indicating that another agonist was capable of inhibiting I_{Ca} in a much greater percentage of these cells under these circumstances.

DISCUSSION

We report here that NPY inhibits an N-type calcium channel in the soma and dendrites of rat hippocampal DGCs by activating predominantly Y_1 receptors. This inhibition results in significantly smaller elevations in $[Ca^{2+}]_i$ during depolarizing electrical activity. To our knowledge, this is the first report of NPY inhibiting an I_{Ca} in the soma of a central neuron, and the first report of NPY inhibiting an I_{Ca} by the activation of a Y_1 receptor. Y_1 -receptor activation has until now been associated with the activation or potentiation of different physiological processes (Michel, 1991; Wahlestedt and Reis, 1993; Wiley et al., 1993). Our observations suggest that the role of Y_1 receptors in the CNS may differ from that in other tissues (Michel, 1991; Wiley et al., 1993; Xiong et al., 1993).

The imaging experiments did not show the elevation in resting $[Ca^{2+}]_i$ of DGCs with NPY that we had first hypothesized, but instead demonstrated the inhibition of depolarization-induced increases in $[Ca^{2+}]_i$ in their somata and dendrites. We did not observe a change in the holding current during the application of NPY to intact cells in slices, making it unlikely that NPY activates or potentiates a potassium conductance in these cells (Zidichouski et al., 1990; Illes et al., 1993), in agreement with previous observations (Klapstein and Colmers, 1993). There was also no evidence of a direct excitatory effect (Brooks et al., 1987). The simplest explanation for these observations is that NPY inhibits a VDCC in DGCs, and the experiments on the acutely dissociated DGCs support this conclusion.

In the NPY-responsive DGCs to which selective agonists were applied, $[Leu^{31}Pro^{34}]NPY$ and PYY always inhibited I_{Ca} . In contrast, NPY_{13-36} was inactive in a majority of NPY-sensitive DGCs. Because NPY_{13-36} is inactive at Y_1 receptors, with the highest activity at Y_2 and less activity at Y_3 receptors (Michel et al., 1991; Foucart et al., 1993), it can be concluded that in a majority of DGCs I_{Ca} is inhibited via a Y_1 receptor-mediated mechanism. In DGCs that responded to NPY_{13-36} , NPY must be activating more than one receptor, but no single agonist used here is entirely selective for any one receptor subtype (Michel, 1991; Dumont et al., 1992). The exact combination of receptors present on these DGCs may involve the combination of any two or perhaps all three receptor subtypes. Because all NPY-sensitive DGCs seem to be responsive to $[Leu^{31}Pro^{34}]NPY$ and most respond to PYY, we believe that Y_1 receptors are likely to be present on the cells that also respond to NPY_{13-36} . These observations agree with binding studies showing a preponderance of Y_1 -binding sites in rat dentate gyrus, with far fewer Y_2 sites (Dumont et al., 1990, 1993; Aicher et al., 1991). The present observations are of potential importance because the majority of the NPY binding in rat cortex is Y_1 (Dumont et al., 1993).

In most PNS neurons in which it has been studied, I_{Ca} inhibition by NPY is mediated by a Y_2 receptor (Bleakman et al., 1991, 1992; Wiley et al., 1993), although in one case, Y_3 receptors also seemed to inhibit I_{Ca} (Foucart et al., 1993). Y_1 -receptor activation, by

Figure 5. NPY inhibits an N-type I_{Ca} in acutely dissociated DGCs. **A**, Details of the experiment are as in Figure 3. The Y_1 agonist $[Leu^{31}Pro^{34}]-NPY$ inhibited I_{Ca} by 44%. Application of $5 \mu M$ ωCTX GVIA reduced I_{Ca} by 60% and entirely occluded the response to the subsequent application of NPY. Similar results were obtained for NPY in seven additional cells and in one other cell for $[Leu^{31}Pro^{34}]-NPY$. **B**, Inhibition of I_{Ca} by NPY in seven neurons (left bars; $42 \pm 6\%$) is completely occluded by $3-5 \mu M$ ωCTX GVIA (+ ωCTX ; $1 \pm 2\%$, $p < 0.02$, Wilcoxon signed rank test). Inhibition of I_{Ca} by NPY (right bars, $26 \pm 5\%$) is not affected by treatment with $3 \mu M$ nimodipine (+Nimodipine, $23 \pm 6\%$, $n = 7$, $p > 0.59$, Wilcoxon signed rank test). **C**, The inhibition of I_{Ca} produced in eight different cells by a second application of NPY (open bar, $16 \pm 3\%$) is reduced by $32 \pm 10\%$ compared with the inhibition produced by the first application of NPY (hatched bar, $24 \pm 3\%$, $n = 8$, $p < 0.02$, Wilcoxon signed rank test). **D**, Estimated inhibition of I_{Ca} by NPY. The value of I_{Ca} inhibition in nimodipine corrected for the rundown of the NPY response ($34 \pm 3\%$; Corrected, hatched bar), compared with the value of I_{Ca} inhibition predicted from the nimodipine-sensitive I_{Ca} ($34 \pm 5\%$; Predicted, open bar). These two values do not differ statistically ($p > 0.39$, Wilcoxon signed rank test).

contrast, usually results in a potentiating effect on I_{Ca} . For example, in rat nodose ganglia, Y_1 -receptor activation potentiates the same I_{Ca} that is reduced by Y_2 -receptor activation (Wiley et al., 1993). Y_1 -receptor activation potentiates α_1 -adrenoceptor actions in vascular smooth muscle (Zukowska-Grojec and Wahlestedt, 1993) and potentiates L-type VDCCs in vascular smooth muscle (Xiong et al., 1993). Such Y_1 -mediated potentiations in I_{Ca} contrast with the observed Y_1 -receptor action in the rat DGCs.

Our data demonstrate that NPY inhibits an N-type I_{Ca} in rat DGCs. In every case, the NPY- or $[Leu^{31}Pro^{34}]-NPY$ -mediated inhibition of I_{Ca} was entirely occluded by ω -CTX GVIA but not by nimodipine. The amount of I_{Ca} inhibited by NPY in the presence of nimodipine, when corrected for the rundown of the response, matches the predicted magnitude of inhibition of I_{Ca} if NPY were to have no effect on nimodipine-sensitive VDCCs. NPY inhibits an N-type current in numerous other cell types (for review, see Bleakman et al., 1993; Colmers and Bleakman, 1994). As an aside, it is interesting to note that ω -CTX GVIA inhibited only 20% of

the I_{Ca} in DGCs isolated from the guinea pig hippocampus (Eliot and Johnston, 1994) compared with the 44% inhibition reported here in the rat, suggesting a substantial species difference in these cells.

In the majority of DGCs studied, NPY inhibited I_{Ca} without changing the time course of I_{Ca} . In $\sim 25\%$ of DGCs, however, NPY inhibition of I_{Ca} was accompanied by a slowing of the time course of activation, as has been seen elsewhere (Bean, 1989; Hille, 1994). Here, NPY did not change the voltage range at which I_{Ca} activated or the V_m at which I_{Ca} reached a maximum. Previous investigations in other types of neurons have demonstrated that other neuromodulators can inhibit I_{Ca} by two distinct mechanisms, as NPY seems to do in DGCs (Beech et al., 1992; Formenti et al., 1993; Toselli and Taglietti, 1993; Luebke and Dunlap, 1994). Most of the DGCs here responded to NPY without an apparent alteration in the time course of their I_{Ca} , as others have observed in a proportion of chick sensory neurons (Luebke and Dunlap, 1994). Because of the relative rarity of the altered time

Figure 6. NPY inhibition of I_{Ca} in acutely dissociated DGCs is sensitive to calcium. The left bar (Ca^{2+}) indicates the percentage of NPY-responsive cells recorded with 5 mM Ca^{2+} in the extracellular solution as the charge carrier and low (0.1 mM BAPTA) intracellular Ca^{2+} buffering. The right bar (Ba^{2+}) indicates the percentage of NPY-responsive cells recorded with 5 mM Ba^{2+} in the extracellular solution as the charge carrier and high (20 mM BAPTA) intracellular Ca^{2+} buffering. Numbers within bars indicate the number of observations.

course, we did not perform the depolarizing prepulse experiments necessary to distinguish between the two mechanisms (Luebke and Dunlap, 1994). Additional studies are needed to elucidate the pathways by which the receptor couples to the calcium channels.

Inhibition of I_{Ca} in rat DGCs by NPY also seems to be sensitive to $[Ca^{2+}]_i$. Recording conditions favoring low $[Ca^{2+}]_i$ (i.e., high intracellular BAPTA concentrations) greatly reduce the proportion of cells responding to NPY. Only 23% of DGCs responded to NPY when Ba^{2+} was the charge carrier and internal Ca^{2+} was strongly buffered. However, baclofen reversibly inhibited I_{Ba} in 81% of DGCs tested under the same conditions. When recording conditions were changed to favor higher $[Ca^{2+}]_i$ (i.e., low intracellular BAPTA concentrations), the proportion of DGCs responding to NPY increased to 84%. Recent studies have shown that inhibition of I_{Ca} in neurons is caused by a number of distinct mechanisms (for review, see Hille, 1994). One such mechanism is sensitive to $[Ca^{2+}]_i$. Buffering $[Ca^{2+}]_i$ to very low concentrations either blocks or reduces the inhibition of I_{Ca} by metabotropic glutamate receptors in cultured hippocampal neurons (Lester and Jahr, 1990) and by M_1 acetylcholine and angiotensin II receptors in rat superior cervical ganglion neurons (SCG) (Beech et al., 1991; Shapiro et al., 1994). The mechanism of this Ca^{2+} and BAPTA sensitivity is unknown; however, in rat SCG neurons the Ca^{2+} sensitivity does not involve intracellular stores of Ca^{2+} (Beech et al., 1991). More recently, it has been shown in chick sensory neurons that α_2 adrenoceptors can activate two pathways that inhibit N-type I_{Ca} , one of which does not alter the time course of current activation and which involves protein kinase C (Diversé-Pierluissi et al., 1995). The DGCs here that responded to NPY with a change in I_{Ca} activation kinetics had a larger inhibitory response to NPY. One potential interpretation of this observation is that all DGCs responding to NPY possess the pathway that does not alter the activation kinetics, whereas some have, in addition, the pathway that alters the kinetics of I_{Ca} activation.

Although NPY inhibits an N-type VDCC in rat DGCs via the activation of Y_1 receptors, the biological purpose of this action is not clear. NPY has no evident effect on the synaptic inputs or excitability of DGCs (Klapstein and Colmers, 1993). The DGCs, however, release not only glutamate but also peptides, particularly dynorphin, which they can release from their dendrites as well as from their terminals (Drake et al., 1994). The release of dynorphin from the dendrites of DGCs depends on L- and N-type channels (Simmons et al., 1995). It is tempting to speculate that

the release of NPY in the dentate gyrus will inhibit the release of dynorphin and possibly other peptides (Morris et al., 1988; Goodman and Sloviter, 1993) from DGCs. This would tend to reduce the influence of opioid peptides when levels of activity in the dentate gyrus were high.

Alternatively, or in addition, the regulation of Ca^{2+} influx by NPY could affect the expression of immediate early genes (Morgan and Curran, 1986; Murphy et al., 1991; Ghosh et al., 1994) and/or could act to prevent damaging levels of Ca^{2+} influx under conditions of elevated levels of excitation.

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