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Conantokins and variants derived from cone snail venom inhibit naloxone-induced withdrawal jumping in morphine-dependent mice

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Abstract

The biochemical processes underlying opiate addiction are complex, but N-methyl-D-aspartate receptor (NMDAR) dysfunction appears to be one contributing factor. NMDAR antagonists, including MK-801 and memantine, have previously been shown to assuage symptoms stemming from opiate withdrawal. The conantokins are a small family of naturally occurring peptide toxins known to specifically antagonize the NMDAR. In the present study, the effects of wild-type and variant conantokins on the suppression of naloxone-induced jumping in morphine-dependent mice were evaluated. Our results demonstrate that NR2B-selective conantokins, *viz*., con-G, con-G[$S^{16}Y$] and con-G[γ^7K], are potent inhibitors of naloxone-induced jumping at low doses (2–15 nmol/kg) compared with con-T, con-R[1-17], and small-molecule antagonists of the NMDAR, including the NR2B-selective agent, ifenprodil. We conclude that the NR2B-selective conantokins may find utility as neuropharmacological tools for probing NMDAR-related mechanisms of opiate dependence.

Keywords: Conantokin; Morphine; Naloxone; NMDA receptor; NR2B; Mice

The N-methyl-D-aspartate receptor (NMDAR) is a ligand-gated ion channel composed of an obligatory NR1 subunit and one or more modulatory NR2(A-D) or NR3(A-B) subunits [10]. Normal NMDAR activity contributes to numerous processes that facilitate learning and memory, while receptor dysfunction is associated with an array of chronic and acute neuropathophysiologies, including ischemic damage, epilepsy and Parkinsonism. Several studies also implicate NMDAR involvement in the development and maintenance of psychological and physical opiate dependence [2,22,23,11,21]. This proposed role of the NMDAR in opiate addiction is reinforced by evidence demonstrating that NMDAR antagonists can attenuate the development, expression and maintenance of opiate addiction [23,12]. Significantly, regional differences in NMDAR subunit expression and/or protein levels in rodent models of morphine-dependence [2,23,31] suggest that subunit-selective NMDAR antagonists may be useful in probing the mechanisms of opiate dependence.

The conantokins are small, γ-carboxylate-rich peptides derived from the venoms of the *Conus* predatory marine snails. Four members of this peptide family are currently known: conantokin(con)-G, con-T, con-R, and con-L. In mammals, they act as potent and selective inhibitors of the NMDAR [25,16]. Despite the high sequence homology that exists among the conantokins, distinct differences in their subunit selective properties have been reported. In particular, con-G exhibits a marked preference for NR1a/NR2B- and NR1b/NR2B receptor combinations compared with the NR2A-containing counterparts [14,13,7]. In the present study, the conantokins, and select derivatives thereof, were examined for their ability to curtail naloxone-induced jumping in morphine-dependent mice. Con-G-based peptides were probed in order to determine if their NR2B subunit-selective properties would result in responses different from those observed with the non-selective conantokins. Our results demonstrate that NR2B-selective peptides are more effective at inhibiting naloxone-induced jumping than those with broader subunit activity.

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Male Kuming mice (20–24 g, Beijing Animal Center, China) were housed in groups of eight on a 12 h light–dark cycle (light cycle from 8 a.m. to 8 p.m.) at a temperature of 23 ± 2 °C and a relative humidity of 50%. Food pellets and water were available *ad libitum*. All experiments were conducted in accordance with the guidelines of the Beijing Institutes for Biological Science Animal Research Advisory Committee and conformed to the European Community directives for the care and use of laboratory animals.

All peptides were synthesized and purified as described previously [6], converted into their ammonium salts through exchange on a Sephadex G-25 gel-filtration column. The primary sequences of the conatokin peptides were employed in this study are as follows:

con-G: GEγγLQγNQγLIRγKSN-NH_2
con-G[γ7K]: GEγγLQKNQγLIRγKSN-NH_2
con-G[S16Y]: GEγγLQγNQγLIRγKYN-NH_2
Ala-con-G: GEγγLGKAQALIRAAYA-NH_2
con-G[γ14A]: GEγγLQγNQγLIRAKSN-NH_2
con-R[1-17]: GEγγVAKMAAγLARγNI-NH_2
con-T: GEγγYQKMLγNLRγAEVKKNA-NH_2
con-T[γ10K,γ14K]: GEγγYQKML**K**NLR**K**AEVKKNA-NH_2

Morphine hydrochloride was purchased from Qinghai Pharmaceutical Factory, China. Naloxone hydrochloride, ifenprodil tartrate, memantine hydrochloride, agmatine sulfate was obtained from Sigma (St. Louis, MO, USA). All drugs were dissolved in 0.9% saline to final concentrations and administered intraperitoneally (i.p.) in a volume of 10 ml/kg or intracerebroventicularly (i.c.v.) in a volume of 1 ml/kg using a 25 gauge needle. For the latter route of administration, the needle shield (0.4 mm outer diameter) was trimmed and placed back on the needle to allow for no more than 2 mm of penetration into the left hemisphere of the brain during injection. The doses of memantine and agmatine, a noncompetitive NMDAR antagonist and partial NMDAR inhibitor, respectively [3,1], were previously reported to be effective in reducing naloxone-induced morphine withdrawal symptoms [18,17]. The doses of ifenprodil (a NR2B subunit selective inhibitor) employed in this study were chosen based on the highest doses used in a previous investigation of ifenprodil suppression of morphine-induced place preference [26].

Morphine hydrochloride was injected subcutaneously (s.c.) into the scruff of the mice three times daily (t.i.d.: 8:30, 14:30 and 20:30) for 7 days according to an escalating dose schedule [8]. The initial dose of morphine was 5 mg/kg. Thereafter, the dose of morphine was doubled every second day. A dose of 160 mg/kg was delivered on the sixth day and this dose was given again only once on the morning of the seventh day.

In all groups of experiments, saline (1 ml/kg, i.c.v.), conantokin peptides (2.5, 5, 10, 15 nmol/kg, i.c.v.), memantine (10 mg/kg, 46.3 μmol/kg, i.p.) [18], agmatine (10 mg/kg; 11 μmol/kg, i.c.v.) [17], and ifenprodil (1.25 μmol/kg, i.c.v.; 25 μmol/kg, i.p.) [26] were administered 2.5 h following the final dose of morphine. Saline or NMDAR antagonists did not induce jumping in morphine-dependent mice prior to naloxone injection. The abstinence syndrome was precipitated by administering an i.p. injection of naloxone (4.5 mg/kg) [9,24] 30 min after injection of saline or NMDAR antagonist. Each mouse was immediately placed in a square observation box (30 cm × 30 cm × 50 cm) and the number of jumps was recorded over a 15 min period. The body weight loss induced after naloxone administration was also recorded and expressed as the weight loss per 100 g body weight in 1 h.

The data expressed as the mean ± S.D. were analyzed by one-way analysis of variance (ANOVA) followed by the Student-Newman-Keul's test. Differences with a P-value <0.05 were considered to be statistically significant.

As shown in Fig. 1, at a dose of 15 nmol/kg (i.c.v.) all the conantokins pertinent to this study were capable of attenuating naloxone-induced jumping in morphine-dependent mice. In the mice treated with con-G, con-G[γ7K], or con-G[S16Y], the jumping response was not only considerably diminished, but essentially eliminated for the latter two treatment groups. The suppression of jumping was dose-dependent for all three peptides (Fig. 2).

Fig. 3A compares the attenuating effects of memantine, agmatine and ifenprodil with con-G and con-G[γ7K] in the context of naloxone-induced jumping. At high doses, memantine (10 mg/kg; 46.3 μmol/kg, i.c.v.) and agmatine (10 mg/kg; 11 μmol/kg, i.c.v.) significantly reduced naloxone-induced jumping. Ifenprodil, on the other hand, had no effect on this behavior at either an i.p.-administered dose of 20 mg/kg (25 μmol/kg) or an i.c.v. dose of 1 mg/kg (1.25 μmol/kg). Since the doses of memantine and agmatine were those reported to be most active in the suppression of naloxone-induced jumping

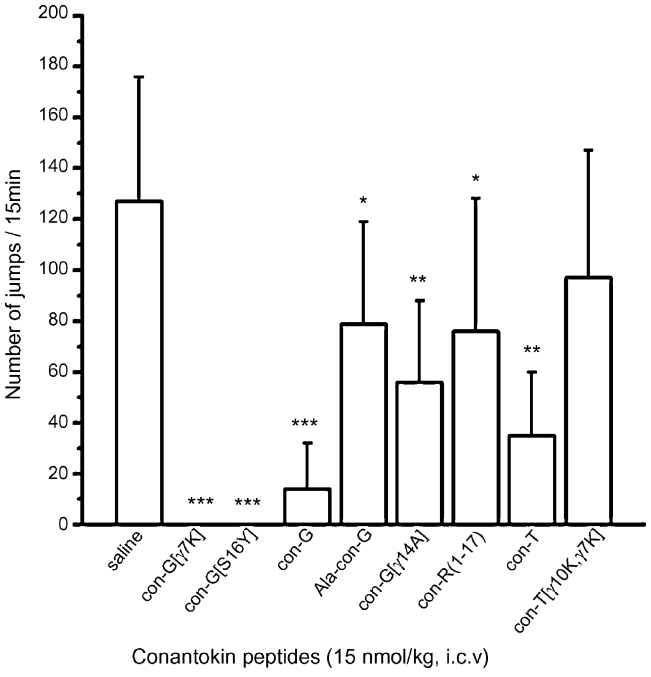

Fig. 1. Effects of the conantokin peptides and select variants on naloxone-induced jumping in morphine-dependent mice. Saline (1 ml/kg, i.c.v.) or peptides (15 nmol/kg, i.c.v.) were injected 30 min prior to naloxone administration. All morphine-dependent animals received a 4.5 mg/kg, i.p., dose of naloxone to induce jumping. Bars indicating number of jumps represent mean ± S.D. Each group was comprised of eight mice. Asterisks represent P-values of *<0.05, **<0.01, and ***<0.001 in comparison with the saline control groups.