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Effects of Calcitonin Gene-Related Peptide on Acute and Chronic Effects of Morphine

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AZAROV, A. V., G. SZABÓ, L. CZAKÓ AND G. TELEGDY. *Effects of calcitonin gene-related peptide on acute and chronic effects of morphine.* PHARMACOL BIOCHEM BEHAV **52**(3) 595–599, 1995.—Calcitonin gene-related peptide (CGRP), which has been observed in different parts of the nervous system, is known to modify pain sensitivity to different stimuli in rats and mice. The aim of this study was to investigate the possible interaction of CGRP with morphine on nociception in adult male NMRI mice after central administration of the peptide. CGRP (20 or 200 ng) did not itself modify pain sensitivity in the tail-flick test and did not affect the acute antinociceptive action of a single dose of morphine in the same test. However, CGRP suppressed the development of rapid tolerance to morphine in a dose-dependent manner, but had no action on the development of chronic tolerance to morphine and on manifestations of naloxone-precipitated withdrawal syndrome.

Calcitonin gene-related peptide CNS Dependence Intracerebroventricular Mice Morphine Naloxone Pain Tail-flick Tolerance Withdrawal

CALCITONIN gene-related peptide (CGRP), a product of alternative splicing of RNA from the calcitonin gene (11), is widely distributed in the central nervous system. High concentrations of CGRP-like immunoreactivity has been detected in the hypothalamus, amygdala, hippocampus, striatum, nucleus accumbens, brainstem nuclei, and superficial layers of the spinal dorsal horn. Localization of this neuropeptide in the sensory neurons suggests a role in sensory processes (7,12,13).

Several findings suggest that CGRP is involved in central regulation of nociception (1,5,6,8,16). When administered into the brain, high doses of human CGRP has been shown to have a significant intrinsic antinociceptive effect in rats (6,10). Rat CGRP, on the other hand, can produce antinociception in mice subjected to noxious chemical stimuli (1,3,16), in hot-plate test, but failed to produce antinociception in tail-flick test in mice (16) and in tail-immersion test in rats (6).

CGRP has the ability to alter the antinociceptive effect of an acute morphine challenge. Rat CGRP can antagonize morphine-induced nociception in tail-flick test upon a shorter (30 min) pretreatment with the peptide; 1-h pretreatment, however, nonsignificantly facilitated the morphine-induced antinociception to thermal, but not to chemical stimuli and doses below 2 μg/animal produced no alterations in any behavioral test used (16). Similarly, conflicting results have been published on intrathecal injection of CGRP. Intrathecal administration of CGRP (80 μg/animal) had no effect on nociception (6), but nociceptive behavioral responses were suppressed by intrathecal administration of anti-CGRP antibody (8). The available data show disparate results on nociception, due in part to differences in procedures and the doses and types of CGRP used (1,5–8,16). The aim of the present study was to measure the effects of rat CGRP on intrinsic pain sensitivity, and after a challenge dose of morphine, on the development of acute/rapid and chronic tolerance to morphine, and on naloxone-precipitated withdrawal symptoms.

METHODS

Animals

Male NMRI mice (33 ± 5 g) of an inbred strain (LATI, Gödöllő, Hungary) were used. They were kept under a standard light-dark cycle (lights on between 0600 and 1800 h) with food and water available ad lib. At least a period of a week was allowed before the beginning of experiments. The animals

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were kept and treated according to the rules of the Ethical Committee for the Protection of Animals in Research (Albert Szent-Györgyi Medical University).

Surgery

For intracerebroventricular (ICV) cannulation, mice were anesthetized with sodium pentobarbital (Nembutal®, CEVA, France; 50 mg/kg IP), and a polyethylene cannula was inserted into the right lateral cerebral ventricle and cemented to the skull with cyanoacrylate-containing instant glue. The experiments were started 4 days after ICV cannulation. Upon conclusion of the experiments, 10 μl methylene blue was injected into the ventricle of decapitated animals and the position of the cannula was inspected visually. Animals with improper cannula placement were excluded from the final statistical analysis.

For chronic tolerance and withdrawal testing, animals were lightly anesthetized with ether (Lek-Chinoin, Budapest, Hungary) and morphine pellets were implanted subcutaneously (SC) into the sacral area through a small section in the neck area.

Treatments

For ICV treatment, the peptide was dissolved in artificial cerebrospinal fluid and injected in a volume of 2 μl. In all experiments involving measurement of the antinociceptive effect, morphine-HCl (Alkaloida, Tiszavasvári, Hungary; 4 mg/kg SC) was used. In rapid tolerance studies, 60 mg/kg morphine-HCl (SC) was used as a tolerance-inducing dose. In chronic tolerance and withdrawal studies, pellets containing 35 mg morphine were implanted. Precipitated withdrawal syndrome was induced with 0.1 mg/kg naloxone-HCl (SC) (Narcanti®, Du Pont, Germany).

Investigation of Morphine Sensitivity

Rapid morphine tolerance was induced by injecting 60 mg/kg morphine. Twenty-four hours later, the antinociceptive effect of a test dose of morphine (4 mg/kg) was used in morphine-pretreated and morphine-naive groups. The latter group had been treated with 0.9% saline 24 h earlier.

Procedures

Four major types of experiments were carried out with CGRP (α-CGRP [rat]; Bachem, Bubendorf, Switzerland). All experiments were started with an initial tail-flick latency measurement, pain sensitivity being measured immediately before and 30, 60, and 120 min after the test morphine challenge. The heat-radiant tail-flick method of D'Amour and Smith (4) was used. The antinociceptive effect was expressed according to the equation:

$$\text{Antinociceptive Effect} = \frac{TF_{30} - TF_0}{TF_{max} - TF_0} \times 100$$

where TF_0 is the tail-flick latency in the preliminary test mentioned above, or (in all tolerance studies) before injection of the test dose of morphine, TF*n* is the value of a repeated corresponding measurement *n* (30, 60, and 120 min) after morphine injection, and TF*max* indicates the cut-off time (20 s). The control tail-flick latencies (TF_0) were between 1.5 and 2.2 s.

1. In the first study, the effect of the peptide itself on pain sensitivity was measured. CGRP was given ICV 15 min before testing of the analgesic effect.
2. In studies with CGRP on the antinociceptive effect of a single dose of morphine, the peptide was given ICV 45 min prior to the test dose of morphine (4 mg/kg, SC).
3. In rapid tolerance studies, animals were pretreated with the peptide and 1 h later a tolerance-inducing dose of morphine (60 mg/kg) was injected; 24 h later, a test dose of morphine was used to assess the antinociceptive effect.
4. In chronic tolerance and withdrawal studies, the peptide was given 1 h before the pellets were implanted. The peptide treatment was repeated three times 24 h apart. On day 4, a test dose of morphine was given, and the antinociceptive effect was determined. Three hours later, the animals received naloxone (0.1 mg/kg, SC) and the precipitated withdrawal signs were assessed. The precipitated abstinence syndrome was measured by scoring the latency of the appearance of stereotyped jumping from a circular platform 35-cm diameter and 70-cm high. A cut-off time of 900 s was used (9). The body temperatures and body weights of all animals were measured 1 h after injection of naloxone, and changes in both parameters were calculated.

Statistical Analysis

Statistical analysis of the data was made by one-way ANOVA, followed by Tukey's test for multiple comparisons with unequal cell size. A probability level of 0.05 was accepted as indicating significant differences.

For statistical analysis of the dose-response curve in the morphine tolerance study, linear regression lines were computed on the basis of Bolton's method (2). The linearity and parallelism of the dose-response lines were calculated, and the relative potency and ED_{50} values with 95% confidence intervals were determined by comparing the dose-response lines. The relative potency was expressed as a ratio between two drugs that give the same response (2).

RESULTS

Development of Rapid Tolerance to Morphine

Repeated administration of morphine shifted the morphine dose-response curve to the right as compared with that for the saline-pretreated control group. In tolerant animals, 1.84 (confidence limits: 1.50–2.25) times more morphine was necessary to produce the same analgesic effect as that in the morphine-naive control animals. The shift of the dose-response curve to the right demonstrates the development of tolerance to morphine in morphine-treated animals (Fig. 1). The ED_{50} value was 2.5 (1.84–3.39) for the control group and 4.60 (3.12–6.76) for the tolerant group.

Effects of CGRP on Acute Morphine Administration

CGRP had no effect on noiception over 4 h after ICV administration and on the acute antinociceptive action of a single dose of morphine (data not shown).

As a next step, the influence of CGRP on the development of rapid morphine tolerance was investigated. The dose of 200 ng peptide had a significant influence at all time points checked (30, 60, and 120 min after the test morphine challenge), producing a higher antinociceptive effect as compared with the tolerant control group, suppressing the development

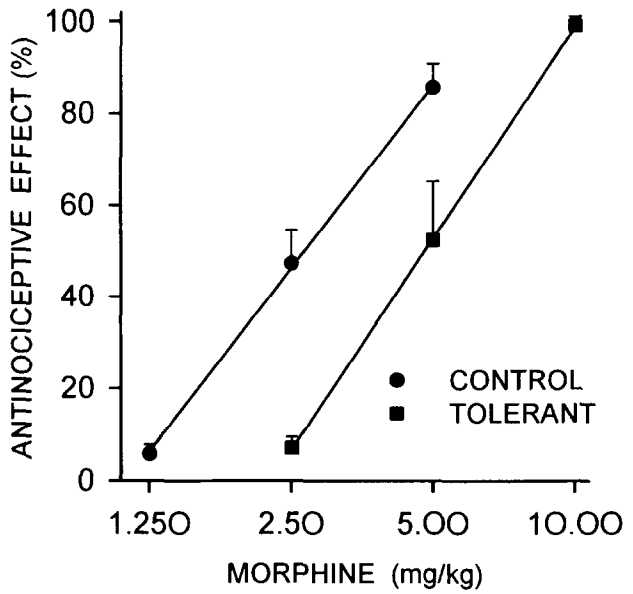

FIG. 1. Dose-response curves for test doses of morphine (1.25, 2.5, 5.0, and 10.0 mg/kg) in mice pretreated with morphine (tolerant) or saline (control). Doses of morphine are depicted on a log scale. Numbers of animals per group: control (42), tolerant (26). Values are means ± SEM.

of rapid tolerance to morphine. At 60 min, the dose of 20-ng CGRP was also effective in blocking the development of tolerance to morphine (Fig. 2). CGRP had a significant tolerance-suppressive influence at 30 min [$F(3, 76) = 74.47$; $p < 0.001$], 60 min [$F(3, 76) = 21.05$; $p < 0.001$], and 120 min [$F(3, 76) = 11.45$; $p < 0.001$].

Effects of CGRP on Chronic Morphine Administration and Withdrawal

In the following experiment, the influence of CGRP on the development of chronic morphine tolerance and on the appearance of naloxone-precipitated withdrawal syndrome was investigated. The peptide had no significant effect on tail-flick latency (data not shown) and naloxone-induced jumping. The withdrawal signs were observed as an increased hypothermic response (Table 1). A marked, but not significant decrease in body weight was also observed.

DISCUSSION

Dose-response studies revealed the development of tolerance to repeated administration of morphine as a parallel shift to the right of the dose-response curves, with changes in relative potency and ED_{50} values. The test dose of morphine chosen for tail-flick studies on the basis of dose-response studies was sufficient to give a significant difference between morphine-naive and morphine-tolerant control groups.

The effects of CGRP on pain sensitivity are the subject of current debate in the literature (6,16). In a previous study (6), synthetic human CGRP was administered ICV in a rather high dose (10.00–20.00 μg/animal) and the peptide itself proved to be analgesic. On the other hand, rat CGRP, similar to human CGRP, with the exception of three amino acids, was ineffective (40–80 μg/animal) in nociceptive reactions to thermal stimuli [e.g., tail-flick and tail-immersion test (6,16)]. In our present study, a similar observation was made by using smaller doses of CGRP. The effects of CGRP on pain sensitization may be dependent upon the noxious stimulus itself, because an analgesic effect was observed in mice pretreated with chemical irritants [e.g., acetic acid (1), formalin (3) and p-phenylquinone (16), and in hot-plate test (16)].

ICV CGRP treatment resulted in a time-dependent, biphasic modulation of morphine-induced antinociception and Ca^{2+} uptake into brain synaptosomes in mice. A 30-min pretreatment shifted the morphine dose-response curve to the left, indicating an antagonism to morphine-induced antinociception. A longer pretreatment (60 min) showed a slight tendency for an enhanced morphine analgesia. The effect was not significantly different from the controls, like in our experiments where CGRP, 45 min after ICV administration did not alter the morphine-induced antinociception. The behavioral observations showed a correlation with in vitro Ca^{2+} uptake in isolated mouse brain synaptosomes. Lack of parallel dose effect curves in behavioral experiments makes unlikely that the peptide can act specifically at the morphine receptor, however, in vitro Ca^{2+} uptake data substantiate the hypothe-

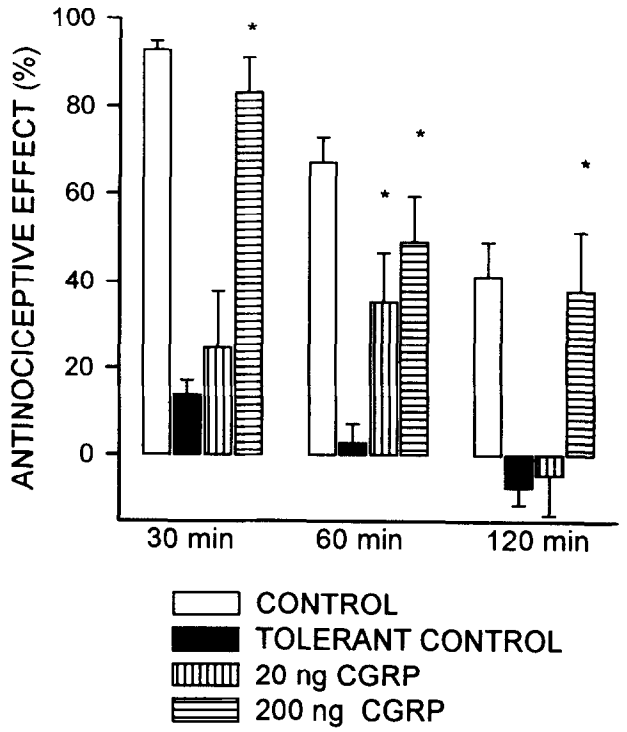

FIG. 2. Effects of CGRP (intracerebroventricular [ICV]) on development of rapid tolerance to morphine. On day 1, the animals were treated with peptide or vehicle, and 1 h later a tolerance-inducing dose of morphine (60 mg/kg) was injected; 24 h later, a test dose of morphine (4 mg/kg) was administered, after a preliminary test of sensitivity to pain; the nociceptive effect was checked 30, 60, and 120 min thereafter. Numbers of animals per group: control, 31; tolerant control, 26; 20 ng CGRP, 10; 200 ng CGRP, 13. There are significant differences ($p < 0.05$) between the control and tolerant control groups at each time point. Asterisks (*) denote significant differences ($p < 0.05$) between the tolerant control and the respective peptide-treated group at a given time point.

TABLE 1

EFFECTS OF CGRP ON NALOXONE-PRECIPITATED WITHDRAWAL

	Control	Tolerant Control	20 ng CGRP	200 ng CGRP
Basal body weight (g)	36.68 ± 0.98	34.68 ± 0.94	34.77 ± 1.09	35.35 ± 0.88
15-min body weight difference (g)	−0.50 ± 0.10	−0.48 ± 0.14	−1.05 ± 0.22	−0.67 ± 0.09
30-min body weight difference (g)	−0.58 ± 0.11	−0.83 ± 0.95	−1.27 ± 0.24	−1.02 ± 0.10
60-min body weight difference (g)	−0.70 ± 0.11	0.94 ± 0.97	−1.40 ± 0.25	−1.24 ± 0.12
Basal temperature (°C)	38.22 ± 0.19	38.56 ± 0.16	37.58 ± 0.21*	38.38 ± 0.22
Temperature difference (°C)	−0.17 ± 0.07	−1.07 ± 0.16*	−0.58 ± 1.79	0.87 ± 0.25*
Jumping latency (min)	N/A	10.10 ± 1.55	10.70 ± 1.79	10.67 ± 1.36

Values are means ± SEM for tested animals. Numbers of animals per group: control, 12; tolerant control, 12; 20 ng CGRP, 10; 200 ng CGRP, 13. N/A = no jumping was detected in the control group. *$p < 0.05$ compared with control group (ANOVA followed by Tukey test).

sis that CGRP acts as a Ca^{2+} modulator in different brain areas (16).

The development of tolerance to morphine was investigated in two experimental designs; rapid and chronic tolerance to morphine was affected in different ways by CGRP treatment. A higher dose of CGRP significantly impaired the development of rapid morphine tolerance over the 2-h investigation period, whereas a smaller dose of CGRP, had only a transitory blocking effect 1 h later. The dose of CGRP modifying the response to morphine was considerably lower than that in previous studies (5,6,10,16) suggesting a possible endogenous role of CGRP in the response to morphine. The development of lessened analgesia in CGRP-treated animals after a challenge dose of morphine support the view that CGRP acts as an endogenous Ca^{2+} modulator in the brain to counteract morphine's physiological effects by antagonizing morphine-induced decreases in Ca^{2+} uptake to neural tissue (16). One might suppose that CGRP treatment transitorily alters opiate binding.

Development of chronic tolerance to morphine and morphine abstinence syndrome, measured as naloxone-precipitated withdrawal jumping was not affected by ICV CGRP. Similar observations were reported on the effect of intrathecal CGRP on development of chronic tolerance. An enhanced frequency of naloxone-precipitated jumps were noted (15). No other withdrawal symptoms were affected and naloxone did not show dose-related effect (15). Chronic morphine administration results in development of tolerance to hypnotic effect of morphine. In our study, temperature of tolerant animals did not differ from control animals at the beginning of the experiments. CGRP is known to produce hypothermia upon ICV administration (14) and a slight hypothermia was observed in animals who received 20 ng CGRP at the beginning of the experiments. The hypothermic effect was not dose-related and upon naloxone administration only a small temperature drop was observed in this group. Although pain sensitivity and jumping latency was not affected by CGRP in chronic tolerance and withdrawal studies, morphine withdrawal signs were evidenced by marked hypothermia and a loss of body weight in these animals.

The above data show that a low dose of CGRP given ICV to mice has no effect on intrinsic pain sensitivity and on acute morphine analgesia, but diminishes the development of rapid tolerance to morphine.

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