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Blockage of narcotic-induced dopamine receptor supersensitivity by cyclo(Leu-Gly)

(addiction/withdrawal/peptides)

RONALD F. RITZMANN*, RODERICH WALTER*, HEMENDRA N. BHARGAVA†, AND LOUIS B. FLEXNER‡

*Department of Physiology and Biophysics, and †Department of Pharmacognosy and Pharmacology, University of Illinois at the Medical Center, Chicago, Illinois 60612; and ‡Department of Anatomy, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19101

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ABSTRACT **We have previously reported that the administration of cyclo(Leu-Gly) to mice prior to morphinization blocked the development of tolerance to the analgesic effects of morphine as well as the development of some signs of physical dependence. In the present series of experiments, the effect of the same peptide treatment on changes in dopamine receptor sensitivity induced by chronic morphine treatment were determined. Changes in dopamine receptor sensitivity were determined by measuring (*i*) the effect of the dopamine agonist apomorphine on locomotor activity and (*ii*) the hypothermic response to another dopamine agonist, piribedil. Mice that had received the chronic morphine treatment were found to require significantly less apomorphine to produce an increase in locomotor activity, and they exhibited a significantly greater hypothermic response to piribedil than did morphine-naive mice. The injection of 0.2 μmol of cyclo(Leu-Gly) per mouse 2 hr prior to morphine treatment prevented this increased response to both dopamine agonists. Administration of the peptide after the tolerance and dependence had developed did not alter morphine tolerant and dependent states or the enhanced response to apomorphine or piribedil. It is concluded that dopamine receptor supersensitivity may be involved in the development of narcotic tolerance and physical dependence.**

Chronic morphine administration leads to the development of tolerance to and physical dependence on the drug. Concomitantly there is an increase in the response to the dopamine agonist apomorphine (1, 2). This increased response to apomorphine has been interpreted as an increase in dopamine receptor sensitivity (2). The significance of this change in dopamine receptor sensitivity in the development or display of the symptoms of physical dependence on and tolerance to morphine is unclear; however, it has been proposed that changes in dopamine systems may mediate many of the overt signs of morphine withdrawal (1–3). Recently we have reported that, if administered prior to chronic morphine treatment, several peptides, including the cyclic dipeptide cyclo(Leu-Gly), prevent the development of tolerance to the analgesic properties of morphine as well as some of the symptoms of opiate withdrawal (4, 5). These effects were obtained without altering either the acute response to morphine or brain morphine levels (4, 5). When the same peptides were injected after tolerance and physical dependence had already developed, tolerant-dependent states remained unmodified (4). To investigate further the role of dopamine receptor sensitivity in morphine tolerance and dependence we determined the effect of cyclo(Leu-Gly) treatment on the morphine-induced increase in the response to the dopamine agonists apomorphine and piribedil.

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MATERIAL AND METHODS

Cyclo(Leu-Gly) used in these studies was synthesized in our laboratories (6). Male Swiss Webster mice [mean (±SD) weight, 26 ± 4 g] were randomly divided into two groups: one group received a subcutaneous injection of water (vehicle); the other group received a subcutaneous injection of cyclo(Leu-Gly) at a dose of 0.2 μmol per mouse. All injections were 0.1 ml in volume. At 2 hr after the injection, the mice were further subdivided, each subgroup was given an implant of either a placebo or a morphine (75 mg of free base) pellet (5). The pellets were removed 72 hr later. To determine the effect of the peptide on the overt response to the agonist after dopamine receptor supersensitivity had already developed, cyclo(Leu-Gly) was injected on the third day of morphine treatment, a time when physical dependence as well as receptor sensitivity could readily be demonstrated (7). Dopamine receptor sensitivity was measured 24 hr after the removal of the pellets. The dose of apomorphine that produced an increase in locomotor activity was determined by comparing pre-injection locomotor activity (Stoelting activity monitor) with that 15 min after intraperitoneal injection of an appropriate dose of apomorphine (0.5–4.0 mg/kg). Mice were first allowed to perambulate for 5 min followed by a 10-min test period. After an interval of 30 min, mice were given the dose of apomorphine. Fifteen minutes later, the 5-min perambulation and 10-min test periods were repeated. Data are expressed as the difference between the test activity scores before and after injection.

Additional groups of mice that had received the same chronic morphine and peptide administration were used to determine the effect of these treatments on the response to another dopamine agonist, piribedil. The hypothermic response to an intraperitoneal injection of piribedil (20 mg/kg) was determined 24 hr after the pellet was removed. Body temperature was determined by using a rectal probe (inserted 2.5 cm into the rectum) and telethermometer; the first measurement was made just prior to the drug injection and was repeated at 15 and 30 min after injection.

RESULT AND DISCUSSION

There was no significant difference in the pre-injection activity among any of the groups of mice ($P > 0.05$), nor did the injection of saline produce any change in locomotor activity ($P > 0.05$). As in previous experiments, chronic morphine exposure resulted in a shift of the apomorphine dose–response curve to the left. The minimal dose of apomorphine that produced a significant increase in locomotor activity in chronic morphine-treated mice (60%) was 1 mg/kg, compared with a minimal dose of 2 mg/kg in placebo-implanted mice. This significant increase in response to the dopamine agonist was not

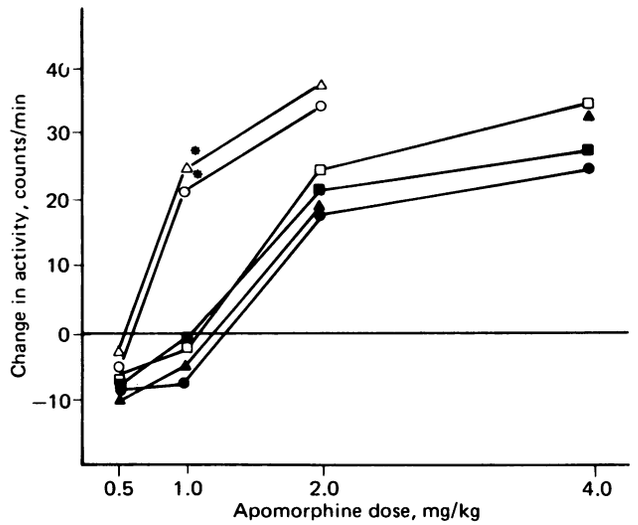

FIG. 1. Effect of apomorphine on locomotor activity in mice. Morphine or placebo pellets were implanted in mice; 72 hr later, the pellets were removed. Locomotor activity was determined 24 hr after pellet removal in a Stoelting activity monitor. Mice were first allowed to perambulate for 5 min and then were tested for 10 min. After an interval of 30 min, mice were injected intraperitoneally with apomorphine (0.5–4.0 mg/kg); 15 min later the 5-min perambulatory period and 10-min test periods were repeated. Data are expressed as the difference between the pre- and post-injection activity scores before and after injection.* $P < 0.05$ (Duncan's multiple range test after significance on analysis of variance. Groups tested were: ○, vehicle/morphine; □, cyclo(Leu-Gly)/morphine; ●, vehicle/placebo; ■, cyclo(Leu-Gly)/placebo; △, morphine/cyclo(Leu-Gly) on day 3; ▲, placebo/cyclo(Leu-Gly) on day 3.

observed in morphine-treated mice given a single injection of cyclo(Leu-Gly) 2 hr prior to pellet implantation. A 1 mg/kg dose of apomorphine failed to elicit any increase in locomotor behavior (Fig. 1); the 2 mg/kg dose produced an increase (57%) that was not significantly different from the increase observed in controls (60–65%). Cyclo(Leu-Gly) did not alter the response to apomorphine in placebo-implanted mice at any of the doses tested. Similar to our earlier findings on the effect of cyclo(Leu-Gly) on morphine tolerance and physical dependence (4, 5), injection of the peptide after the development of dopamine receptor supersensitivity had already occurred (day 3 of morphine treatment) did not alter the morphine-induced increase in the response to apomorphine.

Chronic morphine treatment also produced an increased hypothermic response (−2.2°C) to piribedil (20 mg/kg) compared to −1.2°C in placebo-implanted mice (Table 1). As in the test with apomorphine, morphine-treated mice that had received an injection of cyclo(Leu-Gly) 2 hr prior to the start of morphine treatment did not exhibit enhanced hypothermic response to piribedil.

We have previously shown that cyclo(Leu-Gly), as well as several chemical derivatives, block the development of tolerance to the analgesic properties of morphine and some symptoms of physical dependence (5, 8). One sign of dependence that is blocked by peptide pretreatment in morphine-pellet-implanted mice is the hypothermia that occurs either during abrupt or naloxone-induced withdrawal (4, 5). On the other hand, naloxone-induced stereotyped jumping was not altered by the same peptide treatment (8). Because this peptide treatment also prevents the development of dopamine receptor supersensitivity, it would seem reasonable to conclude that the dopaminergic system may be involved in the hypothermia that occurs during the abstinence syndrome and the development of tolerance to the analgesic properties of morphine but probably not in stereotyped jumping behavior. The correlation between the abilities to produce analgesia and to alter body temperature has also been reported for several endogenous neuropeptides; moreover, the intensity of the effects on body temperature are related to the analgesic potency of these peptides (9). These data indicate that analgesia and thermoregulation may have some common neurochemical mechanisms, possibly involving dopaminergic systems, which are modified by morphine. This modification is prevented by peptides such as cyclo(Leu-Gly). It also appears that these neurochemical systems are involved in the development of tolerance and some aspects of physical dependence on opiate drugs.

Table 1. Effect of piribedil on body temperature in mice

Group*	Body temperature, °C		
	At 0 min	At 15 min	At 30 min
Vehicle/morphine	36.90 ± 0.17	34.83 ± 0.13†	34.71 ± 0.13†
Cyclo(Leu-Gly)/ morphine	36.70 ± 0.16	35.64 ± 0.25	35.58 ± 0.27
Vehicle/placebo	36.60 ± 0.09	35.37 ± 0.21	35.40 ± 0.10
Cyclo(Leu-Gly)/ placebo	36.70 ± 0.15	35.62 ± 0.21	35.73 ± 0.28

* See text for experimental treatment.
† $P < 0.05$ for difference from other groups.

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