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25. We thank V. S. Kalyanaraman for performing the radioimmunoprecipitation assays. B. Kramarsky for electron microscopic examination, F. Bach for lymphocyte tissue typing, and N. Suciu-Foca for valuable comments on the manuscript. We also thank E. Richardson and E. Read for technical help and A. Mazzuca for editorial assistance. Supported in part by an interagency agreement with the Uniformed Services University of Health Sciences (Y01-CP-30500).

21 May 1984; accepted 10 July 1984

Separation of Morphine Analgesia from Physical Dependence

Abstract. *Intravenous infusion of morphine sulfate in rats for 24 hours produced marked opioid dependence, manifested by a series of well-documented signs appearing after injection of the opiate antagonist naloxone. Treatment of rats with naloxonazine significantly reduced the analgesia associated with the morphine infusions for more than 24 hours. Furthermore, 14 of 16 withdrawal signs observed in naloxonazine-treated rats were virtually identical to those in rats that received morphine alone. These results raise the possibility that different receptor mechanisms mediate morphine analgesia and many of the withdrawal signs associated with morphine dependence.*

Repeated administration of morphine produces tolerance and physical dependence. A number of typical signs and symptoms of this dependence are seen after the morphine is withdrawn or an opiate antagonist such as naloxone is administered, Although analgesia and physical dependence are discrete pharmacological effects, they have a close association. A major question in opiate pharmacology is whether analgesia can be dissociated from the production of physical dependence. Binding studies have identified two subtypes of μ receptors: the μ_1 or common high-affinity site, which binds opiates and the enkephalins equally well, and the μ_2 site, which binds morphine far more potently than the enkephalins (*1*). The development of irreversible antagonists of μ_1 binding sites (*2*), such as naloxonazine, has permitted investigations of the pharmacological actions mediated by these sites. Naloxonazine selectively antagonizes in a long-acting manner a variety of opioid actions, including analgesia, prolactin release, catalepsy, acetylcholine turnover, and hypothermia without affecting a number of other actions, such as respiratory depression, growth hormone release, bradycardia, sedation, inhibition of electrically stimulated guinea pig ileum, and dopamine turnover (*3*). We examined the effects of naloxonazine on morphine analgesia and physical dependence.

Male Sprague-Dawley rats (250 to 300 g; Charles River) were surgically prepared with cranial cannula connector pedestals (*4*). Four days later, indwelling jugular cannulas were emplaced (*5*). The animals were then treated intravenously with naloxonazine (20 mg/kg) or saline, and 24 hours later intravenous infusions of morphine sulfate (15 or 50 μg/kg-min) or saline were started at the rate of 6ml/day. Analgesia was assessed with the radiant heat tail-flick method with a maximum cutoff of 10 seconds (*3, 6*). The infusions were discontinued after 24 hours and the animals then received a single dose of naloxone HCl (4 mg/kg, subcutaneously). Rats were monitored continuously for any signs associated with the morphine withdrawal syndrome for 1 hour after the naloxone injection (*7*). Body weights were also measured. Nonparametric statistical techniques were used to assess the significance of differences in withdrawal signs observed among groups (*8*).

Morphine infusion at 15 μg/kg-min (group 4) and 50 μg/kg-min (group 2) significantly increased tail-flick latencies over baseline values (Table 1). Peak latencies were observed 2 hours into the infusion, after which latencies decreased, presumably because of the development of tolerance. After a 24-hour infusion, the mean latency of group 4 returned to baseline (2.8 ± 0.3 seconds) and that of group 2 decreased to 6.3 ± 0.6 seconds. Naloxonazine pretreatment (group 3) markedly attenuated the peak analgesic effect of morphine at 50 μg/kg-min, reducing it to that produced by morphine at the lower dose. The mean latency of group 3 remained much lower than that of group 2 throughout the 24-hour infusion. However, little tolerance was noted, with an average latency at 24 hours of 4.0 ± 0.2 seconds. Tail-flick latencies of rats infused with saline only (group 1) did not change significantly from baseline. Similarly, naloxonazine treatment did not produce a significant change in latency.

Group 2 became dependent on mor-

phine, as shown by the rapid precipitation of classical withdrawal signs after the injection of naloxone (Table 2). Withdrawal was intense and was equivalent to that observed in rats treated with morphine for longer periods by alternative routes of administration, including multiple injections and pellets (*7*). In contrast, the control rats (group 1) showed few signs other than exploratory rearing and self-stimulation. Similarly, very few signs of withdrawal were observed after naloxone injection in rats treated with naloxonazine before being infused with saline for 24 hours. The signs observed with morphine infusion were opiate-specific. Coinfusion of morphine (50 μg/kg-min) with naloxone (12.5 μg/kg-min) yielded withdrawal results indistinguishable from those of the saline control group.

Expression of many of the signs varied depending on the dose of morphine given (Table 2). Weight loss was the most easily quantifiable sign. Group 1 animals gained 4.3 ± 1.0 percent of their body weight after the injection of naloxone, while group 2 animals lost 6.9 ± 1.1 percent and group 4 lost 4.1 ± 0.8 percent. Although the frequency of wet-dog shakes did not differ significantly between groups, there was a trend toward increasing incidence at the higher morphine dose.

We also examined the effect of naloxonazine pretreatment on the withdrawal syndrome in rats given morphine at 50 μg/kg-min (group 3). In view of the significant reduction in analgesia produced by naloxonazine, the incidence of withdrawal signs mediated by receptors sensitive to naloxonazine should be similar to that seen in saline-treated rats receiving the lower dose of morphine (group 4). On the other hand, signs mediated by other subclasses of opioid receptors should be unaffected by naloxonazine, resembling those in rats given the higher dose of morphine (group 2). Penile discharge and salivation were the only two withdrawal signs whose incidence was significantly lowered by naloxonazine treatment, suggesting that they are mediated by the same sites as analgesia. The incidence of all the other signs in group 3 was the same as in group 2. For example, weight loss was identical to that in group 2 and significantly greater than that in group 4, implying that the receptors mediating this effect are not very sensitive to naloxonazine. The similar incidence in groups 2 and 3 of ear blanching, irritability, ptosis, tachypnea, and teeth chattering suggests that they, too, involve a naloxonazine-insensitive site. Little can be said about the remaining signs (diarrhea, defecation, exophthalmos, wet-dog shakes, escape attempts, exploratory rearing, and writhing) since their incidence in groups 2 and 4 was not statistically different. Even if these signs were mediated through a naloxonazine-sensitive site, we would not expect to detect changes.

Previous studies have demonstrated the ability of naloxonazine to antagonize in a long-lasting manner a number of opioid actions, including analgesia, without affecting others, such as respiratory depression (*3*). On the basis of the selectivity of naloxonazine's irreversible actions in binding studies, we propose that μ_1 receptors are probably responsible for these naloxonazine-sensitive actions. Our investigation confirms the sensitivity of morphine analgesia to naloxonazine. Although naloxonazine markedly reduced the analgesic potency of morphine, a small elevation in latency remained. This persistent elevation might be explained by residual μ_1 sites that were not antagonized by naloxonazine or by interactions of this high dose of morphine with other classes of opioid receptors also capable of mediating analgesia (*9*). Our results imply that the most sensitive system involves the naloxonazine-sensitive (μ_1) receptors. Studies in mice indicate a possible role of δ sites in opioid analgesia after blockade of μ_1 sites (*10*).

The withdrawal syndrome might be considered as the summation of a number of separate actions mediated through different classes of opioid receptors. Differences in the dependence profiles ob-

Table 1. Effect of morphine and naloxonazine on tail-flick latency. Groups of rats were treated intravenously with saline or naloxonazine (20 mg/kg) and 24 hours later were infused with saline or morphine sulfate at the doses indicated. Values are means ± standard errors. Baseline values between groups do not differ significantly by analysis of variance; 2 hours after the initiation of morphine infusion, latencies are significantly greater than baseline. The peak elevations seen in groups 3 and 4 do not differ significantly from each other but are significantly lower than the elevation in group 3 ($P < 0.05$).

Group	Treatment	*n*	Tail-flick latency (seconds)	
			Baseline	After 2 hours
1	Saline + saline	9	2.7 ± 0.2	2.6 ± 0.2
2	Saline + morphine (50 μg/kg-min)	37	2.8 ± 1.0	8.7 ± 0.4
3	Naloxonazine ± morphine (50 μg/kg-min)	17	2.6 ± 0.1	3.9 ± 0.3
4	Saline ± morphine (15 μg/kg-min)	27	2.7 ± 0.1	4.1 ± 0.3

Table 2. Incidence of withdrawal signs precipitated by naloxone. After the rats had been infused with saline or morphine for 24 hours, they received naloxone HCl (4 mg/kg, subcutaneously) and were scored for withdrawal signs without knowledge of the group to which they belonged. Values give the incidence (percent) of the signs (number of rats exhibiting the sign divided by the total number of total rats), with the exception of the values in parentheses, which denote the average number of times the sign was shown per rat per hour and body weight change, which denotes the mean change in body weight (percent) 24 hours after naloxone treatment.

Sign	Group 1 (*n* = 9)	Group 2 (*n* = 15)	Group 3 (*n* = 15)	Group 4 (*n* = 14)
Body weight change	+4.3 ± 1.0*	−6.9 ± 1.1	−6.9 ± 0.8	−4.1 ± 0.8*
Ear blanching	0*	93	80	29*
Irritability	0*	87	60	23*
Ptosis	0*	93	87	14*
Tachypnea	11*	80	73	36*
Teeth chattering	0*	100	93	64*
Salivation	0*	87	33*	7*
Penile discharge	0*	47	0*	0*
Diarrhea	0*	73	80	86
Defecation	11*	100	80	86
Exophthalmos	0*	47	53	36
Wet-dog shakes	22	60	73	36
	(0.3 ± 0.3)	(1.4 ± 0.5)	(3.9 ± 1.1)	(1.0 ± 0.4)
Self-stimulation	44	80	93	100
	(0.8 ± 0.5)	(2.8 ± 1.6)	(3.3 ± 0.5)*	(4.5 ± 0.6)*
Escape attempts	22	53	27	29
	(1.1 ± 0.5)	(1.5 ± 0.3)	(0.5 ± 0.3)	(0.8 ± 0.4)
Exploratory rearing	78	100	100	100
	(1.0 ± 4.6)	(8.8 ± 2.2)	(5.1 ± 0.8)	(8.2 ± 1.9)
Writhing	0	27	20	0
	(0)	(0.3 ± 0.2)	(0.2 ± 0.1)	(0)

*Significantly different ($P < 0.05$) from the corresponding value for group 2.

served with opioids might be explained by their ability to interact with the various receptor classes. The ability of naloxonazine to effectively decrease salivation and penile discharge as well as analgesia without affecting the other signs of dependence supports this concept. In addition, the dissociation by naloxonazine of receptor mechanisms mediating analgesia from those involved with many aspects of physical dependence raises the possibility of synthesizing selective analgesics with little dependence liability.

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4. Under enflurane anesthesia, a cannula connector pedestal (Plastic Products) was affixed to each rat's cranium with two 1-mm mounting screws and plastic cement. For drug administration, a metal-jacketed catheter was attached to the pedestal and connected to the jugular vein cannula. The other end of the catheter was passed through the top of the cage, where it was attached to a swivel (Alice King Chatam Medical Arts). An infusion line was passed through the swivel to a multichannel peristaltic infusion pump (Manostat).
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23 April 1984; accepted 14 August 1984

Growth Hormone–Releasing Factor: Direct Effects on Growth Hormone, Glucose, and Behavior via the Brain

Abstract. *Intracerebroventricular administration of human pancreatic growth hormone–releasing factor caused a dose-dependent inhibition of growth hormone secretion, elevated plasma glucose concentrations, and produced marked behavioral and motor effects. Immunoneutralization with antiserum to somatostatin did not reverse the suppression of growth hormone. These findings suggest that hypothalamic growth hormone–releasing factor may regulate its own neurosecretion through an "ultrashort-loop" negative feedback mechanism and may have important neurotransmitter and neuromodulatory functions in the brain.*

Peptides with high growth hormone (GH)–releasing activity were recently isolated from two human pancreatic islet cell tumors (*1*). Synthetic replicates of these peptides are potent and specific stimulators of pituitary GH release when administered systemically (*2*) and are indistinguishable in biological activity from the GH-releasing factor (GRF) present in human and rat hypothalamus (*3*). There is evidence that the hypothalamic releasing and inhibiting hormones not only regulate endocrine function of the adenohypophysis but also exert nonendocrine actions in the central nervous system (CNS) (*4*).

I report that intracerebroventricular administration of the 44-amino-acid peptide, human pancreatic GRF (hpGRF), severely suppresses GH release, elevates plasma glucose concentrations, and produces marked behavioral and motor effects. The findings suggest that hypothalamic GRF may regulate its own neurosecretion through an "ultrashort-loop" feedback mechanism and that it may, in addition to its endocrine role as a hypophysiotropic hormone, regulate glucose and behavior by direct action on the brain.

Adult male Sprague-Dawley rats (300 to 350 g) were implanted with intracerebroventricular and intracardiac venous cannulas (*5*). After surgery the animals were placed in isolation chambers (lights on between 0600 and 1800 hours) and given unlimited Purina Rat Chow and water until their body weights returned to preoperative levels. During this time (usually 1 week) the rats were handled daily to minimize any stress associated with handling on the day of the test. In the first experiment, with six groups of rats, a baseline blood sample was obtained at 1000 hours; immediately afterward the rats were administered 10 μl of hpGRF at various doses or normal saline through the left lateral ventricle of the brain. The hpGRF had been synthesized by solid-phase techniques (*6*) and diluted in normal saline to attain concentrations of 10, 5, 2.5, 1.25, and 0.5 μg per 10 μl.

Blood samples were withdrawn 5 minutes after the injection and subsequently every 15 minutes for 6 hours (1000 to 1600 hours). All samples were immediately centrifuged and the plasma was separated and stored at −20°C for subsequent assay of GH, prolactin, and glucose (*7*). The apparatus used allowed the animals to behave freely during the removal of blood samples. After the injections, behavior and motor control were continuously monitored through a one-way observation port. The results were evaluated by analysis of variance for