ELSEVIER

European Journal of Pharmacology 280 (1995) R1–R3

ejp

Rapid communication

Inhibition of protein kinase C, but not of protein kinase A, blocks the development of acute antinociceptive tolerance to an intrathecally administered μ-opioid receptor agonist in the mouse

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Received 24 May 1995; accepted 29 May 1995

Abstract

A specific protein kinase C inhibitor, calphostin C, which injected alone had no effect on the antinociception induced by intrathecal (i.t.) administration of a selective μ-opioid receptor agonist, [D-Ala^2,NMePhe^4,Gly(ol)^5]enkephalin (DAMGO), dose-dependently attenuated the development of acute tolerance to the i.t. DAMGO-induced antinociception in male ICR mice. On the other hand, a selective protein kinase A inhibitor, KT5720, did not have any effect on the development of acute tolerance to DAMGO antinociception. These findings suggest that protein kinase C, but not protein kinase A, plays an important role in the development of acute tolerance to the μ-opioid receptor agonist-induced antinociception.

Keywords: Protein kinase A; Protein kinase C; Opioid tolerance

The mechanism responsible for antinociceptive tolerance to opioids has been the subject of speculation for several decades, but there is still much uncertainty concerning the nature of adaptive processes involved. In recent years, increasing understanding of intracellular messenger pathways has provided an experimental framework for studies of molecular mechanisms underlying opioid tolerance (Nestler, 1992; Narita et al., 1994a, b, c). The development of opioid tolerance may be achieved by receptor phosphorylation or uncoupling of the receptor from its effector system, resulting in a loss of agonist affinity and activity. The aim of the present study was to investigate the effects of a highly selective protein kinase C inhibitor, calphostin C (Kobayashi et al., 1989), and a specific protein kinase A inhibitor, KT5720 (Kase et al., 1987), on the development of acute tolerance to antinociception induced by an intrathecally (i.t.) administered μ-opioid-receptor agonist, [D-Ala^2,NMePhe^4,Gly(ol)^5]enkephalin (DAMGO), in the mouse.

Male ICR mice weighing 25–30 g (Sasco, Omaha, NE, USA) were used. The drugs used and their suppliers were as follows: DAMGO (Peninsula Laboratory, Belmont, CA, USA), calphostin C (Calbiochem-Novabiochem International, San Diego, CA, USA) and KT5720 ((8*R*,9*S*,11*S*)-(−)-9-hydroxy-9-*n*-hexyloxycarbonyl-8-methyl-2,3,9,10-tetrahydro-8,11-epoxy-1*H*, 8*H*,11*H*-2,7*b*,11*a*-triazadibenzo[*a*,*g*]cycloocta[*cde*]-trinden-1-one, Calbiochem-Novabiochem International). Calphostin C and KT5720 were dissolved in dimethyl sulfoxide 0.5% in saline. All drugs were injected i.t. in mice. The injection volume for i.t. injection was 5 μl. Groups of mice were pretreated i.t. with DAMGO (10 ng), DAMGO (10 ng) in combination with calphostin C (1, 2 and 4 ng) or DAMGO (10 ng) in combination with KT5720 (4 ng). A second injection of DAMGO at 10 ng was given i.t. 3 h after the first i.t. injection of DAMGO, DAMGO and calphostin C or DAMGO and KT5720. The antinociceptive assay was performed using the tail-flick test 10 min after the first or second injection. For measurement of the latency of the tail-flick response, mice were gently hand-held with their tail positioned in the apparatus (Model TF6, EMDIE Instrument Co., Maidens, VA, USA) for radiant heat stimulation of the dorsal surface of the tail.

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SSDI 0014-2999(95)00322-3

Antinociception was expressed as percent maximal 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 injection of the opioid receptor agonist and T_2 was the cutoff time, which was set at 10 s for the test to avoid injury to the tail. The data are expressed as means and S.E.M. The statistical significance of differences between groups was assessed with a one-way analysis of variance (ANOVA) followed by the Newman-Keuls test.

An i.t. injection of DAMGO (10 ng) induced inhibition of the tail-flick response. The inhibition of the tail-flick response started in 5 min, reached a peak in 10–20 min. The tail-flick inhibition then gradually decreased and the tail-flick latencies returned to their control levels in 60 min (data not shown). Concomitant i.t. injection of calphostin C (1–4 ng) or KT5720 (4 ng) did not have any effect on antinociception induced by i.t. administered DAMGO (Table 1). Intrathecal pretreatment with DAMGO markedly attenuated the antinociception effect induced by the second i.t. injection of DAMGO (61.4% inhibition). The attenuation of DAMGO-induced antinociception produced by DAMGO pretreatment was dose-dependently blocked by i.t. pretreatment with calphostin C. On the other hand, KT5720 at 4 ng, which can inhibit protein kinase A activity (Kase et al., 1987), given as treatment concomitantly with DAMGO had no effect on the development of desensitization to DAMGO antinociception.

Table 1
Effects of specific protein kinase A and protein kinase C inhibitors on the i.t. DAMGO-induced antinociception and the development of acute tolerance to the i.t. DAMGO antinociception

First injection	Second injection challenge	%MPE ± S.E.M.	Number of animals
DAMGO, 10 ng	None	81.0 ± 5.8	15
DAMGO, 10 ng + calphostin C 1 ng	None	84.2 ± 6.9	10
DAMGO, 10 ng + calphostin C, 2 ng	None	76.4 ± 8.2	14
DAMGO, 10 ng + calphostin C, 4 ng	None	81.7 ± 8.8	15
DAMGO, 10 ng + KT5720, 4 ng	None	87.7 ± 6.8	10
DAMGO, 10 ng	DAMGO, 10 ng	31.3 ± 4.1 [b]	16
DAMGO, 10 ng + calphostin C 1 ng	DAMGO, 10 ng	49.8 ± 9.0 [a]	10
DAMGO, 10 ng + calphostin C 2 ng	DAMGO, 10 ng	65.8 ± 6.8 [c]	20
DAMGO, 10 ng + calphostin C 4 ng	DAMGO, 10 ng	77.1 ± 7.2 [c]	15
DAMGO, 10 ng + KT5720 4 ng	DAMGO, 10 ng	33.7 ± 7.2 [b]	10

Calphostin C and KT5720 are specific protein kinase C and protein kinase A inhibitors, respectively. All drugs were injected intrathecally in mice. Calphostin C or KT5720 was concomitantly injected i.t. with DAMGO. The second i.t. injection was performed 3 h after the first injection. The tail-flick latency was measured 10 min after the first or second injection.
[a] $P < 0.05$, [b] $P < 0.01$, compared to mice with a single i.t. injection of DAMGO. [c] $P < 0.01$, compared to mice injected with DAMGO + DAMGO.

In the present study, a single i.t. injection of a μ-opioid receptor agonist, DAMGO, produced acute antinociception tolerance to a second i.t. injection of DAMGO in mice. Tolerance to antinociception induced by i.t. injection of DAMGO occurred rapidly; the effect became apparent within 3 h after the first injection. The present data were supported by previous findings that desensitization to opioid-induced pharmacological actions is rapidly induced by single in vivo or in vitro treatment with opioids (Jung et al., 1994; Nomura et al., 1994). This acute tolerance to DAMGO antinociception is reversible and recovers in 24 h (Narita et al., manuscript in preparation). These findings suggest that opioid receptor down-regulation is not likely to play a role in this short-term desensitization.

We found in the present study that a specific protein kinase C inhibitor, calphostin C, at low doses, which injected alone has no effect on antinociception induced by i.t. injection of DAMGO, caused a dose-dependent blockade of the development of tolerance to DAMGO antinociception. These results indicate that the development of acute antinociception tolerance to DAMGO may result from the phosphorylation of μ-opioid receptors by protein kinase C. This contention is supported by recent findings that repeated administration of morphine to rats produced upregulation of the protein kinase C system with a time course that closely paralleled the time course by which antinociception tolerance to morphine developed (Narita et al., 1994a,c). It was also observed that concomitant i.c.v. infusion of H-7, which is a potent inhibitor of protein kinase C and protein kinase A, dose-dependently inhibited the development of tolerance to i.c.v. morphine-induced antinociception in rats (Narita et al., 1994b). On the other hand, a selective protein kinase A inhibitor, KT5720, did not have any effect on the development of DAMGO tolerance. It has been reported that opioids acutely inhibit adenylate cyclase and thus lower cAMP levels (Nestler, 1992). It seems unlikely that protein kinase A activity is increased during short-term exposure of opioid agonists. Thus, protein kinase A appears not to play an important role in the development of acute tolerance to μ-opioid receptor agonist-induced antinociception.

In conclusion, concomitant i.t. injection of a specific protein kinase C inhibitor, but not of a protein kinase

A inhibitor, attenuates the development of acute tolerance to antinociception induced by an i.t. injection of the μ-opioid receptor agonist, DAMGO, in mice.

Acknowledgments

This work was supported by U.S. Public Health Service Grant, DA 03811 from the National Institute on Drug Abuse, National Institutes of Health.

References

Jung, J.-S., D.-K. Song, H.-W. Suh and Y.-H. Kim, 1994, Effects of intraventricular injection of morphine and β-endorphin on serotonin release from the spinal cord in rats, Pharmacol. Biochem. Behav. 49, 1037.

Kase, H., K. Iwahashi, S. Nakanishi, Y. Matsuda, K. Yamada, M. Takahashi, C. Murakata, A. Sato and M. Kaneko, 1987, K-252a compounds, novel and potent inhibitors of protein kinase C and cyclic nucleotide-dependent protein kinase, Biochem. Biophys. Res. Commun. 142, 436.

Kobayashi, E., H. Nakano, M. Morimoto and T. Tamaoki, 1989, Calphostin C (UCN-1028C), a novel microbial compound, is a highly potent and specific inhibitor of protein kinase C, Biochem. Biophys. Res. Commun. 159, 548.

Narita, M., M. Makimura, Y.-Z. Feng, B. Hoskins and I.K. Ho, 1994a, Influence of chronic morphine treatment on protein kinase C activity: comparison with butorphanol and implication for opioid tolerance, Brain Res. 650, 175.

Narita, M., Y.-Z. Feng, M. Makimura, B. Hoskins and I.K. Ho, 1994b, A protein kinase inhibitor, H-7, inhibits the development of tolerance to opioid antinociception, Eur. J. Pharmacol. 271, 543.

Narita, M., Y.-Z. Feng, M. Makimura, B. Hoskins and I.K. Ho, 1994c, Repeated administration of opioids alters characteristics of membrane-bound phorbol ester binding in rat brain, Eur. J. Pharmacol. 271, 547.

Nestler, J., 1992, Molecular mechanisms of drug addiction, J. Neurosci. 12, 2439.

Nomura, K., E. Reuveny and T. Narahashi, 1994, Opioid inhibition and desensitization of calcium channel currents in rat dorsal root ganglion neurons, J. Pharmacol. Exp. Ther. 270, 466.