ELSEVIER

European Journal of Pharmacology 311 (1996) R3–R5

ejp

Rapid communication

The mouse vas deferens: a pharmacological preparation sensitive to nociceptin

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Received 12 July 1996; accepted 16 July 1996

Abstract

The newly discovered neuropeptide, nociceptin (alias orphanin FQ), was tested for its potential direct effects, as well as for its ability to modify the electrically evoked contractions in several isolated organs suspended in vitro. The electrically stimulated mouse vas deferens is a sensitive preparation on which nociceptin exerts an inhibitory effect which is not affected by naloxone. The mouse vas deferens is therefore proposed as a bioassay for nociceptin and related compounds.

Keywords: Nociceptin receptor; Bioassay; Vas deferens, mouse

A 17-amino-acid-long peptide was identified simultaneously in the rat brain by Meunier et al. (1995) and in porcine brain by Reinscheid et al. (1995). The peptide was named nociceptin (Meunier et al., 1995) because it increases the reactivity to pain in animals and orphanin (Reinscheid et al., 1995) because it is the endogenous ligand of the orphan receptor named ORL1 by Mollereau et al. (1994) or LC132 by Bunzow et al. (1994). The sequence of this new receptor is similar to that of opioid receptors. The new peptide has been found to inhibit forskolin-stimulated cAMP accumulation in Chinese hamster ovary cells transfected with ORL1/LC132 in vitro and to interfere with brain functions in the mouse in vivo, by influencing nociception and locomotor activity (Meunier et al., 1995; Reinscheid et al., 1995).

The present study was undertaken to identify a pharmacological preparation with a functional ORL1/LC132 site in order to find (a) if the receptor is expressed in peripheral tissues; (b) which kind of biological effect the receptor may mediate; and (c) how specific and selective nociceptin action is with respect to the receptors of other agents, especially opioids. Nociceptin and some of its fragments (Table 1) were prepared by solid phase synthesis and purified by high pressure liquid chromatography. The potential biological activity of nociceptin was tested in different pharmacological preparations. The peptide was found to be inactive as smooth muscle stimulant in the human umbilical and renal veins, in the rabbit vas deferens and carotid artery, in the mouse vas deferens, in the guinea pig ileum, and in the pig pulmonary and coronary arteries; it is inactive as inhibitor in the rabbit carotid and the pig coronary and pulmonary arteries precontracted with KCl 30 mM. Nociceptin however exerts inhibitory effects in electrically stimulated preparations such as the guinea pig ileum and the mouse vas deferens, which are classical preparations for studying respectively the pharmacology of μ-opioid (Paton, 1957) and δ-opioid (Hughes et al., 1975) receptors. Nociceptin induces a concentration-dependent inhibition of the electrically stimulated mouse vas deferens which reaches $-76 \pm 4\%$ as a maximal effect. The affinity of nociceptin is in the range of 10 nM (pIC_{50} 7.88) (Table 1). Nociceptin also inhibits the electrically stimulated guinea pig ileum. In this tissue, nociceptin induces a concentration-dependent inhibition (pIC_{50} 8.12) which, however, reaches only $-48 \pm 6\%$. The classical κ-opioid receptor preparation, the rabbit vas deferens (Oka et al., 1980), was also carefully investigated and nociceptin was

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PII S0014-2999(96)00563-8

Table 1
Effects of nociceptin and its fragments on various electrically stimulated pharmacological preparations

	Mouse vas deferens		Guinea pig ileum		Rabbit vas deferens	
	pIC_{50} ($CL_{95\%}$)	E_{max}	pIC_{50} ($CL_{95\%}$)	E_{max}	pIC_{50} ($CL_{95\%}$)	E_{max}
Nociceptin	7.88 (7.76–8.00)	$-76 \pm 4\%$	8.12 (8.02–8.22)	$-48 \pm 6\%$	Inactive	
Nociceptin-(1–13) amide	7.56 (7.36–7.76)	$-84 \pm 5\%$	7.94 (7.77–8.11)	$-36 \pm 5\%$	Inactive	
Nociceptin-(1–9)	Inactive		Inactive		Inactive	

pIC_{50}: the negative logarithm to base 10 of the molar concentration of an agonist that produces 50% of the maximal inhibitory effect.
E_{max}: the maximal effect induced by an agonist expressed as percent inhibition of electrically induced twitches.
$CL_{(95\%)}$: 95% confidence limits.
Nociceptin (H-Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH).

found to be inactive in this tissue. Nociceptin does not affect the inhibitory effects mediated by μ-opioid receptors in the guinea pig ileum or by δ-opioid receptors in the mouse vas deferens, suggesting that nociceptin does not interact with opioid receptors and is not a partial agonist.

Moreover, fragments of nociceptin were designed to eliminate C-terminal sequences and obtain derivatives ending with the cationic couple, Arg-Lys, (nociceptin-(1–13) amide and nociceptin-(1–9)). Nociceptin-(1–13) amide was found to be as effective (in terms of maximal effect) and almost as potent (in terms of affinity) as the natural peptide, while nociceptin-(1–9) was inactive (Table 1).

The nociceptin receptor of the mouse vas deferens and guinea pig ileum was further characterized with antagonists. The broad-spectrum opioid receptor antagonist, naloxone, was applied 15 min before testing nociceptin as well as a submaximal concentration of deltorphin I (1 nM) in the mouse vas deferens and of dermorphin (1 nM) in the guinea pig ileum. Naloxone (1 μM) was able to block the effects of deltorphin I (in the mouse vas deferens) and dermorphin (in the guinea pig ileum) without modifying the inhibitory effects of nociceptin. A compound of a new series (Salvadori et al., 1995), N(Me_2)Dmt-Tic-OH, which has been found to act as a selective δ-opioid receptor antagonist (Salvadori and Bianchi, personal communication), was found to block the effect of deltorphin I in the mouse vas deferens (pA_2 9.5) without affecting that of nociceptin. Furthermore, nociceptin up to 1 μM did not have any effect on the rabbit vas deferens also when applied in the presence of the selective κ-opioid receptor antagonist, nor-binaltorphimine (10 nM). This compound was, however, found to reduce (pA_2 10.3) the inhibitory effect of the selective κ-opioid receptor agonist U69593 (5α,7α,β-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]benzene acetamide) in this preparation.

The results described above and summarized in Table 1 indicate that the nociceptin receptor, ORL1/LC132, which has been found in the central nervous system of several species is also expressed in both adrenergic (mouse vas deferens) and cholinergic (guinea pig ileum) peripheral motor fibers. The nociceptin receptor exerts a biological effect that is similar to those of the opioid receptors, namely it inhibits the electrically induced contraction of two isolated organs, the mouse vas deferens and guinea pig ileum. Because of the finding of Meunier et al. (1995) and Reinscheid et al. (1995) that ORL1/LC132 mediates inhibition of forskolin-stimulated cAMP accumulation as do opioid receptors, this mechanism is proposed to explain the inhibition by nociceptin of the electrically induced contractions of both preparations. We suggest that the ORL1/LC132 receptor which is present in the mouse vas deferens and guinea pig ileum is selectively activated by nociceptin. This interpretation is supported by the results obtained with the antagonists which leave no doubt as to the existence of a specific nociceptin receptor, sensitive to nociceptin and nociceptin-(1–13) amide and not blocked by opioid receptor antagonists which are effective against deltorphin I (on the δ-opioid receptor) or dermorphin (on the μ-opioid receptor).

In conclusion, the present results demonstrate the presence in the mouse vas deferens and the guinea pig ileum of a functional site (receptor) which is sensitive to nociceptin and to nociceptin-(1–13) amide and is not antagonized by compounds that interact with the opioid receptors. The nociceptin receptor, ORL1/LC132, mediates inhibition of the electrically induced contractions in the two tissues. The receptor appears to be more abundant and/or efficient in the mouse vas deferens, which is therefore proposed as a sensitive and useful preparation for future studies on nociceptin.

Acknowledgements

This work was supported by a grant from the Medical Research Council of Canada and a 60% Italian Ministry of University grant. We thank Mirian Bodin and Giuliano Marzola for technical assistance.

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