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Rapid communication

[Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ is an agonist of the nociceptin (ORL1) receptor

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Abstract

[Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$, a pseudopeptide analog of nociceptin, has been shown to be a selective 'antagonist' of the nociceptin receptor in the isolated guinea pig ileum and mouse vas deferens preparations (Guerrini et al., 1998. Br. J. Pharmacol. 123, 163–165). However, in recombinant chinese hamster ovary cells expressing the human nociceptin receptor, we find that the pseudopeptide is a potent (IC_{50} = 7.5 nM) and fully efficacious inhibitor of forskolin-induced accumulation of cAMP, thus behaving as a pure 'agonist' rather than an antagonist of the receptor. The contrary behaviour of the pseudopeptide in smooth muscle and transformed cells may suggest that different nociceptin receptor types are being addressed in the two systems.

Keywords: Nociceptin pseudopeptide analog; Nociceptin receptor, human brain; cAMP synthesis inhibition

Nociceptin (Meunier et al., 1995), also known as orphanin FQ (Reinscheid et al., 1995), and the ORL1 receptor (Mollereau et al., 1994) are the fundamental elements of a novel peptidergic communication pathway in the nervous system. Nociceptin has been shown to produce numerous effects in vivo (reviewed in Meunier, 1997), including spinal analgesia, motor impairment, suppression of spatial learning, and stimulation of food intake. Yet, the peptide is motivationally neutral, and most remarkably, is endowed with supraspinal pronociceptive/anti-opioid properties. Nociceptin is also active at the periphery as a smooth muscle relaxant, and potent anti-natriuretic and diuretic. The broad pharmacological spectrum of nociceptin underlines the therapeutic importance of this system. However, although the in vivo nociceptin effects are generally not prevented by opioid receptor antagonists, it has not yet been proven (a specific ORL1 receptor antagonist was not available at the time) that they are mediated by the ORL1 receptor. Very recently, the pseudo-peptide [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$, a derivative of nociceptin, was shown to prevent inhibition by nociceptin of the electrically-induced contractions of the guinea pig ileum and mouse vas deferens (pA_2 values 7.02 and 6.75, respectively) (Guerrini et al., 1998), and was therefore presented as new selective antagonist of the nociceptin receptor. This important finding prompted us to evaluate further the utility of this novel compound as a molecular probe and an antagonist of the ORL1 receptor, in transformed chinese hamster ovary (CHO) cells expressing the receptor.

[^{3}H]nociceptin (0.85 TBq/mmol) was supplied by Amersham, UK. [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ (ref. SC1266, lot no. KF14-150) was purchased from Neosystem (Strasbourg, France). The manufacturer's experimental data indicated that the pseudopeptide was 99% pure and had the expected molecular mass. Binding and competition studies were carried out as described previously (Butour et al., 1997) using a crude membrane fraction from transformed CHO cells stably expressing the ORL1 receptor, and a rapid filtration procedure on polyethyleneimine-treated glass fiber disks to isolate and quantify bound radioligand. Forskolin-induced accumulation of intracellular cyclic AMP was monitored as described in Butour et al. (1997) using intact recombinant CHO cells stably expressing the ORL1 receptor, and selective batch elution of tritium-labeled cAMP on acidic alumina (Alvarez and Daniels, 1992). Fitting of the data to a

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single sigmoid curve was done using Prism (GraphPad Software, San Diego, USA).

Fig. 1A shows that [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ is a potent inhibitor of equilibrium binding of [^{3}H]nociceptin (1 nM) in the crude membrane fraction from transformed CHO cells expressing the human ORL1 receptor. The IC$_{50}$ values were 1.9 and 1.7 nM in two separate experiments. The average calculated K_i value was 0.2 nM, close to that for nociceptin (0.13 nM, Butour et al., 1997). Surprisingly, the pseudo-peptide was found, in intact cells, to potently inhibit forskolin-induced accumulation of cAMP (Fig. 1B), with IC$_{50}$ values of 7.4 and 7.7 nM (two separate experiments), only slightly lower than that for nociceptin (approx. 1 nM, Meunier et al., 1995) in the same assay. The pseudopeptide was fully efficacious, producing the maximum attainable inhibition (approx. 85% of control) usually recorded in the transformed cell line with nociceptin. Also shown as a control in Fig. 1B, the pseudopeptide was totally inactive (at concentrations up to 1 μM) in wild-type CHO cells which do not express the ORL1 receptor.

Thus, [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ binds tightly the ORL1 receptor, and therefore appears to be suited for development as a receptor probe. However, the pseudopeptide, rather than behaving as a nociceptin receptor antagonist, as has been convincingly shown in guinea pig and mouse isolated smooth muscle preparations (Guerrini et al., 1998), is a potent and pure agonist of the ORL1 receptor in transformed CHO cells expressing the human receptor. In this respect, it is of importance to establish whether the pseudopeptide acts as agonist or antagonist in behavioral paradigms in vivo, and in other in vitro assays, such as the nociceptin-induced activation of a K^+ current in the transfected *Xenopus* oocyte (see, for example, Kobayashi et al., 1997). The reasons for the contrary behaviour of the pseudopeptide in smooth muscle and transformed CHO cells are presently unknown. They may be trivial, yet it should be considered that different nociceptin receptor types may have been addressed in the two systems.

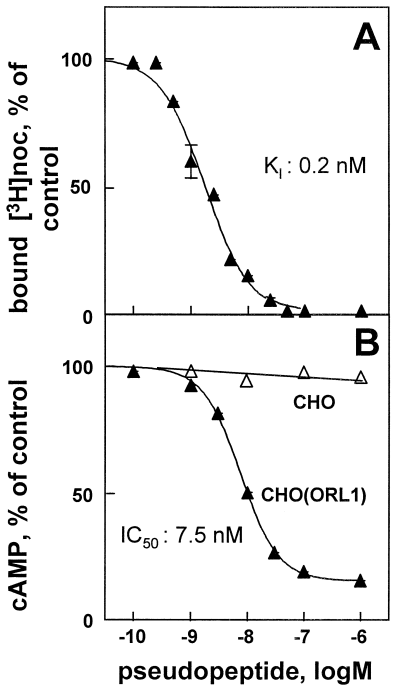

Fig. 1. Inhibition of [^{3}H]nociceptin ([^{3}H]noc) binding (A), and of forskolin-induced accumulation of cAMP (B) by [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ in transformed CHO cells expressing the human ORL1 receptor (solid triangles), and wild-type CHO cells (open triangles). A crude cell membrane preparation was used in (A), and intact cells in (B). Percent of control is calculated as the ratio of specifically bound radioligand (A) and cellular cAMP content (B) in the presence and absence of pseudopeptide. Each value is the mean ± S.E.M of six values obtained in two separate experiments.

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