Influence of Dynorphin A (1-13) and Dynorphin A (1-10) Amide on Stress-Induced Analgesia

M. STAREC, J. ROSINA1, J. MÁLEK2, M. KRŠIAK

Institute of Pharmacology, Third Faculty of Medicine, Charles University, Prague, 1Department of Biophysics and 2Clinic of Anaesthesiology, Faculty Hospital, Prague, Czech Republic

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Summary
Dynorphin A (1-13) and its analog dynorphin A (1-10) amide were applied intracerebroventricularly in male ICR mice. Both dynorphins did not reveal any analgesic activity in tail-flick test under normal (non-stressed) conditions. However, in combination with stress (forced swimming or whole body vibration) both dynorphins prolonged tail-flick latencies when compared with stressed saline controls. Naloxone inhibited the effect of dynorphins in forced swimming test. Neither dynorphin A (1-13) nor dynorphin A (1-10) amide increased tail-flick latencies when combined with weak immobilization stress. Our results suggest that the analgesic effects of dynorphins are potentiated by strong stressors.

Key words
Dynorphin – Stress – Analgesia – Kappa opioids – Mice

Introduction
The role of the μ-opioid receptor in supraspinal antinociception is universally accepted. However, the development of novel analgesics with activity exclusively at this site is limited due to many undesirable side-effects associated with activation of the μ-opioid receptor including respiratory depression, constipation etc. Thus one of the most promising routes for the development of novel analgesics is to influence supraspinal mediation of analgesia through the κ-opioid receptor. It is now generally accepted that κ agonists are effective antinociceptive agents at both supraspinal (Unterwald et al. 1987, Leighton et al. 1987) and spinal sites in a variety of models (Piercey and Einspahr 1989, Millan 1989, Horan et al. 1991). Dynorphin A (1-13) and dynorphin A (1-17) were shown to have unique binding characteristics among the kappa-opioids, to play a physiological role as an endogenous pain modulators (Millan et al. 1986) and especially to have a modulatory effect on the expression of opioid withdrawal and tolerance (Hooke et al. 1995). They are gaining much attention in this respect. Dynorphin A (1-10) amide was reported to be a potent analog of dynorphin A (1-13) given to morphine-tolerant animals (Woo et al. 1982). Dynorphin can be released at spinal sites by certain analgesic compounds given intracerebroventricularly (i.c.v.) (Fujimoto and Rady 1989, Fujimoto et al. 1990, Fujimoto and Holmes 1990). By itself, dynorphin produces analgesia in the tail-pincher test (Herman et al. 1980, Kaneko et al. 1983, Nakazawa et al. 1985), the hind paw pressure test (Hayes et al. 1983, Kishioka et al. 1992), the acetate-induced writhing test (Nakazawa et al. 1985, Gairin et al. 1988, Kishioka et al. 1992) and the cold water tail-flick test (Tiseo et al. 1988, Tiseo et al. 1990), but not in the radiant tail-flick test (Friedman et al. 1981, Pentel et al. 1995).

On the other hand, various stressors produce opioid or non opioid-mediated analgesia. In the opioid mediated analgesia, μ but also δ and/or κ-opioid receptors are involved (Yamada and Nabeshima 1995). Stress- induced analgesia can be influenced by many drugs and also drug-induced antinociception can be modified by different stressors. Analgesia produced by continuous cold-water swimming (non-opiate analgesia) was altered by pretreatment with morphine (Grisel et al. 1993). Short cold-water swimming which did not result in analgesia in mice, shifts the dose-response curve for morphine analgesia to the left.
(Vanderah et al. 1993). Restraint – another frequently used stressor – potentiates the magnitude and duration of analgesia following both peripheral and i.c.v. administration of several opioid agonists (morphine, enkephalin analog DAGO) as compared to non-stressed controls. (Calcagnetti et al. 1992, Levesque and Holtzman 1993, Woolfolk and Holtzman 1993).

Thus, we decided to study the analgesic effects of i.c.v. administration of dynorphin A (1-13) and its analog dynorphin A (1-10) amide under normal (non-stress) conditions and in combination with warm-water swimming, whole body vibration or immobilization stress.

**Materials and Methods**

**Animals**

Male, white ICR mice weighing 25 to 30 g were used in all experiments. Ten mice per cage had free access to food and water. They were housed under natural day/night conditions for at least two weeks before experimentation.

**Stressful manipulations**

Water swim stress consisted of immersion of mice in water at 32 °C using a cylindrical container, 25 cm in diameter and 25 cm high, with water filled to a depth of 15 cm. Mice were placed in the water for a 3-min period and upon extraction dried with a towel. Analgesic activity was tested immediately, 15 and 30 min after the mice had been taken out of the water (i.e. in the 15th, 30th and 45th min after i.c.v. injection). Because dry mice had significantly shorter tail-flick latencies than dunked mice (O'Connor and Chipkin 1984), implying that the dampness of the mouse's tail contributed to the increase in the tail-flick latency, we used mice whose tails had previously been moistened and then dried as the relevant controls.

Mice were placed in a plastic cage 15 cm x 20 cm x 20 cm covered with an iron mash so as not to escape. Mice were exposed to whole body horizontal vibration with a frequency of 200/min, i.e. 3.33 Hz for 15 min.

Immobilization stress was produced by placing the animal in a snug-fitting plexiglass restraining tube for 5 min. Adequate ventilation was provided by means of holes in the sides of the tube.

All stressful manipulations ended in the 15th min after the i.c.v. administration of the compounds or saline and the analgesic activity was immediately measured and then again in the 30th and 45th min after i.c.v. injection.

**Analgesic assay**

Pain sensitivity was measured by the tail-flick assay in which a beam of light was focused on the dorsal surface of the tail of the mouse approximately 2.5 cm from the tip of the tail. The intensity of the heat stimulus was adjusted so that the baseline tail-flick latencies (TFL) averaged between 2–3 s in intact dry control animals. To avoid tissue damage, the stimulus was discontinued after 15 s.

**Drugs**

The following drugs were used: dynorphin A (1-13) and dynorphin A (1-10) amide obtained through the courtesy of the Tsumura Inc. (Japan) naloxone hydrochloride (Narcanti inj., Du Pont).

**Injections**

Compounds or vehicle were delivered into the lateral cerebral ventricle using a modification of the method of Haley and McCormick (1957) as previously described by Porreca et al. (1984). The mice were lightly anaesthetized with ether, an incision was made in the scalp and the bregma was located. Compounds or saline were injected in awake, hand-held mice directly through the skull at a point 2 mm caudal and 2 mm lateral to bregma at a depth of 3 mm using a 10 microlitre Hamilton syringe. All i.c.v. injections were made in a volume of 3 µl. Control experiments were made to verify the correct site of the i.c.v. administration by injecting methylene blue at the same speed and volume as for drug administration. After sacrificing the mice in ether, the brains were removed, sectioned frontally and examined microscopically for the presence of dye particles in the ventricles. In 85% of mice the injections were correctly located. Naloxone was injected subcutaneously 15 min before i.c.v. administration of drugs.

**Data analysis**

Statistical significance of the results was determined by analysis of variance (ANOVA). In all cases p<0.05 was considered statistically significant. Means ± S.D. are shown.

The experiments were approved by the Ethical Committee of the Third Faculty of Medicine, Charles University, Prague. They were always performed between 9.00 and 13.00 h.

**Results**

**Swimming stress**

In non-stressed animals there was no statistically significant difference between saline controls and mice treated with dynorphin A (1-10) amide or dynorphin A (1-13). The analgesic effect of dynorphin A (1-10) amide or dynorphin A (1-13) was significantly higher in stressed animals forced to swim, than in unstressed animals (p<0.01). Compared to the stressed control mice (.injected with saline and forced to swim), stressed mice treated with dynorphin A (1-13) or with dynorphin A (1-10) amide exhibited an attenuated analgesic response (p<0.01).
**Stress- and Dynorphin-Induced Analgesia**

1996

**Fig. 1**

Effect of dynorphin A (1-13) and dynorphin A (1-10) amide 15 min after their intracerebroventricular administration under normal (non-stressed) and forced swimming stress conditions. 0 = intact non-stressed controls, S = saline i.c.v. non-stressed controls, D13 = dynorphin A (1-13) i.c.v., D10 = dynorphin A (1-10) amide i.c.v., D13+N = dynorphin A (1-13) i.c.v. + naloxone s.c. D10+N = dynorphin A (1-10) amide i.c.v. + naloxone s.c. * p<0.05, ** p<0.01. The results are means ± S.D. of TFL values, n=20 in non-stressed mice, n=20 or 30 in stressed mice.

**Fig. 2**

Effect of dynorphin A (1-13) and dynorphin A (1-10) amide 15 and 30 min after their intracerebroventricular administration under normal (non-stressed) and 15-min whole body vibration stress conditions. S = saline i.c.v. non-stressed controls, D13 = dynorphin A (1-13) i.c.v., D10 = dynorphin A (1-10) amide i.c.v. * p<0.05, ** p<0.01. The results are means ± S.D. of TFL values, n=15 in all groups.
Subcutaneous injection of naloxone (0.5 mg/kg) 15 min before i.c.v. application of dynorphins caused a significant decrease of the TFL in both swimming groups of animals treated with dynorphin A (1-13) or dynorphin A (1-10) amide (p<0.05).

The mean TFL of swimming mice with and without saline injection did not differ significantly, but there was a statistically significant difference (p<0.05) between intact mice and saline controls indicating that also i.c.v. application of saline is slight stressful manipulation (Fig. 1).

Whole body vibration stress
Further, we tested the influence of dynorphins on vibration stress. We observed that dynorphin A (1-13) in the dose of 25 nmol/mouse increased TFL in stressed mice compared with the vehicle controls (p<0.05). Dynorphin A (1-10) amide in the dose of 60 nmol/mouse was still more potent (p<0.01). Forty-five minutes after the i.c.v. application (i.e. 30 min after the termination of stress), analgesia decreased to the baseline value (Fig 2).

Immobilization stress
Three minutes of immobilization caused an inconsistent non-significant increase of tail-flick latencies in mice injected with dynorphin A (1-13) and had practically no effect in mice with dynorphin A (1-10) amide either 15 nor 30 min after i.c.v. administration of the drugs (Fig. 3).

Discussion
The major finding of this study is that i.c.v. administered dynorphin A (1-13) and dynorphin A (1-10) amide exhibited analgesic activity under stress conditions when measured using the tail-flick method, although they did not exert any antinociceptive effects themselves even with the high doses used.

It was shown that dynorphin could modify morphine- or β-endorphin-induced analgesic activity. Our results are in agreement with previous findings (Nakazawa et al. 1985) that i.c.v. injections of these
kappa agonists cause weak or no analgesia in radiant heat tail-flick test. However, both dynorphin A (1-13) and dynorphin A (1-10) amide did have an analgetic action using the same test in combination with stress. We can thus suppose that in this study dynorphin modified the effect of opioids released under stress conditions.

Warm-water swim stress (32 °C) has been shown to be opioid-mediated (O'Connor and Chipkin 1984, Terman et al. 1986, Marek et al. 1992, Mogil et al. 1995), or to have both an opioid and a non-opioid component (Vaccarino et al. 1992). Restraint (immobilization) stress produced opioid-mediated analgesia (Levesque and Holtzman 1993, Woolfolk and Holtzman 1993). Whole-body vibration of 3 min duration produced analgesia which was not blocked by naloxone which suggests non-opioid mediated analgesia (data not shown). Nevertheless, both vibration and warm-water swimming potentiated dynorphin analgesia. Immobilization stress that we employed (animals were only partially restrained, they could not turn round but could move their extremities in a restrainer) had only a weak non-significant effect in this respect. We thus suggest that the intensity of the stressor and its significance for the animals is more important than the opioid or non-opioid nature of stress. Moreover, interaction between opioid and non-opioid systems in nociception has been demonstrated (Grisel et al. 1993).

The analgesic effect of both dynorphins potentiated by stress lasted approximately 30 min. The literary data support this finding since dynorphin A (1-13) was described to be susceptible to the rapid degradation by aminopeptidases and carboxypeptidases (Herman et al. 1980, Tulunay et al. 1981).

Naloxone given in high doses at least partially blocked the effect of dynorphins, suggesting the significant role of the opioid system in this antinociceptive effect. The present results also imply that not only μ or β, but also κ-opioid agonists could be potentiated by stress. This could have some importance for these compounds in clinical use.

References


