ELSEVIER

European Journal of Pharmacology 302 (1996) R1–R2

ejp

Rapid communication

Determination of activity for nociceptin in the mouse vas deferens

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Received 11 March 1996; accepted 15 March 1996

Abstract

The recently discovered neuropeptide nociceptin was found to inhibit electrically induced contractions of the mouse vas deferens. Nociceptin and its 14-Tyr analog were each partial agonists, but with high affinity (ED_{50} of 20 nM). This activity was not opioid in nature, as it was not inhibited by either selective or non-selective opiate antagonists.

Keywords: Nociceptin; ORL_1; Vas deferens, mouse

Cloning of the δ-opioid receptor was quickly followed by identification of the cDNA for the μ- and κ-opioid receptors as well as that for a fourth receptor with very high structural homology to the other opioid receptors. This fourth receptor, named ORL_1 (for *o*pioid *r*eceptor-*l*ike), required very high concentrations of opioid compounds to mediate the expected inhibition of cAMP accumulation in transfected CHO cells (Mollereau et al., 1994). Very recently, two groups (Meunier et al., 1995; Reinscheid et al., 1995) independently identified a novel 17-amino acid neuropeptide that inhibited cAMP accumulation with an IC_{50} in the nanomolar range. Just as ORL_1 has striking structural similarities to the opioid receptors, the peptide has structural similarities to opioid peptides, particularly dynorphin. This peptide was found to have nociceptive properties in hot plate and tail flick tests, and the names suggested for this peptide are nociceptin by Meunier et al. (1995) and orphanin FQ by Reinscheid et al. (1995).

In an effort to better characterize receptor activity, the ability of nociceptin and its 14-Tyr analog to inhibit electrically induced contractions in the mouse vas deferens assay was studied. Swiss Webster mice weighing 30–35 g were used. The vasa deferentia were prepared according to the method of Hughes et al. (1975) and bathed at 31°C in Mg^{2+}-free Krebs solution bubbled with a mixture of oxygen and carbon dioxide (95 : 5) in an organ bath of 8 ml capacity. An initial tension of 200 mg was applied. The parameters of field stimulation were slightly modified from those originally described (Ronai et al., 1977). Paired shocks with a 100 ms delay between supramaximal rectangular pulses of 1 ms duration, delivered at a rate of 0.1 Hz, were used. The electrically induced contractions were recorded using an isometric transducer (Metrigram) and a Grass 7D multichannel polygraph. A Grass S-88 electrostimulator was used for the electrical stimulation.

The agonist potencies of compounds were determined from concentration response curves and characterized by ED_{50} values. The percent inhibition of the stimulation-induced contraction produced by each agonist was plotted against the log agonist concentration. ED_{50} is defined as the concentration of the agonist that produces 50% of the maximum effect attainable by that agonist.

The mouse vas deferens possesses all three opioid receptor types but is particularly sensitive to δ-opioid agonists (Lord et al., 1977). The activity of selective agonists (such as DAMGO ([D-Ala^2,(Me)Phe^4,Gly-ol^5]enkephalin), DPDPE ([D-Pen^2,D-Pen^5]enkephalin), and U69593 ([5α,7α,8β]-(+)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxa-spiro(4,5)-dec-8-yl])) can be inhibited by the appropriate selective antagonists (CTAP ([D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2]), naltrindole, and nor-binaltorphimine, respectively). At 200 nM, the nonselective antagonist naloxone can inhibit the activity of all opioid agonists at μ-, δ-, and κ-opioid receptors.

Nociceptin inhibited electrically induced contractions in the mouse vas deferens at reasonably low concentrations. As seen in Table 1, the ED_{50} of nociceptin was about 20

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PII S0014-2999(96)00238-5

Table 1
Relative activity of nociceptin and 14-Tyr-nociceptin in the mouse and rat vasa deferentia

Compound	Tissue	ED_{50} (nM)		Maximum inhibition (%)	
Nociceptin	MVD	18.74 ± 3.85	($n = 8$)	64.99 ± 3.93	($n = 8$)
	RVD	46.56 ± 14.4	($n = 4$)	40%	
14-Tyr-nociceptin	MVD	16.39 ± 0.93	($n = 4$)	59.90 ± 3.89	($n = 4$)
	RVD	100	($n = 2$)	40%	

ED_{50} determinations were made on the electrically stimulated mouse (MVD) and rat vas deferens (RVD) as described in the text. The number of determinations are in parentheses.

nM. On the other hand, unlike opioid ligands, it acts as a partial agonist in this system, maximally inhibiting approximately 65% of the twitch height. The 14-Tyr analog of nociceptin was also tested and found to have virtually identical activity to the parent compound. This is consistent with the binding affinities of the two compounds to ORL_1, which are also virtually identical (Toll et al., manuscript in preparation). The activity of either nociceptin or 14-Tyr-nociceptin was not inhibited by the selective antagonists CTAP, naltrindole, or nor-binaltorphimine nor by naloxone, an indication that these compounds are not acting through an opioid receptor.

Preliminary experiments were also conducted in the rat vas deferens, a tissue with poor sensitivity to opioid compounds (Schulz et al., 1979). Investigations using the rat vas deferens followed essentially the same procedure described for the mouse vas deferens; the only exception was the initial tension used, 1.0 g. In this tissue, nociceptin also acted as a partial agonist, but the sensitivity (ED_{50} of approximately 100 nM) was less than that found in the mouse vas deferens.

The ability to measure ORL_1 activity in the mouse vas deferens should facilitate the characterization and determination of the function of this receptor and the development of biologically active agonists and antagonists.

Acknowledgements

This work was supported by Grant DA06682 to L.T. from the National Institute on Drug Abuse.

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