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Effects of Neuropeptide Y on the Discriminative Stimulus and Antinociceptive Properties of Morphine

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PICKER, M. J., R. M. ALLEN, D. MORGAN, A. S. LEVINE, E. O'HARE AND J. P. CLEARY. *Effects of neuropeptide Y on the discriminative stimulus and antinociceptive properties of morphine.* PHARMACOL BIOCHEM BEHAV **64**(1) 161–164, 1999.—Previous research indicates that opioid receptor blockade diminishes the effects of neuropeptide Y (NPY) on feeding and memory. Conversely, NPY attenuates naloxone-precipitated morphine withdrawal. The present study evaluated the effects of NPY on the discriminative stimulus and antinociceptive effects produced by the prototypical mu opioid, morphine. Rats were trained to discriminate 5.6 mg/kg morphine (IP) from saline using a standard two-lever, food-reinforced, drug discrimination procedure. Across a range of doses (3.0, 5.0, and 10 μg), intracerebroventricular (ICV) injection of NPY failed to substitute for, antagonize, or potentiate the discriminative stimulus effects of morphine. A warm-water tail-withdrawal procedure was used to examine the antinociceptive effects of morphine and NPY, alone and in combination. NPY (3.0 and 10 μg, ICV) failed to alter tail-withdrawal latencies from 52° and 56°C water, whereas morphine (1.0–30 mg/kg, IP) produced a dose-related increase in latencies at both water temperatures. A 10-μg dose of NPY also failed to alter the antinociceptive effects of morphine. This study does not support the idea that the discriminative stimulus and antinociceptive effects of morphine are dependent on an NPYergic pathway. © 1999 Elsevier Science Inc.

Morphine Opioids NPY Drug discrimination Antinociception Rats

NEUROPEPTIDE Y (NPY) is a peptide widely distributed in the nervous system, and has potent effects on a variety of behaviors including feeding, locomotion, sex, anxiety, and memory (2–6,9,14,19). Studies with opioid antagonists suggest that some of NPY's actions are influenced by activity in the opioid system (3,11,13). For example, feeding stimulated by an injection of NPY into the paraventricular nucleus of the hypothalamus is diminished by an injection of the opioid antagonist naltrexone into the nucleus of the solitary tract (12). Also, the opioid antagonist naloxone blocks NPY's memory-altering (3) and discriminative stimulus effects (17).

Recent studies also suggest that NPY neurons and binding sites are localized in brain areas associated with the expression of mu opioid withdrawal (10,15) and ICV administration of NPY can dose dependently reduce some signs of naloxone-precipitated withdrawal in morphine-dependent rats (20). In addition, chronic administration of morphine reduces levels of NPY in both the hypothalamus and striatum (18). Such findings suggest that the NPY system may mediate and/or modulate some effects produced by mu opioids.

In contrast, some of the behavioral effects shared by mu opioids and NPY do not share a common mechanism of action. For example, intrathecal administration of NPY produces antinociceptive effects in the hot plate test in rats (7) and the acetic acid writhing test in mice (1); however, these effects are not antagonized by the opioid antagonist, naloxone. The present study further characterizes the relationship between NPY and the mu opioid, morphine, by examining the effects of NPY on the discriminative stimulus and antinociceptive effects of morphine. Both operant (discriminative stim-

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ulus) and reflexive (antinociceptive) behavioral endpoints were chosen to increase the generality of the conclusions.

METHOD

Subjects

Eight experimentally naive male Long–Evans hooded rats were individually housed and maintained on a 12L:12D cycle (light from 0600 to 1800 h) in a temperature- and humidity-controlled room with continuous access to water. During the experiment proper, rats were maintained at approximately 80% of their free-feeding body weight and fed a daily ration of food following the completion of each experimental session.

Apparatus

Drug discrimination sessions were conducted in standard operant conditioning chambers. All eight chambers contained two operant levers, stimulus lights, a house light, and a food pellet dispenser; an exhaust fan and white noise were used to mask extraneous sounds. Scheduling of experimental events and data collection were accomplished through the use of a microcomputer, using software and interfacing supplied by MED Associates Inc. (Georgia, VT). In the antinociception test (warm-water tail-withdrawal), rats were restrained in plastic restraint tubes (Fisher Scientific, Pittsburgh, PA), and tail-withdrawal latencies were measured with a hand-operated digital stopwatch with a time resolution of 0.01 s. Water was maintained at either 52° and 55° C via separate thermostat-controlled water baths (Fisher Scientific).

Drug Discrimination Procedure

Rats were initially trained to press both levers under a fixed-ratio 1 (FR 1) schedule of food delivery. During subsequent preliminary training sessions, the number of responses required to produce food delivery was gradually increased to 20 (FR 20). When all rats responded reliably under the FR 20 schedule discrimination training was initiated, and during these sessions each rat received an injection (IP) of either 1.0 ml/kg saline or 5.6 mg/kg morphine 30 min prior to the start of each session. A random sequence was used to determine which injection was administered, with the restriction that morphine and saline were not given for more than two consecutive sessions, and that across each 30 training sessions the number of morphine and saline injections was approximately equal. This sequence of injections was employed to ensure equal exposure to both training stimuli (morphine and saline). When morphine was administered, 20 responses on the right lever resulted in food delivery, whereas 20 responses on the left lever resulted in food delivery when saline was administered. Lever press responses on the injection-inappropriate lever had no programmed consequences. Sessions were 20 min in duration and conducted 5 days per week.

Once the discrimination was established, which required an average of 30 (range across rats of 25 to 38) saline and 30 (range of 24 to 39) morphine training sessions, substitution and antagonism tests were conducted in which doses of NPY (3.0, 5.0, and 10 μg) or naloxone (0.5, 5.0, and 50 μg) were administered ICV, alone or in combination with IP administered morphine (1.0, 3.0, 5.6, and 10 mg/kg). During test sessions, 20 responses on either response lever resulted in food delivery. During this phase of the experiment, testing typically occurred on Tuesdays and Fridays, while training sessions were continued on Mondays, Wednesdays, and Thursdays.

Antinociception Procedure

A warm-water tail-withdrawal procedure, as described by Morgan et al. (16), was used to measure the antinociceptive effects of morphine and NPY. Briefly, the distal portion of each rats tail was immersed in 36°, 52°, or 56°C water and the tail-withdrawal latency recorded. A 20-s cutoff time was used to prevent tissue damage. The order of water temperature presentation was counterbalanced across rats, with 3 min separating each trail. After baseline latencies were determined, morphine was administered, and 30 min later rats were tested with the 52° and 56°C water. Morphine was administered cumulatively such that each successive injection increased the total drug concentration by 0.5 log unit. For all rats, these tests started at a 1.0-mg/kg dose and terminated at a 30-mg/kg dose. By using this type of testing procedure it was possible to obtain a complete dose–effect function for morphine in a single session [e.g., see (16)]. For combination studies, a single dose of NPY (3.0 and 10 μg) was administered (ICV) immediately prior to the first dose of morphine (1.0 mg/kg, IP). For the time-course experiment, a single dose of NPY (3.0 and 10 μg, ICV) was administered and tests of antinociception conducted at 30, 60, 90, and 120 min post injection. Previous experiments conducted in our laboratory indicate that testing using the warm-water tail-withdrawal procedure can be conducted on multiple occasions (when tests are separated by at least 1 week) without changes in baseline tail-withdrawal latencies or evidence of tissue damage.

Surgery

Following an average of 113 (range across rats of 102 to 126) training and testing sessions on the drug discrimination procedure, rats were anesthetized with sodium pentobarbital (55 mg/kg, IP) and fitted with a 22-gauge guide cannula terminating in the right lateral ventricle. Stereotaxic coordinates were 1.0 mm posterior, 1.5 mm lateral, and 3.5 mm below the horizontal plane of bregma (incisor bar set a 3 mm posterior, 1.5 mm lateral, and 3.5 mm below the horizontal plane of bregma (incisor bar set a 3 mm below the interaural line). Rats were allowed to recover for several days following surgery. Cannula patency was verified periodically by the observation of vigorous drinking 10–15 min after an ICV injection of 10 μg angiotensin II.

Drug Preparation and Administration

NPY (Sigma, St. Louis, MO), naloxone HCl, and angiotensin II (Research Biochemicals Inc., Natick, MA) were diluted with 0.9% sterile saline. NPY and angiotensin II were stored frozen in sealed plastic containers. NPY, naloxone HCl, and angiotensin II injections were given with a Hamilton syringe (5-μl volumes over 30 s). Morphine sulfate (National Institute on Drug Abuse, Rockville, MD) and sodium pentobarbital (Abbott Laboratories, North Chicago, IL) were dissolved in saline (0.9%) and administered IP at an injection volume of 1.0 ml/kg.

Data Analysis

In the drug discrimination procedure, the percentage of injection-appropriate responses emitted prior to the delivery of the first reinforcer, and rate of responding on both levers was calculated during training and testing sessions. In the tail-withdrawal assay, antinociception was computed as follows:

$$\% \text{ Maximal Possible Effect} = \frac{(\text{test latency} - \text{baseline latency})}{(20\text{ s} - \text{baseline latency})} \times 100$$

ED_{50} values (95% confidence limits, C.L.) were determined by fitting a regression line to the ascending limb of the morphine dose–effect curves and deriving the dose that produced a 50% effect by log-linear interpolation.

RESULTS

Drug Discrimination Procedure

During training sessions preceding the determination of the morphine dose–effect curve, administration of the training dose of morphine produced 85% (SE, ±5.4) drug-appropriate responding, whereas administration of saline produced 9% (±3.4%) drug-appropriate responding. Figure 1A shows that morphine produced a dose-related increase in the percentage of drug-appropriate responding. In contrast, NPY produced predominantly saline-appropriate responding with the maximal level of drug-appropriate responding (37%) obtained at the intermediate (5.0 μg) dose. Pretreatment with 10 μg NPY did not alter the morphine dose–effect curve. The ED_{50} values (95% C.L.) for morphine alone and in combination with 10 μg NPY were 2.10 (1.50–2.96) and 2.16 (0.92–5.10) mg/kg, respectively. No drug or drug dose combination tested altered response rate (data not shown).

Figure 1B shows the effects of the training dose of morphine, alone and in combination with selected doses of NPY or naloxone, on the percentage of drug-appropriate responding. Across the dose range examined, NPY failed to antagonize the stimulus effects of morphine, although at the highest dose of NPY (10 μg) the level of drug-appropriate responding produced by the training dose of morphine was reduced from 87 to 71%. Naloxone produced a dose-related antagonism of the morphine stimulus. At the highest dose of naloxone tested (50 μg), the training dose of morphine produced exclusively saline-appropriate responding.

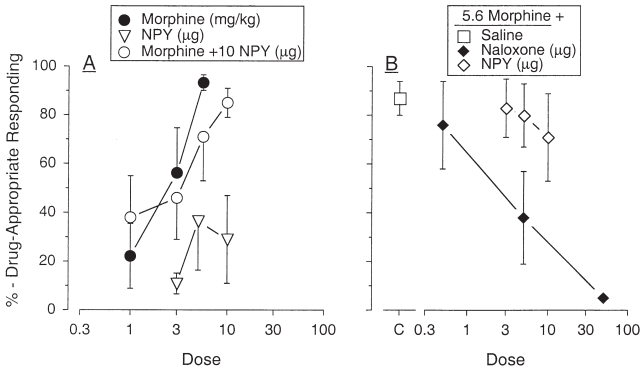

FIG. 1. (A) Effects of morphine (IP) (n = 8 rats per dose) and NPY (ICV) (n = 6 rats per dose) alone and 10 μg NPY in combination with morphine (n = 7 rats per dose) on the percentage drug-appropriate in rats trained to discriminate 5.6 mg/kg morphine from saline. (B) Effects of the 5.6 mg/kg training dose of morphine alone (n = 6 rats per dose) and in combination with NPY (ICV) (n = 6 rats per dose) and naloxone (ICV) (n = 5 rats per dose) on the percentage of drug-appropriate responding in rats trained to discriminate 5.6 mg/kg morphine from saline. The open square above "C" represents the effects of the training dose of morphine in combination with saline (ICV). Ordinates: percentage of drug-appropriate responding. Abscissas: dose expressed in mg/kg or μg/rat. Error bars represent the standard error of the mean; where not indicated the standard error fell within the data point.

TABLE 1

EFFECTS OF NPY (3.0 & 10 μg) AT 30, 60, 90 AND 120 MINUTES FOLLOWING ICV ADMINISTRATION IN RATS TESTED IN THE WARM WATER TAIL-WITHDRAWAL PROCEDURE (ANTINOCICEPTION) USING 52° AND 55°C WATER TEMPERATURES

Dose:	3.0 μg		10 μg	
Temperature:	52°	56°	52°	56°
30 min	7.7 (4.8)	7.6 (2.5)	23.7 (16.3)	12.0 (2.3)
60 min	4.1 (2.2)	3.5 (1.7)	7.9 (5.0)	13.6 (5.7)
90 min	13.6 (7.9)	4.6 (2.2)	3.8 (2.7)	2.3 (1.0)
120 min	1.6 (1.7)	2.4 (0.8)	21.0 (16.0)	10.3 (7.3)

The values represent the percentage of maximal possible effect (±SE) at each time point (all tests were conducted in six rats).

Antinociception Procedure

Baseline tail-withdrawal latencies prior to testing with 3.0 μg NPY alone were 20.0 s (SE, ±0.0) at the 36°C water, 6.7 s (±1.1) at the 52°C water, and 2.6 s (±0.2) at the 56°C water. Similar latencies were obtained prior to testing 10 μg NPY alone, morphine alone, and morphine in combination with 10 μg NPY. Table 1 shows the effects of NPY alone on tail-withdrawal latency at the 52° and 56°C water. At the doses tested, NPY failed to alter tail-withdrawal latencies for up to 120 min following administration.

Figure 2 shows the effects of morphine alone and in combination with NPY on tail-withdrawal latency. When administered alone, morphine dose dependently increased tail-withdrawal latencies at both water temperatures with maximal effects obtained at the 30 mg/kg dose. At both water temperatures, the 10 μg dose of NPY failed to alter the morphine dose–effect curve. The ED_{50} value (95% C.L.) for morphine alone was 5.38 (3.87–7.50) mg/kg at the 52°C water and 7.78 (5.01–12.07) mg/kg at the 56°C water. When morphine was ad-

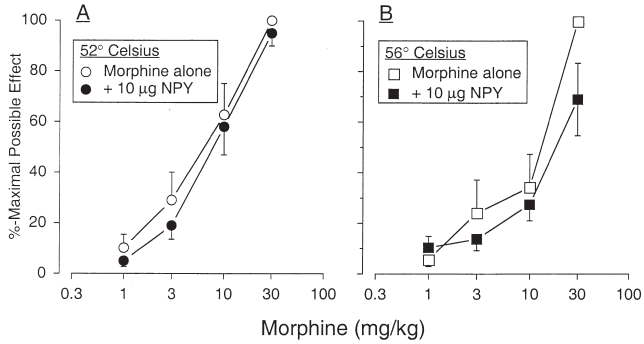

FIG. 2. The effects of morphine (IP) alone and in combination with of a 10μg dose of NPY (ICV) on tail-withdrawal latencies in rats tested on the tail-withdrawal procedure using 52° (A) and 56° (B) C water (n = 7 rats per dose). Ordinates: percentage of maximal possible effect. Abscissas: dose expressed mg/kg. Error bars represent the standard error of the mean; where not indicated the standard error fell within the data point.

ministered in combination with 10 μg NPY, these values were 6.78 (5.19–8.85) and 16.85 (9.58–29.62) mg/kg, respectively.

DISCUSSION

In the present study, doses of NPY that have been shown to markedly enhance feeding behavior (14) failed to produce an antinociceptive response in the warm-water tail-withdrawal procedure. This effect was obtained even under conditions (52°C) in which opioids with minimal efficacy at the mu opioid receptor produce an antinociceptive response (16). Similarly, intrathecal administration of NPY was ineffective at producing antinociception in the rat paw pressure test (7). In contrast, previous studies show that NPY produces antinociceptive effects following intrathecal administration in the rat hot-plate test (7) and following ICV administration in the mouse acetic acid writhing test (1). Collectively, these findings indicate that NPY's antinociceptive effects are dependent on the type of nociceptive stimuli and possibly the route of administration.

As observed in previous investigations [e.g., (16)], morphine produced a dose-dependent increase in antinociception with maximal effects obtained at a 30-mg/kg dose. At the dose examined, NPY failed to alter the antinociceptive effects of morphine. Similarly, NPY failed to generalize to, potentiate, or antagonize the discriminative stimulus effects produced by morphine. It is unlikely that the failure to obtain an interaction between NPY and morphine was a consequence of the poor diffusion of NPY to sites with a high density of NPY receptors, as a major locus on NPY's action is the paraventricular nucleus of the hypothalamus (17). Because this site is in close proximity to the ventricles, it would be expected that sufficient concentrations of NPY at this site would be apparent during testing. Similarly, both systemically and centrally administered morphine is effective in producing an antinociceptive response, and it is well established that this effect is mediated by spinal and supraspinal sites (e.g., periaqueductal gray).

Although NPY's effects are blocked by both systemically and intrahypothalamically administered opioid antagonists (3,12,14,17), the present study suggests that the stimulus effects of NPY are not morphine-like, and although NPY can suppress some symptoms of withdrawal from chronic morphine, it does not alter morphine's discriminative stimulus or antinociceptive effects. Because different anatomical loci and pathways mediate the discriminative stimulus, antinociceptive and withdrawal effects produced by mu opioids [e.g., (8,15, 21)], it remains a possibility that the interaction between NPY and the mu opioid system is specific to certain brain sites.

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