Opioid-Like Actions of Neuropeptide Y in Rat Substantia Gelatinosa: Y1 Suppression of Inhibition and Y2 Suppression of Excitation

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Moran, Timothy D., William F. Colmers, and Peter A. Smith. Opioid-like actions of neuropeptide Y in rat substantia gelatinosa: Y1 suppression of inhibition and Y2 suppression of excitation. J Neurophysiol 92: 3266–3275, 2004. First published August 4, 2004; doi: 10.1152/jn.00096.2004. Neuraptic pain that results from injury to the peripheral or CNS responds poorly to opioid analgesics. Y1 and Y2 receptors for neuropeptide Y (NPY) may, however, serve as targets for analgesics that retain their effectiveness in neuropathic pain states. In substantia gelatinosa neurons in spinal cord slices from adult rats, we find that NPY acts via presynaptic Y2 receptors to attenuate excitatory postsynaptic currents (EPSCs) and predominantly on presynaptic Y1 receptors to attenuate glycnergic and GABAergic inhibitory postsynaptic currents (IPSCs). Because NPY attenuates the frequency of TTX-resistant miniature EPSCs and IPSCs, perturbation of the neurotransmitter release process contributes to its actions at both excitatory and inhibitory synapses. These effects, which are reminiscent of those produced by analgesic opioids, provide a cellular basis for previously documented spinal analgesic actions mediated via Y1 and Y2 receptors in neuropathic pain paradigms. They also underline the importance of suppression of inhibition in spinal analgesic mechanisms.

INTRODUCTION

Nociceptive pain, evoked by tissue damage, serves to protect an organism from further injury. By contrast, neuropathic pain, which may be initiated by damage to the nervous system, persists long after the original injury has healed. This type of pain serves no obvious biological purpose. While opioid analgesics effectively manage nociceptive pain, they are of limited use in the management of neuropathic pain. This clinical observation is reflected in experimental models of neuropathic pain where peripheral nerve axotomy decreases μ-opioid receptor expression on dorsal root ganglia (DRG) neurons and decreases immunoreactivity for μ- and δ-opioid receptors in the spinal dorsal horn (de Groot et al. 1999; Zhang et al. 1998). Furthermore, the effect of morphine on N-type Ca2+ currents (I\text{Ca,N}) in DRG cell bodies is reduced after sciatic nerve axotomy (Abdulla and Smith 1998).

By contrast, nerve injury upregulates NPY Y2 receptor expression and increases the ability of Y2 agonists to suppress I\text{Ca,N} in DRG (Abdulla and Smith 1999; Mantyh et al. 1994; Zhang et al. 1994b). Moreover, expression of NPY and its mRNA increase in DRG and dorsal horn after axotomy or chronic constriction injury (Munglani et al. 1996; Wakisaka et al. 1991). Because NPY acts as an analgesic when applied intrathecally, its receptors represent an attractive target for analgesics that may retain their effectiveness in neuropathic pain states (Hua et al. 1991; Xu et al. 1994). Furthermore, the cellular actions of opioids, which involve suppression of calcium currents, decreased transmitter release, and activation of G-protein-coupled, inwardly-rectifying K+ (GIRK) currents (Grudt and Henderson 1998, 1999; Grudt and Williams 1993, 1994; Hori et al. 1992; Yoshimura and North 1983), are shared by NPY (Bleakman et al. 1991; Colmers and Bleakman 1994; Colmers et al. 1991; Qian et al. 1997; Toth et al. 1993; Zidichouski et al. 1990). We have therefore examined the effects of NPY and NPY receptor-selective ligands on dorsal horn neurons of adult rats. Actions of NPY were also compared with those of the μ-opioid agonist, [d-Ala², NMe-Phe⁴, Gly⁻⁹⁷]-enkephalin (DAMGO). We find that NPY acts on a presynaptic Y2 receptor to attenuate excitatory transmission and on a presynaptic Y1 receptor to attenuate both GABAergic and glycnergic inhibitory transmission. Postsynaptic mechanisms may also contribute to the attenuation of inhibition. The majority of excitatory synapses affected by NPY are affected similarly by DAMGO. Both drugs also have similar effects at inhibitory synapses. Because Y1 agonists produce analgesia when administered intrathecally, (Taiwo and Taylor 2002), selective suppression of inhibition in the substantia gelatinosa (SG) may produce an anti-nociceptive effect that is shared by other agonists including opioids (Grudt and Henderson 1998).

METHODS

All experimental procedures complied with the guidelines of the Canadian Council for Animal Care and the University of Alberta Health Sciences Laboratory Animal Services Welfare Committee. Methods for recording from SG neurons in 20- to 40-day-old Sprague-Dawley rats were similar to those described previously (Moran and Smith 2002) except the slices were incubated at 36°C for 1 h prior to recording and then stored at room temperature. As before, animals were anesthetized with an overdose of intra-peritoneal urethan (1.5 g/kg). Spinal cord slices (300 μM) were transferred to the recording chamber and superfused (flow rate: ~1–2 ml/min) at room temperature (~22°C) with 95% O₂-5% CO₂ saturated artificial cerebrospinal fluid (ACSF, in mM) 127 NaCl, 2.5 KCl, 1.2 NaH₂PO₄, 26 NaHCO₃, 1.3 MgSO₄, 2.5 CaCl₂, and 25 d-glucose, pH 7.4. The SGs appeared as translucent bands under infrared differential-interference optics, and cells were patched under visual control. For recording excitatory evoked postsynaptic currents (eEPSCs), bicuculline (10 μM) and strychnine (1 μM) were included to block inhibitory synaptic inputs. For recording evoked inhibitory postsynaptic currents (eIPSCs), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM) and D-2-amino-5-phosphopentanoic acid (d-AP5; 50 μM) were included to block excitatory synaptic inputs. Tetrodotoxin (TTX; 1 μM) was included when recording miniature EPSCs and IPSCs (mEPSC and mIPSC). Pipettes for recording postsynaptic K⁺ currents had resistances of 5–10 MΩ when filled with an internal solution containing (in mM)
130 potassium glutonate, 1 MgCl₂, 2 CaCl₂, 10 HEPES, 10 EGTA, 4 Mg-ATP, and 0.3 Na-GTP, pH 7.2, 290–300 mosM. For recording synaptic currents, a Cs⁺-based internal solution containing (in mM): 140 CsCl, 5 HEPES, 10 EGTA, 2 CaCl₂, 2 Mg-ATP, and 0.3 Na-GTP, pH 7.2, 290–300 mosM was used.

When recording eEPSCs and eIPSCs, the voltage-gated ion channel blocker QX-314 (5 mM) (Strichartz 1973) was included in the internal solution to prevent action potential discharge. QX-314 was omitted from the internal solution when recording postsynaptic K⁺ currents. Membrane potential was clamped at –60 or –70 mV for recording eEPSCs and eIPSCs when a CsCl-based internal was used. The E_Cl was therefore ~0 mV, and eIPSCs appeared as inward currents at a holding potential of –60 or –70 mV. When a K⁺-glucuronate-based internal solution was used, membrane potential was clamped at –60 or –70 mV for recording eEPSCs and postsynaptic K⁺ currents and 0 mV for recording eIPSCs. Under these conditions, E_Cl = –78 mV, so eIPSCs appear as outward currents. eEPSCs were evoked at 0.05 Hz with a bipolar concentric stimulating electrode (FHC, Bowdoin, ME) or a custom-made bipolar, Teflon-coated nichrome stimulating electrode. These were placed on the dorsal root or near the dorsal root entry zone to activate primary afferent fibers. eIPSCs were generated at 0.05 Hz by focal stimulation (positioned 50–100 μm from the cell body) with a patch pipette containing 2 M NaCl. The stimulating electrode was repositioned until a reliable synaptic input to the cell body was found. The orientation of the stimulating electrode, relative to the cell body, varied in the dorsoventral and lateromedial axes. Stimulus intensity was found. The orientation of the stimulating electrode, relative to the cell body was found. The orientation of the stimulating electrode, relative to the cell body, varied in the dorsoventral and lateromedial axes. Stimulus intensity was found. The orientation of the stimulating electrode, relative to the cell body was found. The orientation of the stimulating electrode, relative to the cell body, varied in the dorsoventral and lateromedial axes. Stimulus intensity was found. The orientation of the stimulating electrode, relative to the cell body was found. The orientation of the stimulating electrode, relative to the cell body, varied in the dorsoventral and lateromedial axes. Stimulus intensity was found.

Rapid axonal transport of synaptic vesicles between presynaptic terminals and the cell body was deemed effective at a particular synapse when they produced >20% reversible or partly reversible depression of the evoked synaptic responses. For statistical analysis of drug effects, mean percentage suppression of eEPSCs or eIPSCs was calculated only from responsive cells (i.e., nonresponsive cells that displayed 0 to <20% change in synaptic events during drug exposure were excluded from the calculation of the mean effect). Because relatively high concentrations of peptides were used (300–1000 nM), bona fide responses were easily distinguished from alterations in recording conditions. This was because most peptide responses involved a clear 40–60% suppression of the eEPSC or eIPSC. To avoid possible consequences of receptor desensitization after multiple drug applications, only one neuron per slice was exposed to NPY or its analogues.

Data were acquired and analyzed using pCLAMP 8.0 (Axon Instruments, Burlingame, CA). Figures were produced with Origin 5.0, 6.1 or 7.0 (OriginLab, Northampton, MA) and Adobe Illustrator 10 (Adobe Software, San Jose, CA). Statistical comparisons were made with paired t-test, unless otherwise specified, using GraphPad InStat 3.05 (GraphPad Software, San Diego, CA). Statistical significance was taken as P < 0.05.

Mini Analysis Program software (Synaptosoft, Decatur, GA) was used to distinguish mEPSCs and mIPSCs from baseline noise. Details of this program and its implementation may be found at www.synaptosoft.com. Miniature postsynaptic currents were detected automatically using an amplitude threshold of 10 pA and an area threshold of 15 fC. All detected events were then re-examined and visually accepted or rejected based on subjective visual examination. Mini Analysis Program was used to further analyze the data and to generate figures.

Cumulative distribution plots were generated to compare the effects of various drugs on the amplitude and interevent intervals of mEPSCs and mIPSCs. Cumulative distribution plots ranked individual amplitudes or interevent intervals in order of increasing size and plotted this rank value against the amplitude or interevent interval size. The Kolmogorov-Smirnov two-sample test was used to compare control and drug distributions of amplitudes and interevent intervals. The Kolmogorov-Smirnov method tested the null hypothesis that two independent samples come from populations that are identical with respect to location and distribution. The Kolmogorov-Smirnov test was used because it compared the entire distribution of amplitudes or interevent intervals rather than mean amplitude or mean interevent intervals (Van der Koot 1991). Distributions were considered statistically significant if P < 0.05. For each cell, typically 500 to 2000 events were analyzed. Ten minutes was allowed for stabilization of the cell after patch-rupture. Baseline miniature events were recorded for a further 10–15 min prior to application of NPY (or NPY analogues) for 5–6 min. An obvious effect of the peptide on mEPSC or mIPSC frequency could usually be seen 3 min after the start of wash-in. Miniature events were recorded for a further 5–10 min in the presence of NPY (or NPY analogue).

Neuropeptide Y was from Tocris (Ballwin, MO) or from Peptide Technologies (Montreal, PQ, Canada). The Y1-agonist [Leu³¹, Pro³²]NPY and the Y2-agonist NPY 13–36 were from Tocris. The Y1-selective agonist [F²⁷,P³⁴]NPY and the Y2-selective agonist [ahx⁵–2⁴]NPY were kindly provided by Dr. Annette Beck-Sickinger (Institute for Biochemistry, University of Leipzig, Leipzig, Germany). CNQX, 2-amino-5-phosphonoveric acid (d-AP5), bicuculline and ruthenium red were from Tocris. QX-314 was from Astra Zeneca. Strychine Hydrochloride was from RBI. All other chemicals from Sigma (Oakville, ON, Canada).

**RESULTS**

**Effects of NPY on eEPSCs**

eEPSCs were recorded at a holding potential (V_h) of –70 mV after stimulation of the dorsal root or dorsal root entry zone in the presence of bicuculline (10 μM) and strychine (1 μM). NPY (300 nM or 1 μM) reduced eEPSC amplitude by 45.5 ± 4.6% in 17/24 cells tested (n = 17; P < 0.0001, paired t-test). In the cell illustrated in Fig. 1A, left, the effect of NPY took ~10 min to develop and recovered over a period of 10 min (Fig. 1A, right). However, the time course of action of NPY varied from cell to cell; wash in times ranged from 5 to 10 min and washout times ranged from 10 to 60 min or longer.

The remainder of Fig. 1 illustrates representative recordings of the actions of various Y1- and Y2-selective agonists (left) and the respective time course of each substance on each neuron (right). Data points on these graphs reflect amplitudes of three to six digitally averaged traces. The Y1-selective agonist, [F²⁷,P³⁴]NPY (1 μM; Soll et al. 2001) did not affect eEPSC amplitude in any of the six cells tested (n = 6, P > 0.05, paired t-test; Fig. 1B). By contrast, the mixed Y2/Y5 agonist, NPY 13–36 (300 nM) reduced eEPSC amplitude by 45.5 ± 5.9% (n = 8/14 cells tested, P < 0.005; Fig. 1C). Because NPY 13–36 has been shown to bind to Y5 receptors (Gerald et al. 1996), a more selective Y2 agonist, [ahx⁵–2⁴]NPY (Rist et al. 1996) was also tested. [ahx⁵–2⁴]NPY (500 nM or 1 μM) reduced the amplitude of the eEPSC by 38.6 ± 6.1% (n = 9/13 cells tested, P < 0.005, paired t-test; Fig. 1D). The effects of NPY agonists on eEPSCs are summarized in Fig. 1E.

Although we always attempted to obtain a steady baseline level for eEPSC amplitude for ~10min prior to drug application, some recordings were associated with rundown, and recordings that displayed >40% rundown during 40 min were...
Presynaptic Y2 receptors are involved in NPY suppression of excitatory synaptic transmission

The effect of NPY on paired eEPSCs was examined to characterize further the mechanism by which it reduced eEPSC amplitude. Paired-pulse stimulation can produce either facilitation or depression. Facilitation is thought to reflect an enhancement in neurotransmitter release due to the transient accumulation of Ca^{2+} close to release sites (Zucker and Regehr 2002). By contrast, short-term paired-pulse depression is thought to reflect decreased transmitter release from the presynaptic terminal. This may involve depletion of transmitter stores, failure of the action potential to invade the presynaptic terminal, activation of presynaptic autoreceptors, a reduction in activity-dependent I_{Ca} responsible for transmitter release (Zucker and Regehr 2002) or postsynaptic receptor desensitization (Mennerick and Zorumski 1996; Trussell et al. 1993). A postsynaptic effect of NPY would be assumed to affect the first and second synaptic responses equally, whereas a presynaptic effect would be expected to alter the ratio of synaptic current amplitudes.

In the experiment illustrated in Fig. 2A, two stimuli separated by an interstimulus interval (ISI; 50 ms) resulted in slight paired-pulse facilitation of eEPSCs. In the presence of NPY, however, the amplitude of the second eEPSC was considerably smaller than the first. In Fig. 2B, the eEPSCs have been normalized to the amplitude of eEPSC_1 to better illustrate the change in the paired-pulse ratio. For all neurons studied, the mean ratio of the amplitude of the paired eEPSCs with ISIs of 30–100 ms was 1.6 ± 0.3 (eEPSC_2/eEPSC_1; n = 7). In 7 of 13 cells, NPY (300 nM) decreased the mean ratio of eEPSC_2/eEPSC_1 to 0.8 ± 0.2 (n = 7, P < 0.05, paired t-test, Fig. 2C). This is consistent with a presynaptic effect of NPY.

To test the possibility that NPY exerted an action on the transmitter release process, we examined its effects on TTX-resistant miniature EPSCs (mEPSCs) in the presence of bicuculline and strychnine. NPY (300 nM) reduced the mEPSC frequency (n = 5/7 cells tested; Kolmogorov-Smirnov test, P < 0.05 in all 5 cells, Fig. 2, D and E). By contrast, NPY had no effect on mEPSC amplitude (n = 5/7 cells tested; Kolmogorov-Smirnov test, P > 0.05 in 5 cells, Fig. 2, D and F). Similarly, the Y2 agonist [ahx^5-24]NPY (1 μM) reduced mEPSC frequency in 4/6 cells tested (Kolmogorov-Smirnov test, P < 0.05 in all 4 cells, Fig. 2, G and H), yet had no effect on mEPSC amplitude (n = 4; Kolmogorov-Smirnov test, P > 0.05 in all cells tested, Fig. 2, G and I). This suggests that action potential-independent mechanisms contribute to NPY inhibition of transmitter release and that this effect is mediated via Y2 receptors.

Effects of NPY on evoked inhibitory synaptic transmission

Unless stated otherwise, eIPSCs were recorded at −70 mV after focal stimulation with a patch electrode in the presence of AP5 (50 μM) and CNQX (10 μM). Effects of NPY and Y1- and Y2-selective agonists are illustrated in Fig. 3. Representative recordings of three to six digitally averaged traces are illustrated in the left panels and the time course of effect of each agonist on eIPSCs in each individual neuron is illustrated in the right panels.

FIG. 1. Neuropeptide Y (NPY) and Y2 agonists but not Y1 agonists suppress excitatory evoked postsynaptic currents (eEPSCs) in substantia gelatinosa (SG) neurons. eEPSCs were generated by stimulating the dorsal root or dorsal root entry zone in the presence of 10 μM bicuculline and 1 μM strychnine. Left: averaged current traces; right: graphs to illustrate time course of effect and washout of agonists on each cell. Each point on the plot represents the digitally averaged amplitude of 3–6 eEPSCs generated at 20-s intervals. A: averaged traces (n = 3) of eEPSCs before, during, and after application of NPY (300 nM). B: averaged traces (n = 6) of eEPSCs before, during, and after application of the Y1-selective agonist [F7, P34]NPY (1 μM). Note that [F7, P34]NPY does not affect eEPSC amplitude. C: averaged traces (n = 6) of eEPSCs before, during, and after application of the Y2/Y5 agonist, NPY 13-36 (300 nM). D: averaged traces (n = 6) before, during, and after application of the Y2-selective agonist, [ahx^5-24]NPY (1 μM). Note that [ahx^5-24]NPY strongly suppresses the eEPSC. Neurons were voltage clamped at a holding potential of −70 mV. E: summary bar graph of the change in eEPSC amplitude in response to NPY, Y1, and Y2 agonists. Agonist effectiveness is expressed as a percentage of the baseline eEPSC amplitude recorded just prior to drug application. Note that NPY (300 nM and 1 μM), the Y1/Y5 agonist, NPY 13-36 (300 nM), and the Y2-selective agonist, [ahx^5-24]NPY (1 μM), reduce eEPSC amplitude by −50% (***P < 0.0001, *P < 0.005). The Y1 agonist, [F7, P34]NPY was ineffective.

rejected. In other cells, rundown was quite variable, and because data were collected from cells that displayed 0 to 33% rundown during 40 min of recording, the reported values of the effectiveness of NPY and Y-2 agonists likely reflect an overestimate of their ability to attenuate eEPSC amplitude.
FIG. 3. Neuropeptide Y and Y1 agonists, but not Y2 agonists, suppress eIPSCs in SG neurons. Evoked inhibitory postsynaptic currents (eIPSCs) were generated by focal stimulation with a patch electrode in the presence of 50 μM 2-amino-5-phosphopentanoic acid (AP5) and 10 μM 6-cyano-7-nitroquinoxalene-2,3-dione (CNQX). Left: averaged current traces; right: graphs to illustrate time course of effect and washout of agonists. Each point on the plot represents the digitally averaged amplitude of 3–6 eIPSCs generated at 20-s intervals.

A: averaged traces (n = 10) of eIPSCs before, during, and after application of NPY (300 nM).

B: averaged traces (n = 6) of eIPSCs before, during, and after application of the Y1-selective agonist [F7, P34]NPY (1 μM).

C: averaged traces (n = 6) of eIPSCs before, during, and after application of the Y2/Y5 agonist, NPY 13–36 (300 nM). Note that NPY 13–36 does not affect eIPSC amplitude.

D: averaged traces (n = 6) before during and after application of the Y2-selective agonist, [ahx5–24]NPY (1 μM). Note that [ahx5–24]NPY does not affect eIPSC amplitude. Neurons were voltage clamped at a holding potential of −70 mV.

E: summary bar graph of the change in evoked IPSC amplitude in response to NPY, Y1 and Y2 agonists. Agonist effectiveness is expressed as a percentage of the "baseline" eIPSC amplitude recorded just prior to drug application. Note that NPY (300 nM) and the Y1 agonists, [F7, P34]NPY (1 μM) and [Leu31,Pro34]NPY (1 μM) reduced eIPSC amplitude by ~50%. The Y2/Y5 agonist, NPY 13–36 (300 nM) and the Y2 agonist, [ahx5–24]NPY (1 μM) were ineffective. ***P < 0.001, **P < 0.002, *P < 0.01.

FIG. 2. A–C: suppression of the eEPSC by NPY is accompanied by a change in the paired-pulse ratio and a change in miniature EPSC (mEPSC) frequency. eEPSCs were recorded at −70 mV after dorsal root or dorsal root entry zone stimulation in the presence of 10 μM bicuculline and 1 μM strychnine. A: averaged traces (left; n = 10) of pairs of eEPSCs (50-ms interstimulus interval). NPY (300 nM; right) reduces the amplitude of the eEPSCs and produces a marked change in the paired-pulse ratio. B: the change in the paired-pulse ratio caused by NPY is seen more clearly after normalizing the data traces in A to the amplitude of the 1st eEPSC. C: summary bar graph of the change in paired-pulse ratio (eEPSC2/eEPSC1) induced by NPY (n = 7). D–F: effects of NPY on mEPSCs. D: recordings of mEPSCs in the same cell before and during the application of NPY (300 nM). E and F: cumulative distribution plots of the mEPSC interevent interval and amplitude distribution from the cell illustrated in D. NPY significantly increased the interevent interval without affecting the amplitude distribution. G–I: effects of the Y2-selective agonist, [ahx5–24]NPY on mEPSCs in another cell. G: recordings of mEPSCs in this cell before and during application of [ahx5–24]NPY (1 μM). H and I: cumulative distribution plots of the mEPSC interevent interval and amplitude distribution from the cell illustrated in G. [ahx5–24]NPY significantly increased the interevent interval without affecting the amplitude distribution. For all illustrations in this figure, V_h = −70 mV using a CsCl-based pipette solution.
NPY (300 nM) reduced eIPSC amplitude by 42.7 ± 6.5% in 7/9 cells tested (n = 7; P < 0.0001, paired t-test; Fig. 3A). The Y1-selective agonist, [F7,P34]NPY (Soll et al. 2001) (1 μM) reduced eIPSC amplitude by 52.0 ± 4.7% in 6/12 cells tested (n = 6; P < 0.0002, paired t-test; Fig. 3B). Similarly, the Y1/Y5 agonist [Leu31,Pro34]NPY (500 nM) (Gerald et al. 1996) reduced eIPSC amplitude by 48.1 ± 13.3% in 5/9 cells tested (n = 5; P < 0.01, paired t-test, data not shown). By contrast, the Y2/Y5 agonist, NPY 13–36 (300 nM; n = 14, Fig. 3C) and the selective Y2 agonist [ahx3–24]NPY (n = 4, Fig. 3D) did not affect eIPSC amplitude. Figure 3E summarizes the effects of NPY agonists on eIPSCs.

The amplitude of eIPSC displayed a certain amount of rundown, but this was generally less than that seen with eEPSCs. Because data were collected from cells in which eIPSC rundown ranged from 0 to 25% in 40 min, our measurements of the effectiveness of NPY and Y1 agonists represent a slight overestimate.

**Mechanism of Y1 receptor suppression of inhibitory synaptic transmission**

Paired-pulse experiments with eIPSCs were complicated by the duration of the events studied. With interevent intervals >100 ms, little or no paired-pulse facilitation or suppression could be seen. It was therefore necessary to use shorter intervals but this meant that the second eIPSC occurred before the first had completely abated. The amplitudes of the first and second eIPSCs were therefore measured from “foot to peak.” Even with short interevent intervals, responses were associated with considerable inter-trial variability. For example, in a trial where the first pair of stimuli exhibited paired-pulse facilitation, the next pair of stimuli exhibited either paired-pulse depression or facilitation. When 10–20 successive paired eIPSCs were averaged, the response usually exhibited overall paired-pulse facilitation, but the mean ratio of the amplitude of the paired eIPSCs was only 1.04 ± 0.11 (eIPSC2/eIPSC₁; n = 5).

In 5/8 cells, NPY (300 nM) decreased the mean ratio of eIPSC2/eIPSC₁ to 0.79 ± 0.11 (n = 5, P < 0.003, paired t-test). These data are consistent with a presynaptic action of NPY. In the cell illustrated in Fig. 4A, there is an initial pronounced paired-pulse facilitation that is clearly attenuated in the presence of NPY. In Fig. 4B, the eIPSCs have been normalized to the amplitude of eIPSC₁ to better illustrate the change in the paired-pulse ratio. Figure 4C summarizes the effect of NPY on the paired-pulse ratio of eIPSCs.

NPY reduced the TTX-resistant mIPSC frequency (n = 6/9 cells tested; Kolmogorov-Smirnov test, P < 0.05 for all 6 cells, Fig. 4, D and E). In 4/6 cells responding to NPY, there was no effect on mIPSC amplitude distributions (n = 4; Kolmogorov-Smirnov test, P > 0.05 for all cells studied, Fig. 4F). However, in two of these six cells, NPY also reduced mIPSC amplitude (n = 2; Kolmogorov-Smirnov test, P < 0.05 for both cells, data not shown). These observations suggest that in some cells, NPY may exert both post- and presynaptic actions, and the latter effects may involve a direct effect of NPY on the release process.

The Y1 agonist [F7,P34]NPY (1 μM) reduced both mIPSC frequency (n = 4/7 cells tested; Kolmogorov-Smirnov test, P < 0.05 for all 4 cells, Fig. 4, G and H), and mIPSC amplitude (n = 4/7 cells tested; Kolmogorov-Smirnov test, P < 0.05 for all 4 cells, Fig. 4I). This is consistent with the presence of Y1 receptors on presynaptic inhibitory terminals and likely also postsynaptically on SG neurons. (Zhang et al. 1994a, 1999).
NPY actions on GABA- and glycine-mediated synaptic currents

Because GABA- and glycine-mediated events are generated by focal stimulation in dorsal horn (Chery and de Koninck 1999; Grudt and Henderson 1998), it is possible NPY may have differentially affected GABA- and glycine-mediated eIPSCs. We therefore compared its effect on pharmacologically isolated GABA-mediated eIPSCs with its effect on glycine-mediated eIPSCs.

Focal stimulation in the presence of strychnine (1 μM), AP5, and CNQX generated eIPSCs that were GABAergic as they were antagonized by bicuculline (Moran 2003). NPY (300 nM) reduced the amplitude of these GABAergic eIPSCs by 58.5 ± 2.0% (n = 3; P < 0.001, paired t-test; Fig. 5A). Focal stimulation in the presence of bicuculline (10 μM), AP5, and CNQX generated glycine-mediated eIPSCs that were antagonized by strychnine (Moran 2003). NPY (300 nM) reduced the amplitude of the glycine-mediated eIPSC by 35.3 ± 4.2% (n = 3; P < 0.005, paired t-test; Fig. 5B). The effect of NPY on GABA- and glycine-mediated eIPSCs is consistent with the observation that GABA and glycine are often co-released from interneurons in the spinal cord (Chery and de Koninck 1999; Jonas et al. 1998; Li et al. 1999).

Comparison of the actions of NPY with the μ-opioid agonist, DAMGO

Because intrathecal application of NPY produces analgesic effects (Naveilhan et al. 2001; Taiwo and Taylor 2002), we compared its actions with those of the μ-opioid agonist, DAMGO.

The effects of NPY and DAMGO on the eEPSC were similar. NPY reduced the amplitude of the eEPSC by 45.5 ± 4.6% (n = 17/24; P < 0.0001, paired t-test) and DAMGO reduced it by 59.4 ± 7.4% (n = 9/9; P < 0.0005, paired t-test). Figure 6A illustrates superimposed recordings of digitally averaged eEPSCs before, during, and after application of 1 μM NPY, and B illustrates the effect of 1 μM DAMGO on eEPSCs in the same cell. Time course of effects of the two agonists on this cell are illustrated in Fig. 6C.

For cells tested with both NPY and DAMGO, DAMGO reduced eEPSC amplitude in every cell tested (n = 9/9), whereas NPY reduced eEPSC amplitude in almost all of the cells tested (n = 7/9). Thus no cell responded exclusively to NPY and not to DAMGO.

Postsynaptic actions of NPY

NPY activates an inwardly-rectifying K+ conductance in a variety of neuron types (Sun and Miller 1999; Sun et al. 2001; Zidichouski et al. 1990), and a similar effect was seen in some SG neurons. NPY (300 nM-1 μM) activated an inwardly rectifying conductance in 14/53 cells tested (26.4%). This was reflected by a 49.0 ± 6.0 pA increase in current at −140 mV (Fig. 6, D–F). Because the postsynaptic response to NPY was observed infrequently, it was not feasible to investigate the receptor subtype involved. Similarly and in confirmation of previous reports, DAMGO (1 μM) activated an inwardly rectifying K+ current of 60.5 pA ±10.0 pA at −140 mV in 8/32 (25%) cells tested (Fig. 6, G–I) (Grudt and Williams 1994; Schneider et al. 1998). The magnitude of the current activated by NPY was not statistically different from that activated by DAMGO and the number of cells that responded to either the agonist was similar. However, only 2/27 cells exhibited postsynaptic responses to both NPY and DAMGO, suggesting that there may be differential expression of NPY receptors and μ-opioid receptors on the cell bodies of SG neurons.

Discussion

NPY has analgesic actions when applied intrathecally. The present results suggest that this may arise, at least in part, from effects on synaptic transmission in the SG. We find that NPY acts at a presynaptic Y2 receptor to attenuate excitatory synaptic transmission and at a presynaptic Y1 receptor to attenuate inhibitory synaptic transmission in rat SG. Postsynaptic Y1 receptors may also be involved in this effect on inhibitory transmission, but no evidence was found for involvement of a postsynaptic Y2 receptor in attenuation of excitatory transmission. In addition, NPY suppressed GABAergic and glycine-mediated inhibitory transmission equally. The presynaptic effect of NPY on excitatory transmission was similar to the μ-opioid agonist, DAMGO, both in terms of efficacy and site of action; NPY and μ-opioids had presynaptic effects at the same synapses. NPY also acted on postsynaptic receptors and activated an inwardly rectifying conductance. The magnitude of the NPY response...
was similar to DAMGO. However, NPY- and µ-opioid receptors were rarely co-localized on the same postsynaptic membrane.

**Y2 suppression of excitatory synaptic transmission**

The finding that presynaptic Y2-receptor activation suppressed EPSCs is supported by the paired-pulse data with NPY and by the effect of NPY and/or the Y2-selective agonist, [Ahx<sup>x-24</sup>]NPY, on mEPSC frequency but not amplitude distribution. Although the decrease in eEPSC<sub>2</sub>: eEPSC<sub>1</sub> ratio seen with in paired-pulse experiments NPY could reflect rapid desensitization of AMPA and/or N-methyl-D-aspartate receptors (Trussell et al. 1993; Mennerick and Zorumski 1996), the mEPSC data are less equivocal. Moreover, a putative presynaptic Y2 effect is consistent with binding and in situ hybridization studies that show Y2 receptor expression on primary afferent terminals in lamina II (Mantyh et al. 1994; Zhang et al. 1995) and a report of Y2 suppression of glutamate release from spinal cord synaptosomes (Martire et al. 2000). Because the effects of NPY were slow to develop and very slow to wash out, it was difficult to obtain more than two or three responses in a given neuron. This precluded determination of concentration dependence of NPY effects and complicated studies with antagonists.

Although the precise mechanism of this presynaptic Y2 effect remains to be determined, it is noteworthy that Y2 receptors are found on the cell bodies of small DRG neurons and these receptors are negatively coupled, via G proteins, to N-type calcium channels (Abdulla and Smith 1999; Bleakman et al. 1991; Walker et al. 1988). Therefore it is plausible that these receptors are transported to the central terminals of primary afferents where they couple to N-type calcium channels and thereby regulate transmitter release. NPY decreases Ca<sup>2+</sup> influx through N-type calcium channels in the terminals of sympathetic neurons (Toth et al. 1993). Because NPY and Y2 agonists decreased the frequency of TTX-R mEPSCs (Fig. 2, D and E), they also likely modulate the vesicle release machinery. A similar effect of NPY has been described in the arcuate nucleus of the hypothalamus (Rhim et al. 1997). It should also be mentioned that mEPSCs recorded in our experiments may have originated from local interneurons or descending inputs (Holets et al. 1988) as well as from primary afferent terminals. We therefore cannot rule out the possibility that NPY affects presynaptic effects on terminals of local interneurons, terminals of descending inputs as well as primary afferent terminals via actions on presynaptic Ca<sup>2+</sup> channels and/or release machinery.

**Y1 suppression of inhibitory synaptic transmission**

The finding that presynaptic Y1 receptors suppressed inhibitory synaptic transmission is supported by the paired-pulse experiments with NPY. Because the ratio of eIPSC<sub>2</sub> to eIPSC<sub>1</sub> was decreased, there is again a concern about possibility of rapid GABA/glycine receptor desensitization in the presence of NPY. It was also necessary to evoke the second eIPSP before the first had completely abated (see Fig. 4A) as paired-pulse facilitation could not be reliably attained with longer intervals. Because the currents are additive and eIPSCs were measured from foot to peak, the measurements likely give a reasonable measure of how much transmitter is released. Because the ratio of eIPSC<sub>2</sub> to eIPSC<sub>1</sub> always changed in the presence of NPY, its effects are best explained in terms of a presynaptic site of action. Moreover, NPY and the Y1-selective agonist, [F<sub>7</sub>, P<sub>34</sub>]NPY also affected mIPSC frequency. The mIPSC frequency decrease caused by [F<sub>7</sub>, P<sub>34</sub>]NPY is consistent with a report of presynaptic Y1 receptors in the dorsal horn (Brumovsky et al. 2002). It is also consistent with the possibility that a direct effect on release process contributes to Y1 inhibition of neurotransmitter release.
In some cells, NPY and [F7, P34]NPY also decreased mIPSC amplitude distributions, suggesting an additional postsynaptic action. This is consistent with reports that Y1 receptors are localized to the dendrites of somatostatin (SST)-positive dorsal horn neurons (Zhang et al. 1994a, 1999). SST-containing neurons in the dorsal horn represent a morphologically heterogeneous population that is composed of both stalked and islet cells (Ribeiro-da-Silva and Cuello 1990; Todd and Spike 1993), some of which are thought to be excitatory interneurons (Todd and McKenzie 1989; Spike and Todd 1992). Although the postsynaptic mechanism by which NPY or [F7, P34]NPY affect mIPSC amplitude remains to be elucidated, these agonists may have activated an inwardly rectifying K+ conductance in the dendrites of SG neurons. This mechanism has been reported to modulate dendritic excitability in both hippocampal (Drake et al. 1997; Ponce et al. 1996) and cortical neurons (Takigawa and Alzheimer 1999). Another possibility may involve cross-talk between the Y1 receptor and postsynaptic GABA\(_A\) and/or glycine receptors, as has been described for dopamine G-protein-coupled receptors and GABA\(_A\) receptors (Liu et al. 2000).

It is not known if NPY receptors are differentially expressed on GABAergic or glycineergic terminals, but the ability of NPY to suppress both GABA- and glycine-mediated currents is consistent with studies that show these neurotransmitters are often co-localized and -released from synapses in the superficial dorsal horn (Chery and de Koninck 1999; Li et al. 1999; Todd et al. 1996).

**Comparison of NPY and DAMGO**

The actions of NPY and Y2 agonists on excitatory synaptic transmission were quantitatively very similar to those of the \(\mu\)-opioid, DAMGO (Kohno et al. 1999). Because synapses affected by NPY were also affected by DAMGO, this is consistent with the involvement of (presynaptic) Y2 receptors in the modulation of nociceptive information. Several other lines of evidence support this hypothesis. First, NPY and opioid receptor binding sites in the superficial dorsal horn are reduced after dorsal rhizotomy or by capsaicin treatment (Gouarderes et al. 1985; Kar and Quirion 1992). Second, NPY and morphine inhibit the release of substance P from nociceptive primary afferents (Duggan et al. 1991; Jessell and Iversen 1977). Third, NPY and opioid agonists both produce analgesia when applied intracerethally or intracerebroventricularly (Broqua et al. 1996; Hua et al. 1991; Xu et al. 1994).

NPY and DAMGO activated a GIRK conductance of similar magnitude in \(\sim\)25% of the cells tested. However, unlike the effects on excitatory synaptic transmission, cells that expressed postsynaptic NPY receptors did not respond to DAMGO and vice versa, suggesting that NPY and \(\mu\)-opioid receptors are not co-localized on the same postsynaptic membrane. Given the rather low proportion of NPY-sensitive neurons, it was not feasible to carry out a detailed analysis of concentration dependence or receptor subtype dependence of the postsynaptic effect.

The similarity of Y2-mediated suppression of excitatory transmission to \(\mu\)-opioids provides a mechanistic explanation for the analgesic effect of NPY. However, the observation that Y1 agonists selectively suppress inhibitory transmission but still produce analgesia (Broqua et al. 1996; Naveilhan et al. 2001; Taiwo and Taylor 2002) is difficult to reconcile. DAMGO and Y1 agonists have similar effects in that they both suppress eIPSCs in 50–60% of dorsal horn cells (Grudt and Henderson 1998; Marinelli et al. 2002; Moran and Smith 2002). By contrast, high concentrations of the morphine metabolite, morphine 3 glucuronide (M3G), suppresses all inhibitory synapses in the SG (Moran and Smith 2002) and cause hyperalgesia rather than analgesia (Woolf 1981). This implies that, regardless of the exact circuitry involved, suppression of a fraction of inhibitory synapses, as is seen with opioids or Y1 agonists, leads to an analgesic effect, whereas suppression of all synapses, as seen with M3G, can produce hyperalgesia.

**Comparison with in vivo studies**

The overall spinal actions of NPY are complex (Broqua et al. 1996; Hua et al. 1991; Taiwo and Taylor 2002; Xu et al. 1994) as it has a biphasic dose-effect curve with pro-nociceptive actions at low doses and anti-nociceptive actions at high doses (Xu et al. 1999; Xu et al. 1994). Despite this, the analgesic effect of NPY appears to be mediated via Y1 receptors in intact rats, while Y1 and Y2 mediate its anti-nociceptive actions after axotomy (Xu et al. 1999). Further support for Y1-mediated anti-nociception is provided by Y1 receptor knockout mice, which have increased pain responses to a variety of modalities before and after nerve injury (Naveilhan et al. 2001).

In summary, NPY and receptor-specific agonists potentially modulate synaptic transmission in the SG. Y1 receptors selectively suppress inhibitory transmission by pre- and postsynaptic mechanisms, whereas Y2 receptors suppress excitatory transmission by a presynaptic mechanism. NPY also activates an inwardly rectifying conductance in a population of SG neurons. These effects of NPY are comparable to the actions of \(\mu\)-opioids and suggest NPY has promise as an analgesic for the treatment of neuropathic pain.

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**References**


Drake CT, Bausch SB, Milner TA, and Chavkin C. GIRK1 immunoreactivity is present predominantly in dentinoids, dentinoid spines, and somata in the CA1 region of the hippocampus. *Proc Natl Acad Sci USA* 94: 1007–1012, 1997.


Drake CT, Bausch SB, Milner TA, and Chavkin C. GIRK1 immunoreactivity is present predominantly in dentinoids, dentinoid spines, and somata in the CA1 region of the hippocampus. *Proc Natl Acad Sci USA* 94: 1007–1012, 1997.


Drake CT, Bausch SB, Milner TA, and Chavkin C. GIRK1 immunoreactivity is present predominantly in dentinoids, dentinoid spines, and somata in the CA1 region of the hippocampus. *Proc Natl Acad Sci USA* 94: 1007–1012, 1997.


Drake CT, Bausch SB, Milner TA, and Chavkin C. GIRK1 immunoreactivity is present predominantly in dentinoids, dentinoid spines, and somata in the CA1 region of the hippocampus. *Proc Natl Acad Sci USA* 94: 1007–1012, 1997.


Drake CT, Bausch SB, Milner TA, and Chavkin C. GIRK1 immunoreactivity is present predominantly in dentinoids, dentinoid spines, and somata in the CA1 region of the hippocampus. *Proc Natl Acad Sci USA* 94: 1007–1012, 1997.


Drake CT, Bausch SB, Milner TA, and Chavkin C. GIRK1 immunoreactivity is present predominantly in dentinoids, dentinoid spines, and somata in the CA1 region of the hippocampus. *Proc Natl Acad Sci USA* 94: 1007–1012, 1997.


Drake CT, Bausch SB, Milner TA, and Chavkin C. GIRK1 immunoreactivity is present predominantly in dentinoids, dentinoid spines, and somata in the CA1 region of the hippocampus. *Proc Natl Acad Sci USA* 94: 1007–1012, 1997.


Drake CT, Bausch SB, Milner TA, and Chavkin C. GIRK1 immunoreactivity is present predominantly in dentinoids, dentinoid spines, and somata in the CA1 region of the hippocampus. *Proc Natl Acad Sci USA* 94: 1007–1012, 1997.


Drake CT, Bausch SB, Milner TA, and Chavkin C. GIRK1 immunoreactivity is present predominantly in dentinoids, dentinoid spines, and somata in the CA1 region of the hippocampus. *Proc Natl Acad Sci USA* 94: 1007–1012, 1997.