ELSEVIER

European Journal of Pharmacology 309 (1996) 37–40

ejp

Short communication

Pretreatment with pertussis toxin spinally, but not supraspinally, blocks the cold water swimming-induced antinociception in the mouse

Hirokazu Mizoguchi, Minoru Narita, John P. Kampine, Leon F. Tseng *

Department of Anesthesiology, MEB-462c, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA

Received 14 March 1996; revised 20 May 1996; accepted 29 May 1996

Abstract

Mice exposed to cold water swimming (4°C) for 3 min produced a marked antinociception. Experiments were designed to determine whether pretreatment with pertussis toxin given intrathecally (i.t.) or intracerebroventricularly (i.c.v.) attenuates cold water swimming-induced antinociception in male ICR mice. Antinociception was measured by the tail-flick test 7 min after cold water swimming. I.t. pretreatment with pertussis toxin at a dose of 0.5 μg for 24–96 h caused a time-dependent attenuation of cold water swimming-induced antinociception. Moreover, i.t. pretreatment with pertussis toxin at doses from 0.125 to 0.5 μg for 96 h attenuated cold water swimming-induced antinociception in a dose-dependent manner. However, i.c.v. pretreatment with pertussis toxin at doses from 0.125 to 0.5 μg for 24–96 h did not affect the cold water swimming-induced antinociception. The present results suggest that pertussis toxin-sensitive Gi/Go proteins in spinal cord, but not at the supraspinal sites, are involved in cold water swimming-induced antinociception.

Keywords: Cold water swimming; Antinociception; Pertussis toxin; Gi/Go protein; Opioid receptor; (Mouse)

1. Introduction

It has been well documented that the antinociception induced by environmental stimuli in rodents is mediated in part by the activation of endogenous opioid systems (Tierney et al., 1991; Mayer and Manning, 1995). Of many forms of environmental stimuli which produce antinociception, cold water swimming in mice has been consistently shown to produce opioid-mediated antinociception which is mediated by the activation of δ-opioid receptors either in the spinal cord (Mizoguchi et al., 1995) or at the supraspinal sites (Killian et al., 1995; Vanderah et al., 1992). However, we have recently found that this cold water swimming-induced antinociception is mainly mediated by the stimulation of δ-opioid receptors in the spinal cord (Mizoguchi et al., 1995) but not at the supraspinal sites (Mizoguchi et al., 1996). The finding is inconsistent with reports by Killian et al. (1995) and Vanderah et al. (1992) who reported that the δ-opioid receptors at the supraspinal sites are involved in cold water swimming-induced antinociception.

Opioid receptors such as μ-, δ- and κ-opioid receptors, at both supraspinal and spinal sites couple to pertussis toxin-sensitive Gi/Go proteins. This contention is supported by the findings that antinociceptive effects induced by μ-, δ- and κ-opioid receptor agonists given intracerebroventricularly (i.c.v.) and intrathecally (i.t.) are attenuated by the pretreatment with pertussis toxin given i.c.v. or i.t., respectively (Przewlocki et al., 1987; Sánchez-Blázquez and Garzón, 1988; Wong et al., 1992; Tseng and Collins, 1996). Pertussis toxin interferes with the signal transduction of μ-, δ- and κ-opioid receptors by ADP-ribosylating α subunit of the coupled Gi/Go proteins (Law, 1995).

The present studies were designed to determine whether i.t. or i.c.v. pretreatment with pertussis toxin attenuated cold water swimming-induced antinociception in mice. We found that i.t., but not i.c.v., pretreatment with pertussis toxin attenuated cold water swimming-induced antinociception. Our results provide additional evidence to support the contention that δ-opioid receptors in the spinal cord, but not at the supraspinal sites, are involved in cold water swimming-induced antinociception.

* Corresponding author. Tel.: +1 414 456 5681; fax: +1 414 456 8541; e-mail: ltseng@post.its.mcw.edu

PII S0014-2999(96)00416-5

2. Materials and methods

2.1. Animals

Male ICR mice weighing 23–25 g (Sasco, Omaha, NE, USA) were used. Animals were housed five per cage in a room maintained at 22 ± 0.5°C with an alternating 12 h light-dark cycle. Food and water were available ad libitum. Animals were used only once.

2.2. Assessment of antinociception

Antinociception was determined by the tail-flick test (D'Amour and Smith, 1941). For measurement of the latency of the tail-flick response, mice were gently held by hand with their tail positioned in an apparatus (model TF6; EMDIE Instrument Co., Maidens, VA, USA) for radiant heat stimulation on the dorsal surface of the tail. The intensity of the heat stimulus was adjusted so that the animal flicked its tail after 3–5 s. The inhibition of the tail-flick response was expressed as percent maximum possible effect, % MPE, which was calculated as: $[(T_1 - T_0)/(T_2 - T_0)] \times 100$, where T_0 and T_1 were the tail-flick latencies before and after the cold water swimming and T_2 was the cutoff time which was set at 10 s for the test to avoid injury of the tail.

2.3. Antinociception induced by cold water swimming

A water tank (30 × 20 and 15 cm tall) filled with ice-cold water (4°C) 7.5 cm in depth was used for cold water swimming. After the measurement of the baseline tail-flick latency, the individual mouse was made to swim in the ice water for 3 min and dried immediately after swimming with cloth towels. The preliminary experiment indicated that the inhibition of the tail-flick response developed immediately after cold water swimming, reached its peak in about 7 min, gradually declined and returned to that of the pre-swimming level in 20–25 min. The tail-flick response was then tested 7 min after cold water swimming in all experiments.

2.4. I.t. and i.c.v. injection

I.t. administration was performed following the method described by Hylden and Wilcox (1980) using a 25-μl Hamilton syringe with a 30-gauge needle, and i.c.v. administration was made according to the procedure of Haley and McCormick (1957) using a 10-μl Hamilton syringe. Injection volume for i.t. and i.c.v. injection was 5 μl and 4 μl, respectively.

2.5. Drugs

Pertussis toxin (Research Biochemicals International, Natick, MA, USA) was dissolved in sterile saline solution (0.9% NaCl solution).

2.6. Statistical analysis

The data are expressed as the mean and S.E.M. Statistical analysis of differences between groups was assessed with a one-way analysis of variance (ANOVA) followed by the Newman-Keuls test.

3. Results

Groups of mice were pretreated i.t. with pertussis toxin (0.5 μg) or saline (5 μl) and the antinociception induced by cold water swimming was measured 24, 48, 72 and 96 h after the pretreatment. As shown in Fig. 1A, the antinociception induced by cold water swimming was gradually attenuated following the i.t. treatment with pertussis toxin (0.5 μg) but not saline (5 μl). The % MPE of cold water swimming-induced antinociception in pertussis toxin pretreated mice was time-dependently attenuated to 65.3, 47.7, 33.9 and 27.2% at 24, 48, 72 and 96 h, respectively,

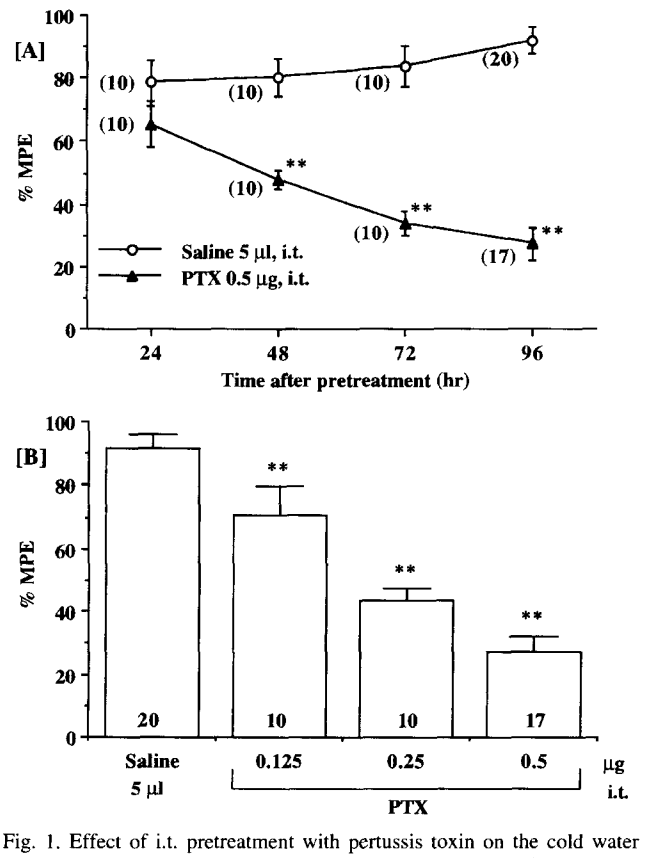

Fig. 1. Effect of i.t. pretreatment with pertussis toxin on the cold water swimming-induced antinociception in mice. A: Time course (24–96 h) of the attenuation of cold water swimming (4°C, 3 min)-induced antinociception following i.t. pretreatment with pertussis toxin (0.5 μg) or saline (5 μl). B: Dose-related effect of 96 h i.t. pretreatment with pertussis toxin (0.125–0.5 μg) or saline on the cold water swimming-induced antinociception. The tail-flick response was measured 7 min after the cold water swimming. The numbers within parentheses (A) or bars (B) indicate the number of mice used and the vertical bars represent the S.E.M. ** $P <$ 0.01, compared to mice pretreated with saline.

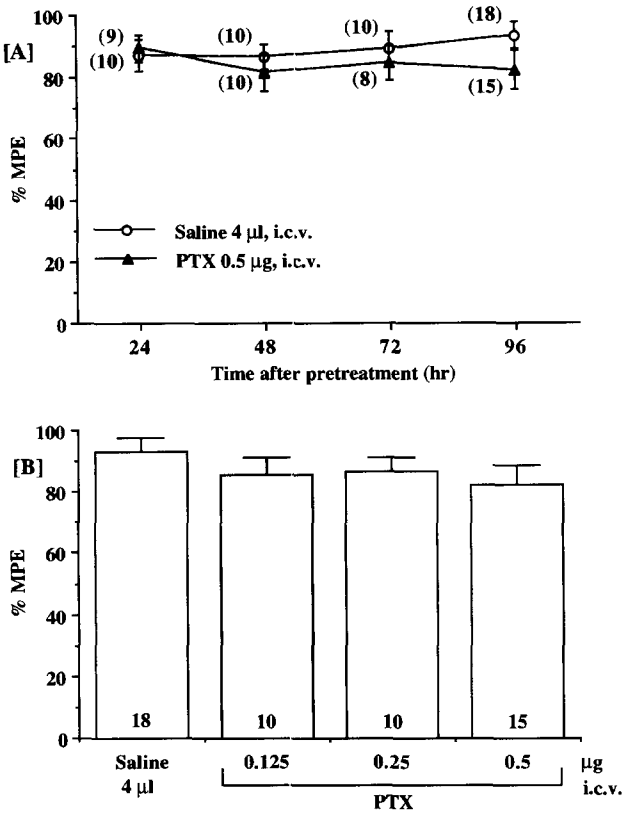

Fig. 2. Effect of i.c.v. pretreatment with pertussis toxin on the cold water swimming-induced antinociception in mice. A: Time course (24–96 h) of the attenuation of cold water swimming (4°C, 3 min)-induced antinociception following i.c.v. pretreatment with pertussis toxin (0.5 μg) or saline (4 μl). B: Dose-related effect of 96 h i.c.v. pretreatment with pertussis toxin (0.125–0.5 μg) or saline on the cold water swimming-induced antinociception. The tail-flick response was measured 7 min after the cold water swimming. The numbers within parentheses (A) or bars (B) indicate the number of mice used and the vertical bars represent the S.E.M.

after pretreatment. On the other hand, saline pretreated mice remained 79–92% MPE of cold water swimming-induced antinociception. Another group of mice was pretreated i.t. with different doses of pertussis toxin (0.125–0.5 μg) 96 h before the exposure to cold water swimming. The i.t. pretreatment with pertussis toxin (0.125–0.5 μg) for 96 h caused a dose-dependent attenuation of cold water swimming-induced antinociception (Fig. 1B). The % MPE of the cold water swimming-induced antinociception was significantly reduced to 70.6, 43.4 and 27.2% MPE by pretreatment with 0.125, 0.25 and 0.5 μg of pertussis toxin, respectively, while mice pretreated with saline remained 91.8% MPE of cold water swimming-induced antinociception.

Groups of mice were pretreated i.c.v. with pertussis toxin (0.5 μg) or saline (4 μl) and the antinociception induced by cold water swimming was measured 24, 48, 72 and 96 h after the pretreatment with pertussis toxin or saline. As shown in Fig. 2A, i.c.v. pretreatment with pertussis toxin (0.5 μg) did not affect cold water swimming-induced antinociception at various times after the pretreatment. Another group of mice was pretreated i.c.v. with various doses of pertussis toxin (0.125–0.5 μg) 96 h prior to the exposure to cold water swimming. I.c.v. pretreatment with various doses of pertussis toxin also did not affect cold water swimming-induced antinociception (Fig. 2B).

4. Discussion

We have recently found that cold water swimming-induced antinociception is mainly mediated by the stimulation of δ-opioid receptors in the spinal cord (Mizoguchi et al., 1995) but not at the supraspinal sites (Mizoguchi et al., 1996). This contention is supported by the finding that the blockade of δ-opioid receptors in the spinal cord by the δ-opioid receptor antagonist naltrindole (NTI) given i.t. or depletion of δ-opioid receptors in the spinal cord by i.t. treatment with antisense oligodeoxynucleotide to δ-opioid receptor mRNA blocks cold water swimming-induced antinociception (Mizoguchi et al., 1995). On the other hand, the blockade of δ-opioid receptors at the supraspinal sites by i.c.v. injection of NTI which significantly blocked the antinociception induced by i.c.v. treated [D-Pen^2,D-Pen^5]enkephalin (DPDPE) or [D-Ala^2]deltorphin II, did not block cold water swimming-induced antinociception (Mizoguchi et al., 1996). The effect is specific to δ-opioid receptors because blockade of μ- or κ-opioid receptors either in the spinal cord or at the supraspinal sites by i.t. or i.c.v. injection of D-Phe-Cys-Tyr-D-Try-Orn-Thr-Phe-Thr-NH_2 (CTOP) or norbinaltorphimine (norBNI) did not block cold water swimming-induced antinociception at doses which blocked i.t. or i.c.v. treated [D-Ala^2,NHPhe^4,Gly-ol]enkephalin (DAMGO)- and U50,488H-induced antinociception, respectively (Mizoguchi et al., 1995, 1996). However, our results are inconsistent with the reports by Vanderah et al. (1992) and Killian et al. (1995). They reported that cold water swimming-induced antinociception was mediated by δ_2-opioid receptor at supraspinal sites. The reason for the discrepancy of the results between their studies and ours is not clear at this time. Besides they did not determine the involvement of spinal δ-opioid receptors on cold water swimming-induced antinociception.

The δ-opioid receptors in both spinal cord and supraspinal sites have been known to couple to pertussis toxin-sensitive Gi/Go proteins (Law, 1995). This is evidenced by the findings that the inhibition of Gi/Go proteins by i.c.v. or i.t. treatment with pertussis toxin blocks, respectively, supraspinal and spinal δ-opioid receptor-mediated antinociceptions (Przewlocki et al., 1987; Sánchez-Blázquez and Garzón, 1988). In the present study, we found that pretreatment spinally with pertussis toxin blocked antinociception induced by cold water swimming. On the other hand, pretreatment supraspinally with pertus-

sis toxin did not affect cold water swimming-induced antinociception. The same pretreatment with pertussis toxin has been shown to significantly attenuate the antinociception induced by the δ_1-opioid receptor agonist DPDPE (Sánchez-Blázquez and Garzón, 1988) and the δ_2-opioid receptor agonist [D-Ala^2]deltorphin II (Tseng and Collins, 1996). Our finding indicates that the antinociception induced by cold water swimming is mediated by the stimulation of pertussis toxin-sensitive Gi/Go protein-coupled receptors in the spinal cord but not at the supraspinal sites. The result is consistent with our previous finding that cold water swimming-induced antinociception is mediated by the stimulation of δ-opioid receptors in spinal cord but not at supraspinal sites (Mizoguchi et al., 1995, 1996).

It has been proposed that the cold water swimming-induced antinociception involves the ascending-descending loops of endogenous pain control system (Mayer and Manning, 1995). The antinociception induced by cold water swimming may involve the receptors at the supraspinal sites which are resistant to pertussis toxin treatment. Kjær et al. (1995) reported that some types of stress induce the release of β-endorphin at the supraspinal sites of rodents. The released β-endorphin activates ϵ-opioid receptors at supraspinal sites and induces subsequently the release of [Met^5]enkephalin in spinal cord which stimulates the δ_2-opioid receptor (Tseng, 1989). Cold water swimming has been considered to be one of the stress. We found in our previous studies that i.c.v. pretreatment with antiserum to β-endorphin or i.t. pretreatment with antiserum to [Met^5]enkephalin attenuated the cold water swimming-induced antinociception (Mizoguchi et al., 1996). This evidence indicates that cold water swimming-induced antinociception is mediated by the release of β-endorphin which stimulates ϵ-opioid receptor at the supraspinal site and the release of [Met^5]enkephalin in spinal cord which stimulates the δ_2-opioid receptor. Tseng and Collins (1996) reported that, unlike i.t. pretreatment with pertussis toxin of mice which blocked i.c.v. injected β-endorphin-induced antinociception, i.c.v. pretreatment with pertussis toxin did not attenuate i.c.v. injected β-endorphin-induced antinociception. These findings indicate that, unlike δ_2-opioid receptor in spinal cord which is coupled with pertussis toxin-sensitive Gi/Go protein, ϵ-opioid receptor at supraspinal sites which is stimulated by β-endorphin is not coupled to pertussis toxin-sensitive Gi/Go protein. This may explain why i.t. pretreatment with pertussis toxin of mice blocked cold water swimming-induced antinociception, whereas i.c.v. pretreatment with pertussis toxin did not attenuate cold water swimming-induced antinociception.

In conclusion, cold water swimming-induced antinociception is blocked by i.t., but not i.c.v., pretreatment with pertussis toxin. The cold water swimming-induced antinociception is mediated by pertussis toxin-sensitive Gi/Go protein in the spinal cord, and this antinociception is not mediated by a mechanism which involves pertussis toxin-sensitive Gi/Go protein at the supraspinal site, like μ-, δ- and κ-opioid receptor at the supraspinal site.

Acknowledgements

This work was supported by US Public Health Service Grant from the National Institute on Drug Abuse (DA 03811).

References

D'Amour, F.E. and D.L. Smith, 1941, A method for determining loss of pain sensation, J. Pharmacol. Exp. Ther. 72, 74.

Haley, M.J. and W.G. McCormick, 1957, Pharmacological effects produced by intracerebral injections of drugs in the conscious mice, Br. J. Pharmacol. 12, 12.

Hylden, J.L.K. and G.L. Wilcox, 1980, Intrathecal morphine in mice: a new technique, Eur. J. Pharmacol. 167, 313.

Killian, P., B.B. Holmes, A.E. Takemori, P.S. Portoghese and J.M. Fujimoto, 1995, Cold water swim stress- and delta-2 opioid-induced analgesia are modulated by spinal γ-aminobutyric acid$_A$ receptor, J. Pharmacol. Exp. Ther. 274, 730.

Kjær, A., U. Knigge, F.W. Bach and J. Warberg, 1995, Stress-induced secretion of pro-opiomelanocortin-derived peptides in rats: relative importance of the anterior and intermediate pituitary lobes, Neuroendocrinology 61, 167.

Law, P.Y., 1995, G-protein and opioid receptor's function, in: The Pharmacology of Opioid Peptides, ed. L.F. Tseng (Hardwood Academic Publishers) p. 109.

Mayer, D.J. and B.H. Manning, 1995, The role of opioid peptides in environmentally-induced analgesia, in: The Pharmacology of Opioid Peptides, ed. L.F. Tseng (Hardwood Academic Publishers) p. 345.

Mizoguchi, H., M. Narita, H. Nagase and L.F. Tseng, 1995, Antisense oligodeoxynucleotide to a δ-opioid receptor blocks the antinociception induced by cold water swimming, Regul. Pept. 59, 255.

Mizoguchi, H., M. Narita and L.F. Tseng, 1996, Ascending-descending loop is involved in antinociception induced by cold water swimming in the mouse, 8th World Congress on Pain, Vancouver, Canada.

Przewlocki, R., T. Costa, J. Long and A. Herz, 1987, Pertussis toxin abolishes the antinociception mediated by opioid receptors in rat spinal cord, Eur. J. Pharmacol. 144, 91.

Sánchez-Blázquez, P. and J. Garzón, 1988, Pertussis toxin differentially reduces the efficacy of opioids to produce supraspinal analgesia in the mouse, Eur. J. Pharmacol. 152, 357.

Tierney, G., J. Carmody and D. Jamieson, 1991, Stress analgesia: the opioid analgesia of long swims suppresses the non-opioid analgesia induced by short swims in mice, Pain 46, 89.

Tseng, L.F., 1989, Intracerebroventricular administration of β-endorphin releases immunoreactive Met-enkephalin from the spinal cord in cats, guinea pigs and mice, Neuropharmacology 28, 1333.

Tseng, L.F. and K.A. Collins, 1996, Pretreatment with pertussis toxin differentially modulates morphine- and β-endorphin-induced antinociception in the mouse, J. Pharmacol. Exp. Ther. (in press).

Vanderah, T.W., K.D. Wild, A.E. Takemori, M. Sultana, P.S. Portoghese, W.D. Bowen, H.I. Mosberg and F. Porreca, 1992, Mediation of swim-stress antinociception by the opioid delta$_2$ receptor in the mouse, J. Pharmacol. Exp. Ther. 262, 190.

Wong, C.-S., Y.-F. Su, K.-J. Chang and D. Watkins, 1992, Intrathecal pertussis toxin treatment attenuates opioid antinociception and reduces high-affinity state of opioid receptors, Anesthesiology 77, 691.