Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 467 (2003) 233–234
www.elsevier.com/locate/ejphar

Rapid communication

Identity of the putative δ_1-opioid receptor as a δ–κ heteromer in the mouse spinal cord

Philip S. Portoghese*, Mary M. Lunzer

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, 308 Harvard Street, S.E., Minneapolis, MN 55455, USA

Received 5 March 2003; accepted 11 March 2003

Abstract

In view of the co-localization of spinal δ- and κ-opioid receptors, we have investigated the interaction of selective opioid receptor agonists and antagonists in the spinal cord of mice in order to determine if these receptors are organized as heteromers. The finding that norbinaltorphimine (κ) antagonized [D-Pen^{2,5}]enkephalin (δ_1), but not deltorphin II (δ_2), strongly suggests that the putative δ_1-subtype is a δ–κ heteromer. Studies with selective opioid receptor (ant)agonists support this conclusion.

Keywords: δ_1-Opioid receptor; δ–κ Heteromer; Norbinaltorphimine

Recently, κ- and δ-opioid receptors have been reported to be co-localized in spinal cord neurons (Wessendorf and Dooyema, 2001). In view of the synergism between κ- and δ-opioid agonists upon intrathecal (i.t.) administration (Miaskowski et al., 1990) and evidence for the association of co-expressed κ- and δ-opioid receptors as heteromers in cultured cells (Jordan and Devi, 1999), we have investigated the interaction between selective opioid receptor (ant)agonist ligands that were administered by the i.t. route in mice. Here we present pharmacological data that support the existence of δ–κ opioid receptor heteromers in the spinal cord and identify the δ–κ heteromer as the putative δ_1-opioid receptor subtype (Sofuoglu et al., 1991; Mattia et al., 1991).

The μ-opioid receptor antagonist, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Phe-Thr-NH_2 (CTOP), and the δ_1- and δ_2-opioid receptor antagonists, 7-benzylidenenaltrexone (BNTX) and naltriben (NTB), all possessed i.t. selectivity in mice consistent with their use as pharmacological tools in vivo (Table 1). However, norbinaltorphimine (norBNI) exhibited an apparent absence of antagonist selectivity. In this regard, the μ-opioid receptor agonist [D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]enkephalin (DAMGO) was most potently antagonized, followed by [D-Pen^{2,5}]enkephalin (DPDPE) and the κ-opioid receptor agonist, 3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidiyl)cyclohexyl]benzeneacetamide (U50488) which served as a reference compound for antagonism by norBNI. The antinociception produced by [D-Ala^2,Glu^4]deltorphin (deltorphin II) was unaffected by norBNI.

Inasmuch as DAMGO has been reported to promote the release of spinal dynorphin-A (Vanderah et al., 2001), we wished to determine if the apparent antagonism by norBNI of DAMGO-induced antinociception was due to antagonism of dynorphin-A. Co-administration of norBNI with dynorphin-A antiserum (5 μg, Peninsula Laboratories, San Carlos, CA) exhibited little, if any, antagonism of DAMGO antinociception [ED_{50} ratio, 2.53 (1.68–4.21)], suggesting that the observed potent antagonism in the absence of antiserum was due to DAMGO-promoted release of dynorphin-A whose acute antinociceptive effect was antagonized at κ-opioid receptors by norBNI.

In contrast to the above results, dynorphin-A antiserum failed to significantly reduce the potent norBNI antagonism of DPDPE antinociception [ED_{50} ratio, 10.84 (6.49–18.83)]. This indicates that dynorphin-A was not released by DPDPE and that some other mechanism for the antagonism of norBNI is involved. Significantly, the observation that DPDPE was more potently antagonized by the δ_1-selective ligand, BNTX, than by the δ_2-opioid receptor antagonist, NTB, is consistent with the action of DPDPE at the putative δ_1-opioid receptor subtype. In this connection, although NTB did not significantly antagonize

* Corresponding author. Tel.: +1-612-624-9174; fax: +1-612-626-6891.
E-mail address: porto001@umn.edu (P.S. Portoghese).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01599-1

Table 1
Antagonism of selective opioid agonist-induced antinociception upon intrathecal administration in mice[a]

Antagonist[b]	Agonist[c] ED_{50} ratio (95% C.L.)[d]			
	DAMGO (μ)	DPDPE (δ_1)	Deltorphin II (δ_2)	U50488 (κ)
norBNI (κ)	26.26 (16.30–46.90)	12.44 (9.91–15.83)	1.04 (0.62–1.77)	5.26 (0.24–16.04)
BNTX (δ_1)	1.50 (0.94–2.83)	10.61 (6.08–18.51)	0.99 (0.46–2.00)	0.94 (0.69–1.27)
NTB (δ_2)	1.20 (0.61–3.26)	0.98 (0.71–1.41)	9.07 (4.79–11.56)	0.81 (0.33–1.50)
CTOP (μ)	9.89 (5.91–15.34)	1.43 (1.06–1.93)	1.91 (0.78–4.47)	0.85 (0.69–1.03)

[a] At least three groups of 10 male CD1 mice (Harlan Sprague Dawley) weighing between 20 and 25 g were employed in a modified tail flick assay (Tulunay and Takemori, 1974). Antinociception was considered positive if the latency to flick its tail was more than the control latency plus 3 S.D. of the mean of the reaction time.

[b] Peak times and doses of i.t.-administered antagonists were as follows: nor-BNI, 2.5 nmol, 16 min; BNTX, 25 pmol, 10 min; NTB, 50 pmol, 10 min; and CTOP, 5.9 pmol, 20 min.

[c] Peak times and control ED_{50} values (nmol/mouse) for the antinociceptive effect of the agonists (i.t.) were as follows: DAMGO, 20 min, 0.011 (0.007–0.015); DPDPE, 10 min, 3.35 (3.05–3.66); deltorphin II, 10 min, 2.91 (2.18–3.93); and U50488, 12 min, 20.97 (18.18–24.71).

[d] The parallel line assay was used to calculate the ED_{50} values and the 95% confidence limits. ED_{50} ratios (ED_{50} with antagonist/control ED_{50}) were considered significant when the 95% confidence intervals of the ratio were >1.0.

DPDPE, it potently reversed the anti-nociception of the δ_2-opioid receptor agonist, deltorphin II. Since the action of deltorphin II was not antagonized by norBNI, we conclude that the putative δ_1-opioid receptor subtype, in contrast to the δ_2-opioid receptor, contains an accessible κ-opioid receptor recognition site.

Taken together with reports on the co-localization of spinal opioid receptors and the synergism between δ- and κ-opioid receptor agonists, we propose that DPDPE interacts with a δ-opioid receptor recognition site in an allosteric δ–κ heteromer. According to our model, the binding of norBNI to the κ-opioid receptor recognition site in the heteromeric complex induces a conformational change in its associated δ-opioid receptor that results in antagonism of DPDPE antinociception. A recent study of the porcine ileum, which contains co-localized δ- and κ-opioid receptors, has afforded similar results, in that norBNI was found to potently antagonize selective δ-opioid receptor agonists (Poonyachoti et al., 2001). Thus, the combined data suggest that the putative δ_1-opioid receptor in the mouse spinal cord is a δ–κ heteromer whose subunits are allosterically coupled.

Acknowledgements

This work was supported by the National Institute on Drug Abuse research grant R37 DA01533. The animal protocols used in these experiments were approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC).

References

Jordan, B.A., Devi, L.A., 1999. G-protein-coupled receptor heterodimerization modulates receptor function. Nature 399, 697–700.

Mattia, A., Vanderah, T., Mosberg, H.I., Porreca, F., 1991. Lack of antinociceptive cross-tolerance between [D-Pen2, D-Pen5]enkephalin and [D-Ala2]deltorphin II in mice: evidence for delta receptor subtypes. J. Pharmacol. Exp. Ther. 258, 583–587.

Miaskowski, C., Taiwo, Y.O., Levine, J.D., 1990. Kappa- and delta-opioid agonists synergize to produce potent analgesia. Brain Res. 509, 165–168.

Poonyachoti, S., Portoghese, P.S., Brown, D.R., 2001. Characterization of opioid receptors modulating neurogenic contractions of circular muscle from porcine-illeum and evidence that delta- and kappa-opioid receptors are coexpressed in myenteric neurons. J. Pharmacol. Exp. Ther. 297, 69–77.

Sofuoglu, M., Portoghese, P.S., Takemori, A.E., 1991. Differential antagonism of delta opioid agonists by naltrindole and its benzofuran analog (NTB) in mice: evidence for delta opioid receptor subtypes. J. Pharmacol. Exp. Ther. 257, 676–680.

Tulunay, F.C., Takemori, A.E., 1974. The increased efficacy of narcotic antagonists induced by various narcotic analgesics. J. Pharmacol. Exp. Ther. 190, 395–400.

Vanderah, T.W., Ossipov, M.H., Lai, J., Malan Jr., T.P., Porreca, F., 2001. Mechanisms of opioid-induced pain and antinociceptive tolerance: descending facilitation and spinal dynorphin. Pain 92, 5–9.

Wessendorf, M.W., Dooyema, J., 2001. Coexistence of kappa- and delta-opioid receptors in rat spinal cord axons. Neurosci. Lett. 298, 151–154.