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Intrahypothalamic Discriminative Stimulus Effects of Neuropeptide Y

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O'HARE, E., J. CLEARY, D. T. WELDON, J. D. POMONIS, C. J. BILLINGTON AND A. S. LEVINE. *Intrahypothalamic discriminative stimulus effects of neuropeptide Y*. PHARMACOL BIOCHEM BEHAV **59**(2) 375–378, 1998.—Neuropeptide Y (NPY) is one of the most ubiquitous neurotransmitters in the CNS and has been implicated in a variety of psychological and physiological functions. The current study investigated whether intrahypothalamic (IH) administrations of NPY were behaviorally discriminable from saline injections. Rats were trained to differentially respond based on whether they received IH injections of NPY (0.5 μg/0.5 μl) or saline (0.5 μl 0.9% NaCl). Subjects demonstrated discriminative control (85% correct in 8 out of 10 consecutive sessions) after a mean of 32 sessions. The ability of subjects to discriminate IH NPY from saline was dose dependent, with the lowest NPY dose tested (0.03 μg/0.5 μl) generalizing to saline. The opioid antagonist naloxone blocked the discrimination of NPY when administered IP (3.0 mg/kg) or IH (50 μg/0.5 μl). © 1998 Elsevier Science Inc.

NPY Neuropeptide Y Discrimination Hypothalamus

DRUG discrimination procedures have traditionally been used in the identification of classes of drugs that appear to affect similar receptor populations (4,9,14,16,18). Under such discrimination procedures, the drug state serves as the discriminative stimulus, and a particular response (e.g., lever press) is reinforced after drug administration; an alternative response is reinforced in the absence of the drug (e.g., saline administration is reinforced following a press on a different lever). If the drug has a discriminable effect, the subject learns to respond differentially, based upon whether the drug or saline has been administered prior to the test session. Although the drug discrimination procedure has been applied to many drugs of abuse, few investigations have focused on the discriminability of endogenous neuroactive agents. Our laboratory was the first to demonstrate that neuropeptide Y (NPY) could be discriminated after intracerebroventricular (ICV) administrations (8). In a second study, we found that the subjective effects of centrally administered NPY seemed to be associated with activation of the Y1 receptor, because the Y1 agonist [Leu^{31}, Pro^{34}] NPY resulted in NPY-appropriate responding, whereas a Y2 agonist did not (7).

Operant discrimination of endogenous peptides might be useful for many reasons. For example, the potential testing of whether the interoceptive states associated with pain, hunger, thirst, etc., generalize to the behavioral response following administration of a given peptide of interest. Also, subjects might be trained to discriminate an endogenous peptide after injection into a specific brain nucleus, to evaluate whether injection into another brain nucleus, or other brain region, might result in similar behavioral responding. Such responding might suggest an interaction between receptors in the regions under investigation, or networking to other common areas. It would also be possible to evaluate whether agonists or antagonists of other families of compounds similarly interacted with the compound that the subject was first trained to discriminate.

NPY affects a host of behaviors, including locomotion (12), sex (2), anxiety (6), memory (3,5), and feeding (1,13,20).

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NPY's orexigenic effects are potent and seem to occur most reliably after administration into hypothalamic areas, such as the paraventricular nucleus (19) or the perifornical area (21). The present study evaluated whether intrahypothalamic (IH) administration of NPY could be discriminated by rats; as was the case following ICV administration of NPY.

It is well known that opioidergic pathways interact with NPY-related phenomena. For example, peripheral and central injections of naloxone (NLX) block NPY-induced feeding (11,13,17). Also, ICV administration of selective antagonists of the mu (β-funaltrexamine) and kappa (nor-binaltorphimine) opioid receptors decrease NPY-induced feeding (10). In the current study, we also investigated blockade of opioid receptors, with peripheral and IH naloxone administrations, on the ability of rats to discriminate IH NPY.

METHOD

Animals

Eleven male Sprague–Dawley rats (Harlan: Madison, WI), approximately 90 days old and weighing 250–275 g at the beginning of the experiment were used. They were maintained at 95% of free-feeding body weights through postsession feeding and were housed individually. Water was continuously available in the home cage. The temperature in the vivarium was maintained at 23°C, with lights on between 0700 and 1900 h. Experimental sessions were conducted using six two-lever rat test chambers (model E10-10, Coulbourn Instruments, Inc., Lehigh Valley, PA) enclosed in sound-attenuating compartments. The reinforcer was one 45 mg food pellet (F0021, Bioserv Holton Industries), which was delivered into a tray situated midway between the levers, 4.0 cm above the test chamber floor. A Zeos 486 computer (Zeos: St. Paul, MN) programmed in MED-PC (Med Associates: Fairfield, VT) controlled the experiment and collected data.

Experimental Procedure

Subjects were trained to press both levers in the operant apparatus and were then fitted with permanently indwelling cannulae. They were anesthetized with sodium pentobarbital (40 mg/kg IP), and 26-gauge guide cannulae were inserted into the right side of the brain. Stereotaxic coordinates, with the incisor bar set at 3.5 mm below the interaural line, were 0.75 mm lateral and 1.8 mm posterior to the bregma, to 8 mm below the surface of the skull [atlas of Paxinos and Watson (15)]. Subjects were allowed to recover for 7 days following surgery. Injection cannulae were 32-gauge stainless steel tubing, cut so that when inserted to maximum depth they extended 1.0 mm beyond the guide cannulae tips. The injection cannulae were attached by polyethylene tubing to a fixed needle microsyringe. Following injection, cannulae were withdrawn and 28-gauge stainless steel stylets were placed in the guide cannulae. NPY was stored lyophilized until solubilization with sterile distilled water, at which time aliquots were made and frozen at −15°C. When required for administration, aliquots were thawed at room temperature immediately prior to injection. NLX was disolved in 0.9% sodium chloride solution.

During discrimination training subjects were injected with either 0.5 μg/0.5 μl NPY or 0.5 μl saline (0.9%) 20 min prior to the start of a session. Injections were performed over a 2-min period to allow for dispersion into tissue. Sessions were conducted daily; food and water were not available during the time period following injections. Sessions preceded by NPY or saline administration alternated randomly, with the restriction that no more than two consecutive sessions of saline or NPY occurred. Responses on the right lever were reinforced following NPY administration and responses on the left lever were reinforced following saline administration. Responses on the inappropriate lever produced an 8-s period of darkness, during which no responses were reinforced. The number of responses prior to the delivery of a reinforcer, or imposition of a time out, was gradually increased to 20 (fixed ratio 20).

Subjects were trained until at least 85% of responses prior to the first consequence (reinforcement or time out) occurred on the appropriate lever in 8 out of 10 consecutive sessions. Sessions ended after 25 reinforcers were delivered. As each subject met the training criterion, test doses of 0.3, 0.1, and 0.03 μg/0.5 μl NPY were administered. Test sessions were separated by four consecutive training sessions. Following the collection of dose–response data, each animal also received test sessions involving combinations of NPY and NLX. NLX was administered IP at 3 mg/kg or IH at 50 μg/0.5 μl. NLX was consistently administered 30 min prior to NPY injections. The NLX test sessions were again separated by four consecutive training sessions.

RESULTS

Figure 1 illustrates that subjects successfully discriminated IH injections of NPY from saline. The mean number of sessions required to reach the discrimination criteria (85% in 8 out of 10 consecutive sessions) was 32 sessions (range = 29–40). Fisher's protected tLSD tests revealed significant differences in NPY appropriate responding between saline and all doses of NPY, except 0.03 μg (ps < 0.01). Under combination injections of NPY and IP or IH NLX, animals responded as if they had received saline instead of NPY (ps < 0.01). There were no differences in rates of responding following saline or

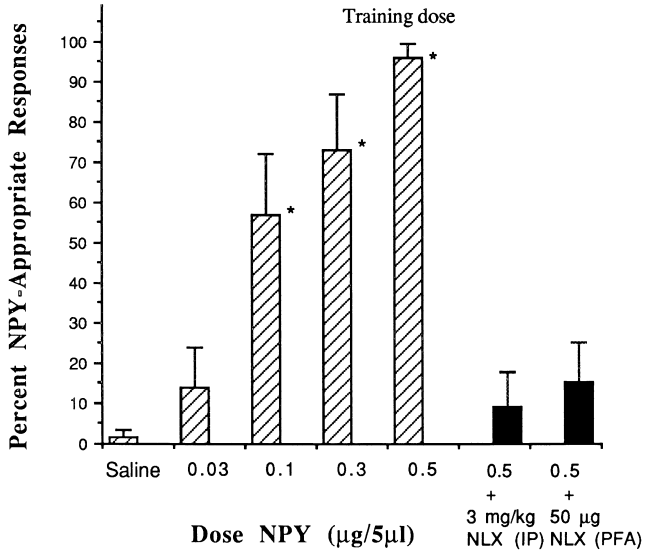

FIG. 1. Mean percent NPY-appropriate responding following IH administration of NPY or saline. Also, mean percent NPY-appropriate responding following administration of 0.5 μg/0.5 μl in combination with peripheral (3.0 mg/kg IP) or central administrations of naloxone (50 μg/0.5 μl). Brackets indicate 1 SEM. Asterisks indicate significant differences from responding under saline administration ($p < 0.01$).

NPY administrations [saline vs. NPY, SEM = 7.83 saline, SEM = 9.41 NPY ($p = 0.88$)].

At the end of this study, after approximately 34 saline and 33 NPY injections, subjects were tested to see if NPY still provoked an enhanced feeding response. All subjects were first injected with 0.5 μl saline at the beginning of the 10th hour of the light period and given free access to food. Under these conditions, food intake 1 h following saline injection was 0.0 g (was unmeasurable). On the following day, under the same conditions, animals were injected with 0.5 μg/0.5 μl NPY and food intake was again measured 1 h after injection. Following NPY administration, subjects consumed an average of 6.3 g lab chow (SEM = 1.5). No subject failed to show enhanced eating under NPY. These data suggest that the cannulae were patent at this time.

Following testing, subjects were overdosed with Nembutal and perfused with ice-cold phosphate-buffered saline. Brains were removed and sectioned (15 μm) through the area of cannula termination, then stained with Nissl or glial fibrillary acidic protein (GFAP) stains. Figure 2 illustrates the effect of multiple IH injections on brain tissue adjacent to cannula termination. Neuron cell density, as measured under Nissl stain in the diffusion area approximate to the cannula termination site, was not significantly different than that on the uncannulated contralateral side. Mean cell count was 175 per 0.1 mm^2 (SE = 15.9) on the cannulated side and 171 per 0.1 mm^2 (SE = 8.9) on the contralateral uncannulated side. GFAP-positive glial response was visible along the cannula tract and at cannula termination, but was less than 5% greater on the cannulated side in the surrounding area of diffusion. In the area surrounding cannula termination, the mean glial count on the cannula side was 136 per 0.1 mm^2 (SE = 9.0), while on the uncannulated side it was 118 per 0.1 mm^2 (SE = 8.5). Figure 3 illustrates the locations of cannulae terminations (filled dots) in a drawing of a coronal section of the rat brain.

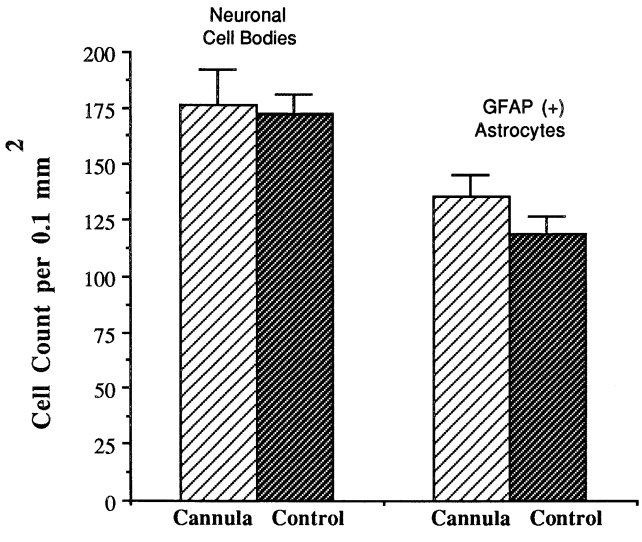

FIG. 2. Mean neuronal cell body counts per 0.1 mm^2 and means positive GFAP astrocyte counts per 0.1 mm^2, on side with cannula placement and on contralateral side without cannula. Brackets represent 1 SEM. Cell counts were taken after a mean of 67 intratissue injections.

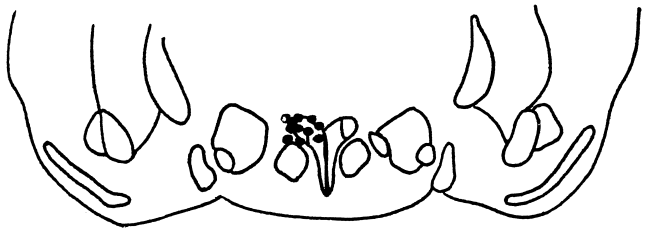

FIG. 3. Drawing of coronal section of rat brain showing cannulae termination areas for 11 subjects. This section is 1.8 mm posterior to bregma.

DISCUSSION

The current study is the first demonstration that discriminative stimulus control can be established by an endogenous neuropeptide injected directly into brain tissue. Previous studies have shown that after drug/saline discriminations are trained using peripheral administration routes, the drugs or their agonists also demonstrate stimulus control after intracerebral injection (14,18). In addition, Jewett and colleagues have shown that NPY can be established as a discriminative stimulus by ICV injection in opposition to ICV saline (7,8).

The establishment of discriminative control following IH administrations of NPY and saline suggests that the hypothalamus contributes to the interoceptive stimuli associated with NPY administration. The current study also found that peripherally and centrally administered NLX blocks the discriminative control apparent following IH NPY administration. NLX has consistently been shown to antagonize the feeding effect elicited following NPY administration (10,11), suggesting that a feeding-related mechanism may contribute to the discriminative stimulus effects of NPY.

Drug discrimination studies are generally conducted to differentiate various drug receptor types. The ability of rats to discriminate central injections of NPY permits investigations as to NPY receptor subtypes, and our laboratory previously reported that an ICV injection of a Y1 agonist was not differentiated from injection of NPY, whereas the effect of a Y2 agonist was different (7). Discrimination of an endogenous regulator, following direct injection into a brain area, may allow for other types of investigations. For example, it might be possible to train subjects to discriminate an injection of a neuropeptide, then inject that peptide, or agonists and antagonists of that peptide's receptor, into other sites to trace possible neural pathways of the discriminative stimulus effect. Also, direct injections permit the use of low drug doses, and potentially reduce actions occurring due to the flooding of multiple brain sites (as is the case with ICV injections).

The current method involves a large number of injections into a selected brain area. Our results show typical injection effects at the termination of the cannulae, but based on neuron and astrocyte cell counts, the multiple injections did not appear to cause noticeable neurotoxicity in the surrounding area of infusion. The fact that NPY still induced a substantial feeding response, even after approximately 70 IH saline or peptide injections, supports this finding. Thus, the current study shows that NPY injected into the hypothalamus is behaviorally discriminable, and that, as both peripheral and IH NLX administrations interfere with the NPY discrimination, this phenomenon appears to be dependent to some extent on an opioidergic mechanism.

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