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SPECIAL REPORT

A new selective antagonist of the nociceptin receptor

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[Phe[1]Ψ(CH_2-NH)Gly[2]]NC(1-13)NH_2 has been tested in the electrically stimulated guinea pig ileum and mouse vas deferens, two nociceptin sensitive preparations. The new compound showed *per se* little or no effect in the two tissues, but it displaced to the right the concentration-response curves of nociceptin in a concentration-dependent manner. Schild analyses of the data indicated a competitive type of antagonism and pA_2 values of 7.02 and 6.75 in the guinea-pig ileum and the mouse vas deferens, respectively. At 10 μM [Phe[1]Ψ(CH_2-NH)Gly[2]]NC(1-13)NH_2 does not modify either the inhibitory effect of deltorphin I (the selective δ opioid receptor agonist) in the mouse vas deferens or that of dermorphine (the selective μ opioid receptor agonist) in the guinea-pig ileum. The present findings indicate that [Phe[1]Ψ(CH_2-NH)Gly[2]]NC(1-13)NH_2 is a selective antagonist of the nociceptin receptor.

Keywords: Guinea pig ileum; mouse vas deferens; nociceptin receptor; [Phe[1]Ψ(CH_2-NH)Gly[2]]NC(1-13)NH_2; antagonist

Introduction The heptadecapeptide nociceptin (NC) has recently been shown (Meunier *et al*., 1995; Reinscheid *et al*., 1995) to be the endogenous ligand of the opioid-like orphan receptor (ORL-1). Despite the structural homology of NC and its receptor with the peptides and receptors of the opioid family this peptide/receptor system appears to be pharmacologically distinct from the opioids. During the last two years several papers have described new biological effects mediated by NC both in the periphery and in the central nervous system. Such effects were not modified by naloxone or other more selective opioid receptor antagonists and were considered to be mediated by the activation of a specific NC receptor (the ORL-1). Lack of selective NC receptor antagonists prevented a definitive pharmacological characterization of ORL-1. In 1997, Kobayashi *et al*. showed that carbetapentane and rimcazole act as antagonists at the NC receptor. However these compounds showed little affinity (IC_{50} about 10 μM) and, more importantly, they were found to interact also with other functional sites such as σ-, μ-, and κ-opioid receptors and M_1-muscarinic receptors. Being non selective, they were therefore considered of little utility for receptor characterization.

In the present study, we describe the chemical structure and the *in vitro* pharmacological effects of [Phe[1]Ψ(CH_2-NH)Gly[2]]NC(1-13)NH_2, a selective NC receptor antagonist.

Methods Male guinea-pigs weighing 280–300 g and male Swiss mice of 25–30 g were used. On the day of the experiment they were killed by decapitation. The guinea pig ileum (gpI) and the mouse vas deferens (mVD) were prepared according to Paton (1957) and Hughes *et al*. (1975), respectively, and suspended in 10 ml organ baths. A resting tension of 0.3 g, was applied to the mVD and of 1 g to the gpI. The preparations were stimulated through two platinum ring electrodes with supramaximal rectangular pulses of 1 ms duration and 0.1 Hz frequency. The electrically evoked contractions were measured isotonically with a strain gauge transducer and recorded on a Linseis multichannel chart recorder (model 2005). After an equilibration period of about 1 h the contractions induced by electrical field stimulation were stable. At this time, cumulative concentration-response curves (CRC) to NC or to opioid selective agonists were performed (0.5 log unit steps) in the absence or in presence of different concentrations of [Phe[1]Ψ(CH_2-NH)Gly[2]]NC(1-13)NH_2. This peptide was prepared by solid-phase condensation of Boc-Phe(CH_2-NH)Gly-Gly-Phe-OH on the amino terminus of NC(5-13)-Rink resin. The modified tetrapeptide was prepared in solution by standard methods (Salvadori *et al*., 1992). All the other peptides used in this study were prepared by solid phase synthesis and purified by high pressure liquid chromatography, as previously described (Guerrini *et al*., 1997). Data are expressed as mean ± s.e.mean of *n* experiments.

Results NC inhibited the electrically induced contractions of the gpI (Figure 1a) and the mVD (Figure 1b) with similar potency (pEC_{50} 7.9 ± 0.2 and 7.6 ± 0.2, respectively) but different maximal effects (about −60% and −80% of control values, respectively). Application of [Phe[1]Ψ(CH_2-NH)Gly[2]]NC(1-13)NH_2 was followed by transient inhibition of the response of the gpI which reached −20% of control values. As shown in Figure 1a, in the gpI [Phe[1]Ψ(CH_2-NH)Gly[2]]NC(1-13)NH_2 (0.1–10 μM) displaces to the right the CRC to NC in a concentration-dependent manner, the curves being parallel to the control and reaching the maximal effects. Figure 1c shows the corresponding Schild plot which is linear ($r = 0.99$) with a slope of 1.02 not significantly different from unity. The extrapolated pA_2 value is 7.0. In the same preparation 10 μM of [Phe[1]Ψ(CH_2-NH)Gly[2]]NC(1-13)NH_2 does not modify the inhibitory effect of the selective μ opioid receptor agonist dermorphin (control: pEC_{50} 8.7 ± 0.2, E_{max} −100%; in the presence of 10 μM [Phe[1]Ψ(CH_2-NH)Gly[2]]NC(1-13)NH_2: pEC_{50} 8.8 ± 0.1, E_{max} −100%). Similar results were obtained in the mVD. In this preparation [Phe[1]Ψ(CH_2-NH)Gly[2]]NC(1-13)NH_2 *per se* did not modify electrically induced twitches but it displaced to the right the CRC to NC in a concentration dependent manner without any modification of the maximal effects (Figure 1b). Schild analysis of the data (Figure 1d) yielded a slope of 1.09 not significantly

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different from unity, a correlation coefficient of 0.99 and an extrapolated pA_2 value of 6.75. In the same preparation, 10 μM [Phe$^1\Psi$(CH$_2$-NH)Gly^2]NC(1-13)NH$_2$ did not modify the inhibitory effect of the selective δ opioid receptor agonist deltorphin I (control: pEC_{50} 9.3$\pm$0.2, E_{max} $-$100%; in the presence of 10 μM [Phe$^1\Psi$(CH$_2$-NH)Gly^2]NC(1-13)NH$_2$: pEC_{50} 9.4$\pm$0.2, E_{max} $-$100%).

Discussion

[Phe$^1\Psi$(CH$_2$-NH)Gly^2]NC(1-13)NH$_2$ was discovered in the frame of structure-activity study (Guerrini *et al.*, 1997) on the NC fragment NC(1-13)NH$_2$, a potent NC receptor agonist (Calo *et al.*, 1996; 1997). Since it has recently been reported that NC is primarily inactivated by cleavage of the peptide

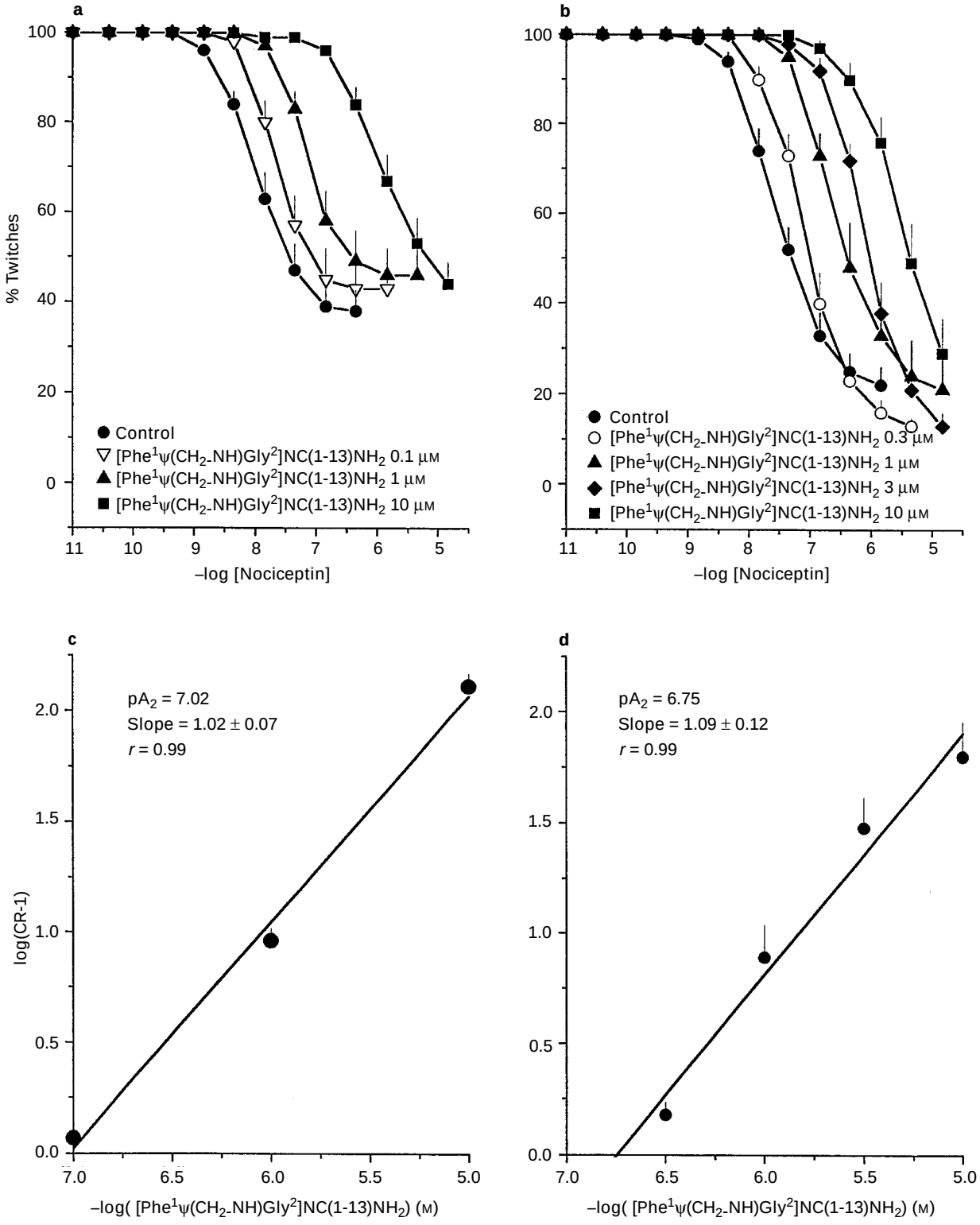

Figure 1 Concentration-response curves for nociceptin (NC) obtained in the absence and in presence of increasing concentrations of [Phe$^1\Psi$(CH$_2$-NH)Gly^2]NC(1-13)NH$_2$ in the electrically stimulated guinea-pig ileum (a) and mouse vas deferens (b). (c) and (d) show the corresponding Schild plots. Points indicate the means and vertical lines the s.e.mean of 5 to 8 experiments.

linkage Phe[1]-Gly[2] (Montiel *et al*., 1997), [Phe[1]Ψ(CH_2-NH)Gly[2]]NC(1-13)NH_2 was designed to protect the NC fragment NC(1-13)NH_2 from degradation by aminopeptidases. Instead, a fairly potent antagonist was obtained which inhibits the effect of NC in two preparations where the NC receptor has been demonstrated and characterized by the use of a variety of agonists (NC, $NCNH_2$, NC(1-13)NH_2) and a large number of analogues of NC(1-13)NH_2 (Guerrini *et al*., 1997).

To our knowledge, [Phe[1]Ψ(CH_2-NH)Gly[2]]NC(1-13)NH_2 is the first specific antagonist of the NC receptor and appears to be suitable as a tool for receptor characterization. In fact [Phe[1]Ψ(CH_2-NH)Gly[2]]NC(1-13)NH_2 acts as a competitive antagonist since it reduces the affinity of NC in the two preparations without depressing the maximal effect and the Schild plots had slopes near unity. [Phe[1]Ψ(CH_2-NH)Gly[2]]NC(1-13)NH_2 is specific for the NC receptor since it does not affect either the μ (dermorphine) or the δ (deltorphin I) opioid receptor selective agonists. Data obtained with [Phe[1]Ψ(CH_2-NH)Gly[2]]NC(1-13)NH_2 support the hypothesis, based on data obtained with agonists (Calo *et al*., 1996; 1997) that the NC receptor is a functional entity different from the opioid receptors.

In conclusion, we have presented an *in vitro* pharmacological evaluation of [Phe[1]Ψ(CH_2-NH)Gly[2]]NC(1-13)NH_2 the first selective NC receptor antagonist. This molecule could therefore be useful in the near future for studying the physiological actions of NC, for clarifying the pathophysiological role of the NC/NC receptor system and for evaluating the therapeutic potential of new drugs designed to block the NC receptor. [Phe[1]Ψ(CH_2-NH)Gly[2]]NC(1-13)NH_2 is expected to be resistant to degradation by aminopeptidases and to show high potency and long duration of action in *in vivo* assays.

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