Neuropeptide Y Attenuates Naloxone-Precipitated Morphine Withdrawal via Y5-like Receptors

DAVID P. D. WOLDBYE, KRISTIAN KLEMP and TORSTEN M. MADSEN

Laboratory of Neuropharmacology, Department of Pharmacology, University of Copenhagen, (D.P.D.W., K.K.); Laboratory for Experimental Neuropsychiatry, Department of Psychiatry, University Hospital, Rigshospitalet, Copenhagen (T. M. M.), Denmark.

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ABSTRACT

The effects of intracerebroventricular injection of neuropeptide Y (NPY) and various NPY-related peptides were studied on naloxone-precipitated withdrawal from morphine in rats. The withdrawal reaction was assessed using an overall motor score, including jumping, wet dog shakes and other motor-related signs as well as a nonmotor score. At doses of 3, 6 or 12 nmol, NPY strongly and dose-dependently reduced the motor score. A less prominent inhibitory effect was revealed on the nonmotor score. At 6 nmol, [Leu31,Pro34]-NPY, NPY 3-36 peptide YY and human pancreatic polypeptide all significantly attenuated the motor score, whereas NPY 13-36 was without effect. This pharmacological profile suggests that the ant withdrawal effect of NPY is mediated via the recently cloned Y5-like receptor. Our data are consistent with a potential role for NPY and Y5-like receptors in basic mechanisms and as a therapeutic target in opioid dependence and withdrawal.

The study of opioid dependence holds the promise of leading to pharmacological treatments for addiction as well as gaining important knowledge of neural mechanisms of motivated behaviors. In experimental animals previously treated with opioids, the precipitation of a range of withdrawal signs by acute injection of opioid antagonists is considered a model for studying opioid dependence (Blasig et al., 1973; Koob et al., 1992; Martin et al., 1963). NPY is a 36 amino acid polypeptide that is widely and abundantly distributed in the central nervous system (Tatemoto et al., 1982). Several characteristics of NPY are suggestive of a potential role in treatment or in basic mechanisms of opioid dependence. For instance, NPY neurons and/or binding sites are located in brain regions (Chronwall et al., 1985; de Quindt and Emson, 1986; Dumont et al., 1992) implicated in expression of opioid withdrawal, e.g., periaqueductal gray, hypothalamus and locus coeruleus (Koob et al., 1992; Maldonado et al., 1992). Central administration of NPY and NPY-related peptides causes analgesia (Broqua et al., 1996; Hua et al., 1991; Pich et al., 1990) and anxiolysis (Wahlestedt and Reis, 1993), effects compatible with an inhibitory effect on opioid withdrawal. Reduced levels of NPY in cerebral cortex and nucleus accumbens resulting from repeated cocaine injections have been suggested to be causally involved in expression of cocaine withdrawal (Wahlestedt et al., 1991). By analogy, reduced levels of NPY in hypothalamus and striatum after chronic morphine or codeine treatment (Pages et al., 1991, 1992) might also be involved in opioid withdrawal. In feeding, which in its basic pattern bears resemblance to that of opioid addiction, there is evidence for a central interaction between NPY and opioids. Thus central administration of NPY or opioids increases feeding, and naloxone decreases NPY-induced feeding (Levine and Morley, 1984; Levine et al., 1985; Stanley and Leibowitz, 1985). Mildly pinching the tail of rats induces eating, a phenomenon known as stress-induced eating (Morley and Levine, 1980). Rats subjected to repeated episodes of stress-induced eating develop an addictive-like state as demonstrated by the display of withdrawal behavior when challenged with naloxone (Morley and Levine, 1980).

NPY receptors are G protein-coupled receptors associated with inhibition of adenylate cyclase (Wahlestedt and Reis, 1993). At least six NPY receptor subtypes have been characterized on the basis of different pharmacological profiles and/or genetic cloning of receptor proteins. The rank order of potency of different NPYergic agonists is as follows (Blomqvist and Herzog, 1997; Gerald et al., 1996; Wahlestedt and Reis, 1993; Weinberg et al., 1996): The Y1 receptor binds NPY, PYY ≈ [Leu31,Pro34]-NPY ≈ NPY 3-36, hPP; Y2 binds NPY, PYY ≈ NPY 13-36 ≈ [Leu31,Pro34]-NPY, hPP; Y3 binds NPY ≈ PYY; Y4 (the PP1 receptor) binds hPP ≈ NPY; Y5 (Gerald et al., 1996) binds NPY, PYY ≈ NPY 3-36, [Leu31,Pro34]-NPY, hPP > NPY 13-36; Y6

ABBREVIATIONS: NPY, neuropeptide Y; hPP, human pancreatic polypeptide; PYY, peptide YY; WDS, wet dog shakes; i.c.v., intracerebroventricular.
(originally also termed Y5; Weinberg et al., 1996) binds NPY, PYY \( \geq \) [Leu31,Pro34]-NPY > NPY 13-36 > hPP.

Here, for the first time, it was examined whether i.c.v. administration of NPY and various NPY-related peptides could be used to inhibit naloxone-precipitated morphine withdrawal.

**Materials and Methods**

Male Wistar rats (Mallegården, DK; 280-320 g) kept under standard laboratory conditions were used in this study. Under equithesin (SAD, Denmark; 3.3 ml/kg) anesthesia, a cannula for i.c.v. injection was positioned into the right lateral ventricle (coordinates: 2.0 mm from bregma on the coronal suture of the skull, 4.5 mm below the surface of the skull) and were secured to the skull with jeweler’s screws and a covering of dental cement (Woldbye et al., 1997). After a postoperative recovery period of 3 to 4 days, morphine withdrawal was induced according to a previous protocol (Lee et al., 1993). All rats were given s.c. injections of morphine hydrochloride (Ncymed DAK, Denmark) twice daily (9 a.m. and 9 p.m.) for 3 days with increasing doses on each day (10, 20, 30 mg/kg). On the fourth day, all animals received a single injection of morphine (30 mg/kg, s.c.) increasing doses on each day (10, 20, 30 mg/kg). On the fourth day, all animals received one single injection of morphine (30 mg/kg, s.c.) at 9 a.m. Then, at 11:30 a.m., the rats received one single i.c.v. injection (10 μl) containing 3, 6 or 12 nmol NPY (human synthetic, #N-5017, Sigma, St. Louis, MO; n = 5 in each group), 6 nmol NPY 3-36 (human synthetic, #H-3326, Bachem, Bubendorf, Switzerland; n = 5), 6 nmol NPY 13-36 (porcine synthetic, #N-6521, Sigma; n = 5), 6 nmol hPP (#P-9903, Sigma; n = 5), or vehicle (0.9% saline and 1% bovine serum albumin; n = 17) administered over a period of 1 min. At 12 a.m., all rats received an i.p. injection of naloxone hydrochloride (Sigma, 10 mg/kg) dissolved in 0.9% saline to precipitate morphine withdrawal.

During the next 2 hr, the rats were observed for signs of withdrawal and subsequently killed. The intensity of the withdrawal reaction was assessed by a point scoring technique based on weighting the signs (Lee et al., 1993) with minor modifications. Two types of withdrawal scores were used: a motor score based on motor-related signs (i.e., jumping, WDS, head shakes, writhing, forelimb tremor, digging, genital licking and mastication) and a nonmotor score based on signs not directly involving motor activity (i.e., irritability, diarrhea and weight loss). Jumping was assigned a score of 2 for 1 to 2 times, 4 for 3 to 4 times, 6 for 5 to 6 times, 8 for 7 to 8 times, 12 for 9 to 12 times, 18 for 13 to 16 times, 24 for 17 to 20 times and 36 for more than 20 times. WDS and head shakes scored 1 for 1 to 2 times, 2 for 3 to 4 times, 4 for 5 to 6 times, 6 for 7 to 8 times, 8 for 9 to 10 times, 12 for more than 10 times. Writhing was assigned a score of 2 for 1 to 5 times and 4 for more than 5 times. Forelimb tremor, digging, genital licking and mastication scored 2 for 1 to 5 times, 4 for 6 to 10 times and 6 for more than 10 times. Irritability and diarrhea were given a score of 4 if present and 0 if absent. Weight loss during the 2-hr observation period was scored 4 for a loss of 6 to 10 g, 8 for a loss of 11 to 15 g, 12 for a loss of 16 to 20 g. The sessions were videotaped to aid in the rating of the withdrawal reaction. Rating was done by an observer ignorant as to whether vehicle or an NPYergic agonist had been injected i.c.v. The withdrawal scores and single signs were analyzed using Kruskal-Wallis one-way analysis of variance by ranks followed by Bonferroni-adjusted Mann-Whitney U tests (each experimental group vs. vehicle) or Bonferroni-adjusted Fisher exact tests. A Bonferroni-adjusted P value of less than 5% was considered significant.

**Results**

**Effect on withdrawal scores.** NPY caused a powerful and significant attenuation of the morphine withdrawal motor score at all tested doses (fig. 1). Subsequent analysis of the three doses (not including vehicle values) with Spearman’s rank order correlation coefficient showed this effect to be dose-related (P < .05, Spearman’s r = −0.57) with increasing effect at increasing doses. Significant reductions in the motor score were also seen with 6 nmol NPY, NPY 3-36, [Leu31,Pro34]-NPY and hPP. In contrast, the effect of NPY 13-36 was far from significant (P < .48, nonadjusted value), consistent with mediation via Y5-like receptors.

In addition, there was a tendency for all tested doses of NPY to decrease the nonmotor score. However, only pooling of the three NPY dose groups revealed an overall significant reduction by NPY (P < .01 vs. vehicle; Bonferroni-adjusted with a factor 9). PYY significantly reduced the nonmotor score while there was no clear effect with other NPYergic agonists.

**Effect on single withdrawal signs.** The reduction in motor and non-motor scores caused by NPY appeared to be matched by reductions in all rated signs except weight loss (table 1). Similarly, significant reductions or tendencies toward reduction in WDS, headshakes, forelimb tremor, digging, genital licking and writhing, were seen with PYY, [Leu31,Pro34]-NPY, NPY 3-36 and hPP, whereas NPY 13-36 appeared either less potent or without effect. In contrast, jumping, irritability and diarrhea, appeared reduced by NPY 13-36 and other NPY-related peptides but not by hPP.

**Discussion**

Our study shows that i.c.v. administration of NPY causes a powerful and dose-dependent attenuation of morphine withdrawal in rats. This effect was most pronounced on the withdrawal motor score. At 6 nmol, NPY 3-36, [Leu31,Pro34]-NPY, hPP, and PYY all significantly reduced the motor score whereas NPY 13-36 was ineffective, suggesting that the overall antiwithdrawal effect is mediated via Y5 receptors. Consistent with this conclusion, several brain regions containing Y5 receptor mRNA (Gerald et al., 1996; Gustafson et al., 1996) have been implicated in morphine withdrawal either because physical withdrawal can be precipitated by focal ischemia.
application of opioid antagonists or because lesioning attenuates withdrawal. Examples of this include anterior and ventromedial hypothalamus (Kerr and Pozuelo, 1971; Maldonado et al., 1992), central amygdala (Calvino et al., 1979; Maldonado et al., 1992; Tremblay and Charton, 1981), midline thalamic nuclei (Maldonado et al., 1992; Tremblay and Charton, 1981; Wei et al., 1972), periaqueductal gray (Yaksh, 1979), nucleus raphe dorsalis (Klatt et al., 1996). Several Y5 mRNA expressing regions (Gerald et al., 1996; Gustafson et al., 1996). Consequently, Y5 receptor levels are probably so low in this region that locus coeruleus is not likely to be important for antidiarrheal effects of NPY.

We recently reported that Y5 receptors also appear to mediate inhibitory effects on seizures as induced by kainic acid (Woldbye et al., 1997). The exact location of these “anti-seizure receptors” remains to be determined, but the hippocampus is one likely possibility, because this region contains a high concentration of Y5 mRNA (Gerald et al., 1996) and is considered a primary focus of kainic acid seizures (Sperk, 1994). WDS were first described in rats during morphine withdrawal (Martin et al., 1963) but also occur in relation to seizures involving the hippocampus (MacLean, 1957). We previously showed that i.c.v. administration of NPY suppresses WDS accompanying hippocampal seizures (Woldbye et al., 1996). In fact, this observation prompted us to examine the effects of NPY on morphine withdrawal in the first place. However, the hippocampus appears not to be involved in mediating withdrawal-related WDS or other signs of opioid withdrawal attenuated by NPY in our study (Mitchell et al., 1990; Tremblay and Charton, 1981). Consequently, hippocampal NPY receptors are not likely to be targets for antidiarrheal actions of NPYergic agents. Consistent with an overall antiwithdrawal Y5-like receptor mechanism, most individual withdrawal signs, including WDS, appeared reduced via Y5-like receptors. Interestingly, as for jumping, irritability, and diarrhea, the “antiwithdrawal pharmacological profile” seemed similar to that of Y6 receptors cloned in mice (Weinberg et al., 1996), NPY 13–36 appearing more potent than hPP. However, the Y6 receptor gene is absent from the rat genome (Blomqvist and Herzog, 1997). One explanation for this discrepancy might be that NPYergic antagonism of morphine withdrawal might also at least in part be related to NPY-induced anxiolysis (Wahlestedt and Reis, 1993). Although previous studies have ascribed this anxiolytic effect of NPY to Y1 receptors (Heilig et al., 1993; Wahlestedt et al., 1993), these studies do not rule out involvement of Y5 receptors.

Our study suggests that Y5-like receptors might form a potentially novel therapeutic target in opioid withdrawal and dependence. Because Y5 receptors are mainly located in the central nervous system (Gerald et al., 1996), Y5 agonists may turn out to have only few peripheral side effects. As a potential central side effect, Y5 agonists might cause weight gain. Thus the powerful feeding stimulatory effect of NPY (Levine and Morley, 1984; Stanley and Leibowitz, 1985) appears to be mediated via hypothalamic Y5 receptors (Gerald et al., 1996). In addition, because NPY administered into the nucleus accumbens causes place preference (Josselyn and Beninger, 1993), NPYergic agonists could themselves be subject to abuse. However, nucleus accumbens does not appear to con-
tain Y5 receptors (Gerald et al., 1996; Gustafson et al., 1996), and, consequently, this might not necessarily be a problem.

Chronic morphine treatment is associated with decreased levels of NPY in hypothalamus (Pages et al., 1991). Decreased brain levels of NPY have been implicated in basic mechanisms of cocaine withdrawal (Wahlestedt et al., 1994). The present data suggest that decreased NPYergic neurotransmission might also be involved in basic mechanisms of opioid dependence. In other words, exogenous application of NPYergic agents might be antagonizing withdrawal by correcting a deficit in NPY in hypothalamus and possibly other areas.

In conclusion, our study demonstrates that NPY causes a powerful attenuation of morphine withdrawal via Y5-like receptors, suggesting that NPY and Y5-like receptors deserve attention with regard to basic mechanisms and possible therapeutic potential in opioid dependence and withdrawal.

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Send reprint requests to: Dr. David P.D. Woldbye, Laboratory of Neuropharmacology, Department of Pharmacology, University of Copenhagen, 3 Blegdamsvej, Bldg. 2200, Copenhagen N, Denmark.