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Electrical Stimulation of Nucleus Paragigantocellularis Induces Opioid Withdrawal-Like Behaviors in the Rat

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LIU, N., R. W. ROCKHOLD AND I. K. HO. *Electrical stimulation of nucleus paragigantocellularis induces opioid withdrawal-like behaviors in the rat.* PHARMACOL BIOCHEM BEHAV **62**(2) 263–271, 1999.—To examine a role for the medullary nucleus paragigantocellularis (PGi) in mediation of the symptomatology of opioid withdrawal, bilateral electrical stimulation of the PGi was performed in conscious, unrestrained, opioid naïve (nondependent) rats. A characteristic series of behaviors was elicited during each 30-min session of PGi stimulation. The profile of these behaviors resembled qualitatively, but was not quantitatively identical with those seen during precipitated withdrawal from opioid dependence. This behavioral syndrome has been termed, opioid withdrawal-like behavior. The opioid withdrawal-like behaviors were voltage-, but not frequency-, dependent. Tolerance to repeated stimulation of the PGi did not develop following a series of 30-min runs of stimulation over 3.5 h. Intracerebroventricular (ICV) injections of the nonselective opioid antagonist, naloxone, significantly decreased (by 40–50%) the intensity of stimulation-induced behavioral responses, as did injections of either the μ-selective (β-funaltrexamine, β-FNA) or the δ-selective (naltrindole, NTI) opioid antagonists. In contrast, similar ICV injections of the κ-selective antagonist, nor-binaltorphimine (nor-BNI), did not block behavioral responses to PGi stimulation. The results indicate that activation of the PGi by electrical stimulation can elicit behaviors similar to those observed during opioid withdrawal. Endogenous opioids, acting through μ- and δ-, but not κ-opioid receptors, participate in mediating opioid withdrawal-like behaviors induced by PGi stimulation. © 1999 Elsevier Science Inc.

Nucleus paragigantocellularis Butorphanol dependence Opioid withdrawal Opioid antagonists Behavior

IDENTIFICATION of brain regions that generate behavioral responses during opioid withdrawal is crucial for understanding the mechanisms of opioid withdrawal. Topographically diverse brain regions, particularly the locus coeruleus (LC), but also including the nucleus paragigantocellularis (PGi) of the rostral ventrolateral medulla, periaqueductal gray (PAG), ventral tegmental area (VTA), amygdala, nucleus accumbens, several hypothalamic nuclei, and the spinal cord, have been suggested to participate in the genesis or expression of the signs and symptoms of opioid withdrawal (27). Recently, Christie et al. (11) have stimulated discussion in this field by emphasizing the importance of many of the regions, in addition to the LC, that contribute to opioid withdrawal behaviors. Because the PGi provides the major excitatory input to the LC (35–37), the LC-PGi connection becomes of major interest to any study of the production of opioid withdrawal behaviors.

The LC has long been considered to play an important role in the development of opioid dependence and withdrawal (40), in part because autoradiographic studies have shown a high density of opioid receptors (mainly of the μ and κ subtypes) to be found on LC neuronal cell bodies and nerve terminals within the LC (44). Electrophysiological studies have demonstrated that acutely applied opioids inhibit the firing rate of LC neurons, and that these inhibitory effects of opioids are reversed by administration of naloxone (1,46). Single neurons within the LC become tolerant to and dependent upon opioids after chronic exposure, a phenomenon that is manifested by precipitation of a dramatic increase in the firing rate of LC neurons following administration of opioid recep-

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tor antagonists to opioid-dependent rats (1,35,47). The role of LC neurons in opioid dependence and withdrawal is supported also by behavioral studies in which the withdrawal-induced activation of LC neurons follows a time course that closely parallels the behavioral signs that accompany opioid withdrawal (36). In addition, electrolytic lesions of the LC reduce withdrawal symptoms in opioid-dependent animals (28). Finally, focal electrical stimulation of the LC has been shown to mimic the behavioral responses noted during opioid withdrawal (21,39).

Although antagonist-induced withdrawal causes a marked increase in LC neuron firing rates in vivo (1,35,47), the withdrawal-induced activation of LC neurons is not seen (4) or greatly reduced (25) in brain slices taken from opioid-dependent rats, suggesting the possibility that the activation seen in vivo is mediated at least in part by afferents to the LC, which are disconnected during the preparation of brain slices. Neuroanatomical studies of afferents to the LC have revealed that LC neurons receive afferents from only a restricted number of brain loci, including the PGi and the nucleus prepositus hypoglossi of the medulla (5,6). Substantial evidence indicates that excitatory afferent projections from the PGi to the LC play a role in mediating opioid withdrawal. For example, focal electrical stimulation of the PGi activates neurons in the LC (14), and radiofrequency lesions of the PGi greatly attenuate withdrawal-induced activation of LC neurons ipsilateral but not contralateral to the PGi lesion (35). Finally, kynurenic acid, a nonselective excitatory amino acid antagonist known to block PGi stimulation-induced excitation of LC neurons, also blocks the withdrawal-induced activation of LC neurons (35). The results from such studies suggest that withdrawal-induced activation of the LC in opioid-dependent animals is mediated, at least in part, by afferents from the PGi.

A characteristic response to narcotic antagonist-precipitated opioid withdrawal is the production of stereotyped behaviors and increased locomotor activity, such as rearing, exploration, wet-dog shakes, etc. (21,48). These are believed to result from opioid withdrawal-induced activation of LC neurons. The present study examined whether focal electrical stimulation of the PGi could reproduce a pattern of behaviors similar to that observed during opioid withdrawal. Such stimulation was found to elicit a pattern of behaviors that qualitatively resembled those commonly noted during withdrawal from opioid dependence. These have been termed, opioid withdrawal-like. The results support the hypothesis that the PGi is involved in the generation of opioid withdrawal-like behaviors.

METHOD

Animals

Male Sprague–Dawley rats (weighing 350–400 g) were purchased from Harlan–Sprague–Dawley Inc. (Indianapolis, IN). The animals were housed in groups of four per cage in a temperature-controlled room with automatic 12 L:12 D cycles for at least 1 week prior to surgery. All procedures involving the rats were performed using protocols approved by the Animal Care and Use Committee of our institution.

Surgical Procedures

Animals were anesthetized with halothane (2.5% halothane in medical grade oxygen) and then placed in a stereotaxic instrument. A midline skull incision was made and the tissues overlying the skull were removed. The skull landmarks, bregma and lambda, were identified and the skull was oriented such that both points were positioned at the same horizontal level. Two bipolar, concentric stimulation electrodes (SNEX-100 mm; David Kopf Instruments, Tujunga, CA), insulated except for bluntly cut tips, were implanted in the PGi [12.2 mm caudal to bregma, 1.5 mm lateral to midline, and 8.8 mm ventral to the cerebellar surface; (32)]. An indwelling stainless steel guide cannula (26 gauge, 10 mm long) was implanted into the right lateral cerebral ventricle [0.5 mm rostral to bregma, 1.3 mm lateral to midline, and 4.5 mm ventral to the skull surface; (32)]. After implantation, the electrodes and guide cannula were fixed firmly to the skull with anchor screws and dental cement. Penicillin G (60,000 U, SC) was then administered to each animal. Postoperative analgesia was provided to each animal by infiltration of the wound site with Sensorcaine® (0.5% bupivacaine with 1:200,000 epinephrine). The animals were housed subsequently in individual cages and were allowed at least 1 week to recover. Sham-operated control rats received the same surgical procedures and implantation of electrodes as experimental rats, but not PGi stimulation.

Induction of Butorphanol Dependence

Under halothane anesthesia, subcutaneous implantation of osmotic minipumps (Alzet 2001, Alza Corp., Palo Alto, CA) between the scapulae was performed. A 4-cm piece of Tygon tubing (0.38 mm inner diameter, Cole-Palmer, Chicago, IL) was employed to connect the minipump to a piece of L-shaped stainless steel injector tubing (32 gauge, 30 mm long) with the length of the guide cannula. Butorphanol tartrate solution (26 nmol/μl) was passed through a 0.2-μm Acrodisk filter (Gelman Scientific, Ann Arbor, MI) before being introduced into the pumps. Minipumps were primed overnight at room temperature in sterile saline so that the nominal flow rate (1 μl/h) was obtained. Animals were infused with butorphanol tartrate (26 nmol/μl/h; ICV) for 3 days via osmotic minipumps. This infusion period and dose paradigm were determined to be optimal for induction of dependence upon butorphanol from previous studies of our laboratory (31). Precipitation of withdrawal was elicited by ICV injection of naloxone (48 nmol/5 μl/rat) in butorphanol-treated rats. Three days after initiation of ICV infusion of butorphanol, the connecting tube between the ICV cannula and the outlet of the minipump was disconnected. Two hours after the termination of butorphanol infusion, naloxone was injected ICV through a hand-held microliter syringe. Sham-operated control rats were given ICV injections of saline (5 μl/rat).

Behavioral Assessment

Animals were placed individually in a stainless steel cage (25 × 20 × 20 cm) and acclimated for at least 1 h prior to behavioral assessment. The opioid withdrawal-like behaviors were evaluated during a 30-min period of focal electrical stimulation of the PGi. Stimuli were monophasic square wave pulses, 1 ms in duration, 0.5 Hz in frequency, and 0.01–10 V in intensity. Ten opioid withdrawal-like behaviors (rearing, sniffing, exploration, teeth chattering, wet-dog shakes, scratching, escape attempts, abnormal posturing, ptosis, and diarrhea), characteristic of those seen in rats during opioid withdrawal (48), were evaluated. The behaviors were recorded and scored by a single trained observer without knowledge of the nature of any drug treatment received by each animal. Behavioral recordings during electrical stimulation alone were, however, not performed in a blinded manner. The following rating scale

was employed to assess the incidence of opioid withdrawal-like behaviors: 0 = not displayed, 1 = 1–5 episodes of a behavior, 2 = 6–10 episodes of a behavior, 3 = 11–15 episodes of a behavior, 4 = 16–20 episodes of a behavior, 5 = 21 or more episodes of a behavior. Behaviors scored in this manner included: rearing, sniffing, exploration, teeth chattering, wet-dog shakes, scratching, escape attempts, and abnormal posturing. Behaviors were defined as follows: rearing, an animal was observed to stand on its hind paws with the forepaws off the bedding; sniffing, an animal sniffed with short audible inhalations, while raising its muzzle and moving the nares and nasal vibrissae; exploration: an animal circled around the cage, thrusting its head in several directions and examining its surroundings; teeth chattering; an animal vigorously moved its jaws together in a chewing movement and chattered his teeth together audibly; wet-dog shakes: an animal shook its head, neck, and body vigorously; scratching: an animal put its two forepaws up and scratched the back of neck or the top of head; abnormal posturing; an animal pressed its abdomen and lower jaw against the floor of the cage; escape attempts; an animal looked apprehensive and attempted to escape from the cage by climbing the walls, with abrupt movements.

The mean value of the rating scale for each opioid withdrawal-like behavior indicates the average incidence of that behavior observed during a 30-min period of PGi stimulation. Two behaviors (ptosis and diarrhea) could not be defined in discrete episodes, and were recorded in an all-or-none manner. Animals were tested each day at approximately 1000 h. Animals were randomly assigned to receive stimulation runs at different voltages and different frequencies. However, each animal used for evaluation of induction of opioid withdrawal-like behaviors received only one 30-min session of PGi stimulation at one voltage or frequency each day. A separate group of animals, used for testing tolerance to repeated electrical stimulation of the PGi, received three sessions of stimulation in 1 day. This protocol included three discrete 30-min runs of stimulation (0.5 Hz, 1 ms, 1 V) over a duration of 3.5 h. Behavioral assessments were performed on the initial 30-min, the 60–90-min, and the final 180–210-min runs. Another group of animals was used to test the effect of naloxone on opioid withdrawal-like behaviors induced by PGi stimulation. In this group, animals received an ICV injection of either naloxone (48 nmol/5 μl/rat) or saline (5 μl/rat), followed by four discrete 30-min periods of PGi stimulation (0.5 Hz, 1 ms, 1 V) commencing at 0–0.5 h, and continuing during the periods of 1–1.5, 3–3.5, and 6–6.5 h after ICV injection. Behavioral responses to PGi stimulation were recorded during each 30-min session of stimulation. The effects of the selective opioid receptor antagonists, β-funaltrexamine (β-FNA), nor-binaltorphimine (nor-BNI), and naltrindole (NTI), on PGi stimulation-induced opioid withdrawal-like behaviors were tested in three separate groups of animals. In these experiments, each animal received a single ICV injection of either β-FNA, nor-BNI, or NTI at a dose of 48 nmol/5 μl/rat. This dose was comparable to that of naloxone in naloxone-injected rats. A 30-min run of PGi stimulation was given 1 h after ICV injection. Behavioral responses to PGi stimulation was recorded during each 30-min period of stimulation.

Histology

At the end of selected PGi stimulation experiments, each rat was perfused through the heart by saline followed by 10% phosphate-buffered formalin. Frozen 40 μm sections were cut through the region of the stimulation sites and stained with cresyl violet. The locations of stimulation sites were plotted onto drawings of the sections, using a microprojector (Fig. 1).

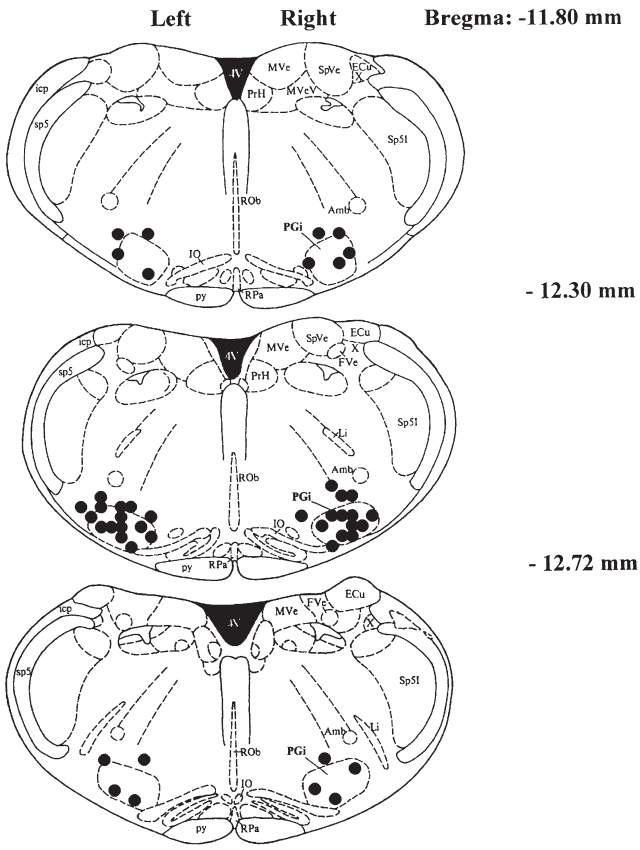

FIG. 1. Locations of electrical stimulation. Solid circles indicate the locations of electrode tips. Amb, nucleus ambiguus; ECu, external cuneate nucleus; FVe, F cell group vestibular; icp, inferior cerebellar peduncle; IO, inferior olive; Li, linear nucleus medulla; MVe, medial vestibular nucleus; MVeV, medial vestibular nucleus, ventral; PrH, prepositus hypoglossal nucleus; py, pyramidal tract; ROb, raphe obscurus nucleus; RPa, raphe pallidus nucleus; sp5, spinal trigeminal tract; Sp5I, spinal trigeminal nucleus, interpolar; SpVe, spinal vestibular nucleus; X, hypoglossal nucleus.

Statistics

The Kruskal–Wallis test and Dunn's test were employed for multiple comparisons. Comparisons between two groups were made using the Mann–Whitney rank-sum test (for non-pairwise tests) or the Wilcoxon signed-rank test (for pairwise tests). Quantal (all or none) behavioral data were analyzed by the chi-square test and the Bonferroni inequality to adjust the *p*-values. Differences were considered to be significant if the *p*-value was less than 0.05.

RESULTS

PGi Stimulation-Induced Opioid Withdrawal-Like Behaviors

Electrical stimulation of the PGi (0.5 Hz, 1 ms, 0.01–10 V) elicited rearing, sniffing, exploration, teeth chattering, wet-dog shakes, scratching, and abnormal posturing in nonopioid-treated rats during each 30-min session of stimulation. The onset of behavioral signs occurred within 10 min of initiation of electrical stimulation in all animals in which the stimulating

TABLE 1

COMPARISON OF BEHAVIORS INDUCED BY PGI STIMULATION AND BUTORHANOL WITHDRAWAL

	Electrical Stimulation of the PGi (0.5 Hz, 1 ms)		Butorphanol Withdrawal	
Behavioral Responses	0V	1V	Saline (5μl/Rat)	Naloxone (48 nmol/Rat)
Rearing	0.1 ± 0.1 (1/8)	1.2 ±0.2 (7/12)**	0.0 ± 0.0 (0/8)	0.4 ± 0.2 (3/8)★
Sniffing	0.1 ± 0.1 (1/8)	1.3 ± 0.2 (8/12)**	0.3 ± 0.2 (2/8)	1.1 ± 0.1 (7/8)††
Exploration	0.9 ± 0.1 (5/8)	1.6 ± 0.2 (12/12)*	0.6 ± 0.2 (4/8)	3.0 ± 0.5 (8/8)†††★
Teeth chattering	0.1 ± 0.1 (1/8)	4.1 ± 0.3 (11/12)***	0.4 ± 0.2 (2/8)	3.8 ± 0.5 (8/8)†††
Wet-dog shakes	0.4 ± 0.2 (2/8)	1.5 ± 0.2 (12/12)**	0.6 ± 0.2 (3/8)	3.4 ± 0.6(7/8)†††★
Scratching	0.3 ± 0.2 (2/8)	1.6 ± 0.2 (10/12)**	0.5 ± 0.2 (3.8)	2.9 ± 0.7 (7/8)††
Abnormal posturing	0.0 ± 0.0 (0/8)	1.6 ± 0.2 (11/12)***	0.6 ± 0.2 (2/8)	2.5 ± 0.6 (7/8)††
Escape attempts	0.0 ± 0.0 (0/8)	0.5 ± 0.2 (3/12)	0.0 ± 0.0 (0/8)	0.0 ± 0.0 (0/8)
Composite score	1.9 ± 0.4	13.0 ± 0.8***	3.0 ± 0.3	17.0 ± 1.3†††★
Ptosis	0/8	7/12*	0/8	5/8†
Diarrhea	1/8	8/12*	1/8	6/8†

Behaviors produced by PGi stimulation (0.5 Hz, 1 ms, 1 V) or naloxone-precipitated butorphanol withdrawal (ICV infusion of butorphanol, 26 nmol/μl/h for 3 days; ICV injection of naloxone, 48 nmol/5 μl/rat). Data represent the mean values ± SEM from 8 rats in the sham-operated group (0 V), 12 rats in the PGi-stimulated group (1 V), and 8 rats in both the saline-injected (5 μl/rat) and naloxone-injected (48 nmol/5 μl/rat) groups. The fraction values given for each individual sign denote the number of rats in which a positive sign was observed, over the total number of rats tested. The Mann–Whitney rank-sum test was used for comparison between the indicated groups. Quantal (all or none) behavioral data were analyzed by the chi-square test and the Bonferroni inequality to adjust the p-values.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (The asterisks denote significance between the sham-operated and PGi-stimulated groups).

†$p < 0.05$; ††$p < 0.01$; †††$p < 0.001$ (The daggers denote significance between the saline-infused and naloxone-infused groups).

★$p < 0.05$ (The star denotes significance between the PGi-stimulated and naloxone-injected groups).

electrodes were found to be within the immediate vicinity of the PGi, bilaterally. In many instances, behavioral activation was observed within seconds of the onset of stimulation. However, increases in behavioral responses tended to be episodic, with quiescent intervals between bouts of locomotor and stereotypic activity.

The incidence of rearing, sniffing, exploration, teeth chattering, wet-dog shakes, scratching, and abnormal posturing was significantly higher in animals that received 1 V of electrical stimulation than in sham-operated animals (Table 1). A composite score that represented the total incidence of quantifiable opioid withdrawal-like behaviors in each animal was recorded as an indicator of the intensity of behavioral responses to electrical stimulation of the PGi. In addition, autonomic signs of withdrawal, such as ptosis and diarrhea, were also measured in PGi-stimulated rats. The PGi-stimulated rats showed a significantly higher incidence of ptosis and diarrhea compared to sham-operated (control) rats (Table 1). Rhinorrhea and lacrimation were not observed in these rats. However, in our pilot experiments in halothane-anesthetized rats, we examined the effect of PGi stimulation on blood pressure and respiration, because the PGi, as a component of the rostral ventrolateral medulla (RVLM), might be implicated in regulation of cardiovascular and respiratory functions. The results revealed that bilateral electrical stimulation of the PGi at low voltage (0.5 Hz, 1 ms, 1 V) did not significantly change blood pressure and respiration. At high voltage (0.5 Hz, 1 ms, 10 V), PGi stimulation produced a significant pressor response and irregular respiration (unpublished data). The incidence of opioid withdrawal-like behaviors was increased in a voltage-dependent manner (Fig. 2). Changes in frequency (from 0.125 to 2 Hz) did not affect significantly the total incidence of stimulation-induced behavioral responses (Fig. 2).

To test whether tolerance could be demonstrated following repeated electrical stimulation of the PGi, animals were given a series of three 30-min episodes of stimulation (0.5 Hz, 1 ms, 1 V) over a period of 3.5 h. The total incidence of opioid withdrawal-like behaviors elicited with an initial 30-min period of stimulation did not differ in rats that also received stimulation between 1–1.5 and 3–3.5 h (Fig. 2).

Comparison of Behaviors Induced by PGi Stimulation and Opioid Withdrawal

The ICV injection of naloxone (48 nmol/rat) precipitated a pattern of behavioral withdrawal signs (including rearing, sniffing, exploration, teeth chattering, wet-dog shakes, scratching, abnormal posturing, ptosis, and diarrhea) in butorphanol-dependent animals (ICV infusion of butorphanol tartrate, 26 nmol/μl/h, for 3 days). The incidence of sniffing, exploration, teeth chattering, wet-dog shakes, scratching, and abnormal posturing was significantly higher in butorphanol-dependent rats that had received naloxone than in sham-operated animals that received comparable ICV injection of saline vehicle (Table 1). The occurrence of ptosis and diarrhea was also significantly higher in butorphanol-dependent rats than in sham-operated rats (Table 1). In comparison to the PGi-stimulated, opioid nondependent animals (0.5 Hz, 1 ms, 1 V), butorphanol-dependent animals that received naloxone exhibited a significantly higher incidence of exploration and wet-dog shakes.

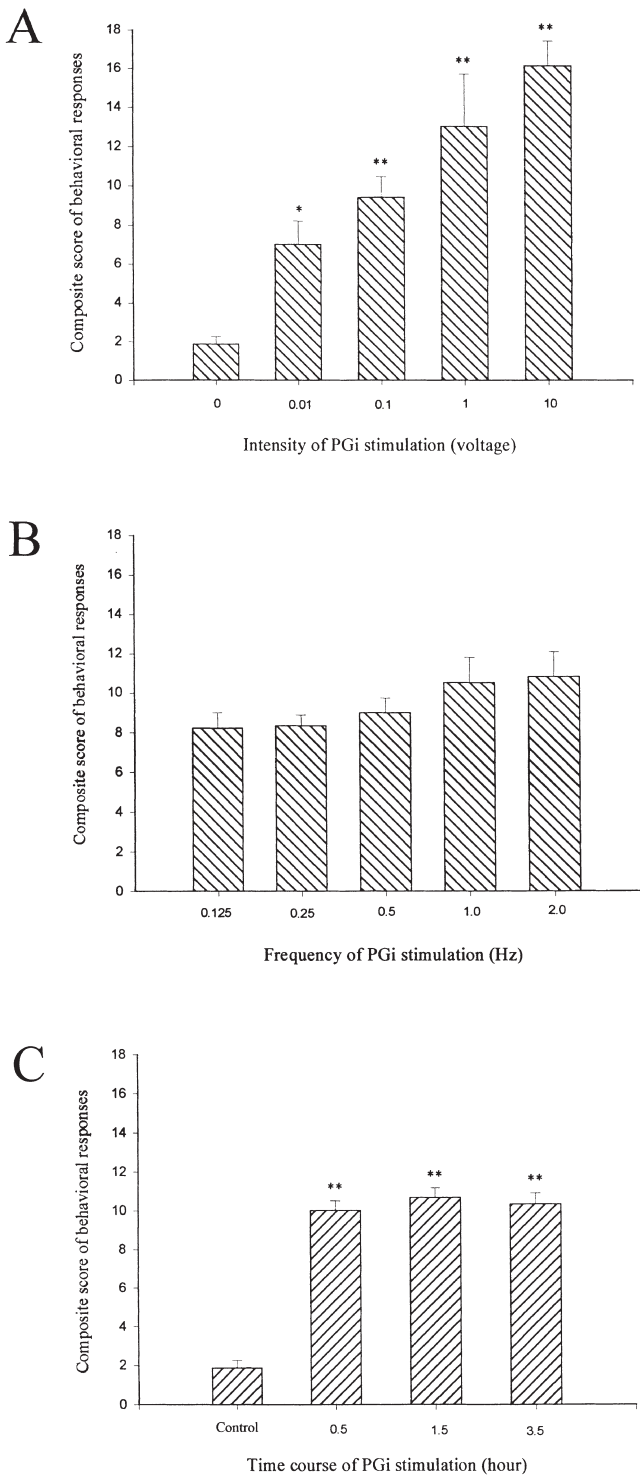

FIG. 2. Effect of voltage (A), frequency (B), or duration (C) on the behavioral responses to electrical stimulation of the PGi (0.125–2 Hz, 1 ms, 0.01–10 V) in nonopioid-dependent rats. Data represent the mean values ± SEM from eight rats. The Kruskal–Wallis test and Dunn's test were used for comparisons among the groups. $*p < 0.05$; $**p < 0.01$.

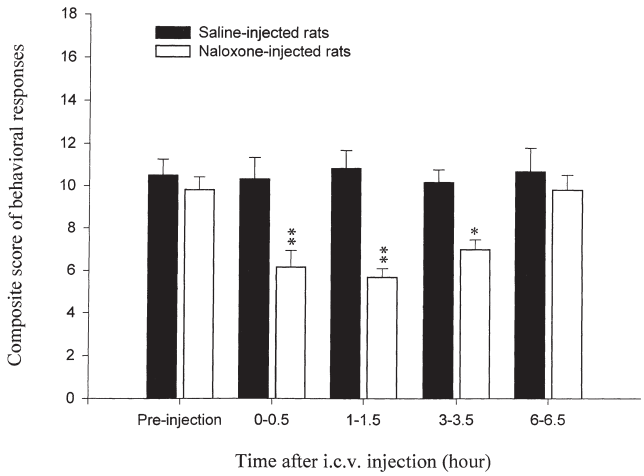

FIG. 3. Effect of naloxone on the behavioral responses to PGi stimulation (0.5 Hz, 1 ms, 1 V) in nonopioid-dependent rats. Data represent the mean values ± SEM from eight rats in each group. The Mann–Whitney *U*-test was used for comparison between saline- and naloxone-injected groups at each time point. Solid bars indicate composite scores of behaviors in saline-injected rats (5 μl/rat, ICV). Open bars indicate composite scores of behaviors in naloxone-injected rats (48 nmol/5 μl/rat, ICV). $*p < 0.05$, $**p < 0.01$.

In contrast, the PGi-stimulated, nonopioid-dependent animals showed a significantly higher occurrence of rearing (Table 1). The composite score of overall behaviors was significantly higher in butorphanol-dependent animals in which withdrawal had been precipitated by naloxone than in PGi-stimulated, opioid nondependent animals (Table 1).

Effects of Opioid Antagonists on PGi Stimulation-Induced Opioid Withdrawal-Like Behaviors

Nonopioid-dependent animals were given ICV injection of either naloxone (48 nmol/5 μl/rat) or saline (5 μl/rat), followed by four discrete 30-min periods of PGi stimulation (0.5 Hz, 1 ms, 1 V) over 6.5 h. The behavioral responses to electrical stimulation of the PGi were significantly reduced in naloxone-injected animals when compared to those observed in saline-injected animals. The inhibitory effect of naloxone on behavioral responses to PGi stimulation was maximal at 1–1.5 h after ICV injection of naloxone and continued to be evident for 2 h (Fig. 3).

The effect of selective μ-, κ- or δ-opioid receptor antagonists on PGi stimulation-induced opioid withdrawal-like behaviors was also examined in separate groups (six rats in each group). Each animal was given a single ICV injection of saline (5 μl/rat) or 48 nmol/5 μl/rat of either β-FNA, nor-BNI, or NTI. One hour after the ICV injection, each animal received a 30-min run of PGi stimulation (0.5 Hz, 1 ms, 1 V). The behavioral responses to electrical stimulation of the PGi were significantly reduced in β-FNA– and NTI-injected rats, but not in nor-BNI–injected rats, when compared to those observed in saline-injected rats (Fig. 4).

DISCUSSION

The principal findings of this study, that electrical stimulation of the PGi elicits increased locomotor activity and stereotypies reminiscent of narcotic antagonist-precipitated opioid

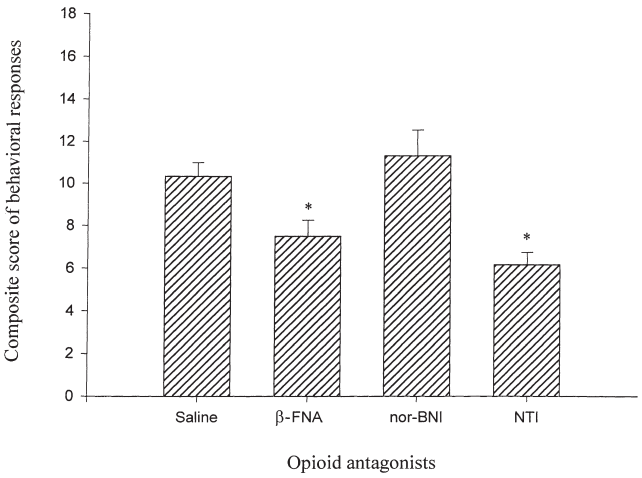

FIG. 4. Effect of β-FNA, nor-BNI, or NTI (48 nmol/5 μl/rat, ICV) on PGi stimulation-induced behaviors in nonopioid-dependent rats. Data represent the mean values ± SEM from six rats in each group. The Kruskal–Wallis test and Dunn's test were used for multiple comparison among saline- (5 μl/rat, ICV), β-FNA-, nor-BNI-, and NTI-injected groups. $*p < 0.05$.

withdrawal, and that ICV administration of narcotic antagonists can inhibit expression of behavioral responses to PGi stimulation, indicate that novel levels of complexity may be present in the neural circuitry subserving behavioral aspects of opioid withdrawal. Two major questions are raised by these findings. The first relates to the putative similarity of PGi stimulation-induced behaviors with those evoked during acutely precipitated opioid withdrawal. The second derives from the seemingly paradoxical ability of narcotic antagonists to inhibit the stimulation-induced behaviors that have been termed opioid withdrawal-like.

The behavioral syndrome that follows acute precipitation of withdrawal from dependence on an opioid can be evoked and is readily quantitated when a narcotic antagonist is administered by systemic (48), ICV (31), or discrete brain region injection (10,27). Evaluation of specific components of the syndrome in the rat varies somewhat among different laboratories [eg., (27,34,48)]. Generally, however, scoring includes the incidence of locomotor activities (digging, sniffing, exploratory behavior, jumping, and/or explosive running behaviors that are often termed escape attempts, wet, or wet-dog shakes), stereotypies (teeth chattering/chewing, grooming, scratching, abnormal posturing or rubbing, rearing, forepaw tremor), and autonomic signs (ear blanching, diarrhea, ptosis, rhinorrhea, salivation, penile erection/seminal emission, urination, and weight loss). The intensity of these is dependent upon the dosage of narcotic antagonist that is used (48), as well as on the type, dosage, route, and duration of administration of the opioid on which dependence is induced. The fact that the spectrum of withdrawal signs and symptoms is dependent on interaction of opioid agonists and antagonists with at least three different opioid receptor subtypes, the μ-, δ-, and κ-opioid receptors, has been recognized for over 30 years (29). Moreover, different profiles of behavioral response in opioid withdrawal can be produced by discrete application of a narcotic antagonist into different brain regions (10,27). These results indicate that activation of a diverse neurobiology, as well as possibly a complex neural network, can elicit the behavioral responses characteristic of opioid withdrawal, and that the precise expression of responses can vary within broadly defined limits.

Our findings demonstrate a qualitative similarity, using 10 commonly evaluated behavioral and autonomic signs of opioid withdrawal, between PGi stimulation-induced behaviors and the profile of responses elicited following naloxone-precipitated withdrawal from dependence on the opioid, butorphanol. Butorphanol is a potent opioid analgesic belonging to the group of morphine derivatives known as morphinans (30). Comparative studies have demonstrated that morphine and butorphanol are equipotent in the induction of dependence, as evidenced by the behavioral signs elicited during acute precipitation of withdrawal by challenge with the nonselective opioid receptor antagonist, naloxone (16,22). It is noteworthy that the pattern of behavioral responses to precipitated withdrawal from dependence on morphine and butorphanol is similar (23). More importantly, crosstolerance between butorphanol and morphine can be produced following ICV infusion of the two agents (15), indicating a fundamental similarity in their actions to cause dependence. In this latter study, chronic ICV administration of butorphanol produced similar rightward shifts of the analgesic response to morphine in both the tail-flick and acetic acid writhing tests (15). A more recent study from this laboratory revealed that equivalent behavioral signs of withdrawal and increases in LC concentrations of glutamate and aspartate were elicited in butorphanol- and in morphine-dependent rats following ICV infusions of either the selective μ-opioid receptor antagonist, CTOP (D-Pen-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2) or the δ-opioid receptor antagonist, naltrindole (17,18). Thus, a pattern of withdrawal signs, similar to those observed during morphine withdrawal, are produced by injection of the nonselective opioid receptor antagonist, naloxone, and by antagonists selective for the μ- and δ-opioid receptors in butorphanol-dependent animals. This indicates a substantive basis for the claim that butorphanol withdrawal is a reasonable model for comparison of opioid withdrawal signs with PGi stimulation-induced behaviors. In the present study, the opioid withdrawal-like behaviors produced by electrical stimulation of the PGi in naive rats were compared with those elicited by naloxone in butorphanol-dependent rats. Butorphanol-dependent rats that received naloxone exhibited a significantly higher incidence of exploration and wet-dog shakes. In contrast, the PGi-stimulated rats showed a higher occurrence of rearing. The composite score of the overall behavioral responses was also higher in butorphanol-dependent rats precipitated by naloxone than in PGi-stimulated rats. These results indicate that electrical stimulation of the PGi produced a pattern of behaviors that is qualitatively similar to, but not quantitatively identical with, that elicited during opioid withdrawal.

The quantitative differences could result from a simple mismatch between the intensity of butorphanol withdrawal (which is dependent on route of administration and dosage) and the intensity or precise location of electrical stimulation of the PGi. This contention is supported by the finding of a correlation between the quantitative degree of behavioral response following bilateral, as opposed to unilateral, stimulation of the PGi is evident (41). In addition, the intensity of behavioral response is proportional to the degree of PGi stimulation-induced elevation of extracellular glutamate levels within the LC [(41); manuscript in preparation]. Thus, the quantitative differences between butorphanol withdrawal and PGi stimulation-induced opioid withdrawal-like behaviors may represent technical difficulties in providing maximally ef-

fective stimulation of the neuronal substrate medicating a common set of behaviors.

Alternatively, the differences might represent the fact that neural projections from the PGi to the LC do not mediate the full expression of opioid withdrawal behaviors. The minor discrepancy of behaviors between rats undergoing withdrawal from butorphanol dependence and those receiving PGi stimulation might be explained by the hypothesis of dual mechanisms of opioid dependence in the LC, as proposed by Aghajanian et al. (2). This hypothesis suggests that both intrinsic (i.e., mediated by opioid receptors within the LC) and extrinsic (due to actions distant from the LC) factors play important roles in the withdrawal-induced activation of LC neurons. Upregulation of the cAMP pathway in the LC neurons is believed to be the most significant of the intrinsic factors mediating withdrawal-induced neuronal hyperactivity that is known to occur within the LC (25). Among the extrinsic factors, activation of an excitatory glutamatergic projection from the PGi to the LC is thought to be particularly important in the withdrawal-induced activation of the LC (3,38). Electrical stimulation of the PGi might not be expected to produce as intense an expression of the withdrawal behaviors as that seen in opioid withdrawal, because such stimulation represents only an extrinsic factor, whereas both intrinsic and extrinsic mechanisms may be required for full expression of withdrawal.

The second issue raised by the present findings is the partial opioid receptor dependency of PGi stimulation-induced behaviors. Of the myriad neurotransmitters present in PGi neurons that project to the LC, glutamatergic projections have been most directly implicated in mediation of withdrawal-induced LC neuronal hyperactivity and behavioral excitation. Other neurotransmitter connections, particularly including enkephalinergic projections, extend from the PGi into the LC (7,37,45). However, no excitatory effects of opioids on LC neurons have been identified. Therefore, it is unlikely that opioid receptor involvement in PGi stimulation-induced behaviors reflects actions within the LC.

The PGi does directly innervate brain regions other than the LC, including the paraventricular hypothalamus (8), and the trigeminal motor nucleus (20), wherein opioid receptors have been found to mediate behavioral responses. Electrical stimulation of the PGi could elicit opioid-dependent components of behavioral responses, including orofacial movements (chewing/teeth chattering), particularly by activating projections to the trigeminal nucleus. For example, oral stereotypic movements, elicited by chronic administration of neuroleptic drugs, are mediated by endogenous opioids and can be blocked by administration of narcotic antagonists (33,43). Moreover, locomotor stereotypies, such as jumping in the bank vole (*Clethrionomys glareolus*), are naloxone sensitive (24). Locomotor responses in tardive dyskinesia have been shown to be sensitive to naloxone blockade (26), although not all groups verify this sensitivity (49). These data provide precedent for the opioid receptor dependency of the behavioral syndrome evoked by PGi stimulation.

Stimulation of the PGi might also result in polysynaptic responses, mediated initially through the LC, that release an opioid peptide at a distant site(s), explaining the ability of narcotic antagonists to reduce PGi stimulation-induced changes in behaviors. The site or sites at which this effect occurs have yet to be identified. However, several brain regions, such as the ventral tegmental area and the nucleus accumbens are recognized as sites where endogenous opioids mediate locomotor behaviors (9,12,13,42). Immunohistochemical studies have confirmed direct connections between the LC and these brain regions (19). It is possible that ICV administration of opioid antagonists attenuated PGi stimulation-induced opioid withdrawal-like behaviors by blockade of the action of endogenous opioids released from secondary or tertiary synapses from the LC within the ventral tegmental area and/or nucleus accumbens.

In the present study, the effects of opioid receptor antagonists on PGi stimulation-induced opioid withdrawal-like behaviors were tested. The results indicated that the nonselective opioid receptor antagonist, naloxone, greatly attenuated opioid withdrawal-like behaviors induced by PGi stimulation, suggesting involvement of opioid receptors and/or endogenous opioids in mediation of such behaviors. To further define this interaction, animals were given ICV injections of selective opioid receptor antagonists prior to PGi stimulation. Again, the μ-selective antagonist, β-FNA, and the δ-selective antagonist, NTI, but not the κ-selective antagonist, nor-BNI, significantly reduced opioid withdrawal-like behaviors induced by PGi stimulation. These results confirm that endogenous opioids are involved in mediating opioid withdrawal-like behaviors induced by PGi stimulation and indicate further that such stimulation evokes behaviors selectively through μ-, and δ-, but not κ-, opioid receptors. However, the data also indicate that only 40–50% of the behavioral response to PGi stimulation can be blocked by pretreatment with a narcotic antagonist. Clearly, opioid peptides/receptors mediate only a portion of the withdrawal-like behaviors. Data from ongoing studies in our laboratory suggest that excitatory amino acids also contribute significantly to the behavioral responses [(41); manuscript in preparation], as has been hypothesized for narcotic antagonist-precipitated withdrawal from opioid dependence. Further study will be needed to fully reconcile the demonstrated responses with the ability of narcotic antagonists to precipitate withdrawal behaviors in opioid-dependent animals.

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