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Pharmacological properties of peptides derived from an antibody against the tachykinin NK_1 receptor for the neuropeptide substance P

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Abstract

Two peptides were derived from the structural analysis of a previously described monoclonal antibody [Mol. Immunol. 37 (2000) 423] against the tachykinin NK_1 receptor for the neuropeptide substance P. Here we show that these two peptides were able to inhibit the inositol phosphate transduction pathway triggered both by substance P and neurokinin A, another high-affinity endogenous ligand for the tachykinin NK_1 receptor. They also reduced the cAMP production induced by substance P. By contrast, only one antagonist peptide was able to prevent substance P and neurokinin A from binding the receptor, as revealed both by biochemical and autoradiographic studies. First, these results illustrate the generality of the antibody-based strategy for developing new bioactive peptides. Second, they indicate that antagonists, even exhibiting very close amino acid composition, can interact with the tachykinin NK_1 receptor at different contact sites, some of them clearly distinct from the contact domains for endogenous agonists.

Keywords: Tachykinin NK_1 receptor; Substance P; Neurokinin A; Peptide receptor antagonist; Hydropathy; Antibody

1. Introduction

Substance P is a neuropeptide, belonging to the tachykinin family, which is involved in various physiological functions (e.g. pain transmission, endocrine and exocrine secretions, immunomodulation and inflammation) (Otsuka and Yoshioka, 1993; Harrisson and Geppetti, 2001) as well as pathological processes like schizophrenia, migraine, epilepsy and depression (Raffa, 1998). Substance P exerts its biological effects through interaction with a G protein-coupled receptor named tachykinin NK_1 receptor. Recent studies have proposed that the tachykinin NK_1 receptor exists in two distinct, non-stochiometrically expressed conformers or isoforms, each one being constitutively associated in vivo either with a Gs or with a $G_{q/11}$ protein (Sagan et al., 1997, 1999; Holst et al., 2001). Substance P seems to be a high-affinity ligand for both conformers, triggering both the cAMP and the inositol phosphate transduction pathways, respectively (Sagan et al., 1996). By contrast, neurokinin A, another important endogenous tachykinin in mammals which possesses a specific tachykinin NK_2 receptor, appears to be a high-affinity ligand (Hastrup and Schwartz, 1996; Bremer et al., 2001) exclusively for the tachykinin NK_1 receptor conformer associated with the $G_{q/11}$ protein. Other peptides, like some C-terminal analogs of substance P, e.g. septide, share with neurokinin A similar pharmacological properties (Sagan et al., 1996). A question which remains largely unanswered and controversial is whether substance P and neurokinin A recognize the same binding site presented with crucial structural differences by the two conformers or, alternatively, recognize two distinct binding sites, present on each conformer, as also suggested (Maggi and Schwartz, 1997; Sagan et al., 1999; Wijkhuisen et al., 1999; Holst et al., 2001).

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What is clear however is that, due to the importance and variety of the physiological roles played by endogenous tachykinins, a large panel of peptide as well as non-peptide tachykinin NK_1 receptor selective antagonists has been developed with view to producing therapeutic agents (Shelton, 2001). These compounds have been obtained through rational optimization of natural ligands or, more recently, through screening of combinatorial peptide libraries (Boyle et al., 1994). Using a very different strategy, we also reported recently the possibility of raising a novel antagonist of the tachykinin NK_1 receptor (Sagot et al., 2000). This strategy consists in taking advantage of the diversity of the immune repertoire, in other words, to develop biologically active peptides based on the structure of monoclonal antibodies directed against the receptor for the hormone (Williams et al., 1991). Starting from the sequence of a monoclonal antibody against the tachykinin NK_1 receptor, which competed with substance P for receptor binding, we synthesized a peptide corresponding to a hypervariable region (or complementarity determining region: CDR) of the light chain and showed that this CDR peptide antagonized in vitro one pharmacological action of substance P, namely the phospholipase C-associated transduction pathway (Sagot et al., 2000). The originality of this finding resulted from the observation that this CDR peptide, contrary to other pharmacologically active compounds obtained following this antibody-based strategy (Pride et al., 1992; Taub et al., 1992; Levi et al., 1993), displayed no sequence homology with the natural ligand for the receptor, i.e. substance P, only a similar hydropathic profile. Using this concept of hydropathy, Villain et al. (2000) have recently described the de novo design of bioactive peptides, reporting in addition the interesting finding that a small difference in the length of these novel compounds resulted in a complete change in their pharmacological (agonist versus antagonist) properties.

In this context, the aim of the present study was twofold: first, we wanted to investigate further the pharmacological properties of the CDR peptide, which we previously obtained through the antibody-based methodology, especially its possible antagonist potency against neurokinin A as well as against substance P. Second, we looked for the possibility of extending this approach by developing a novel bioactive peptide through a modification of the amino acid sequence length. For this purpose, we synthesized a peptide (denoted S-CDR), shorter (11 versus 17 residues) than the long-CDR peptide (denoted L-CDR) which we prepared previously, but long enough to conserve the hydropathic homology with substance P. We found that both peptides behaved as antagonists against substance P and neurokinin A, extending to the tachykininergic system the general interest of the antibody-based approach for a pharmacological purpose. Furthermore, since both peptides exerted their antagonism through clearly different mechanisms, the present results strongly suggest that antagonists with very similar amino acid compositions interact with the tachykinin NK_1 receptor at distinct contact sites that do not overlap with the contact regions for endogenous tachykinins.

2. Materials and methods

2.1. Peptides, reagents and cell cultures

Substance P (RPKPQQFFGLM) and neurokinin A were from Neosystem Laboratoire (Strasbourg, France). L-CDR peptide (17 amino acids: PRLLIYLVSNLESGVPA) and S-CDR peptide (11 amino acids: LIYLVSNLESG), presenting a degree of purity >80%, were synthesized by Chiron (France). S-CDR peptide corresponds to the (4−14) region of L-CDR, i.e. the 11-amino-acid sequence hydropathically similar to substance P (Sagot et al., 2000). Three unrelated 11- to 16-amino-acid peptides, synthesized in the laboratory (purity>90%), were systematically used as controls: a peptide derived from the prion protein (Pri7: TIKNHTVVTTTKG), a peptide derived from cyclooxygenase (CX: VGFNIVKTATLKKLVC) and a peptide derived from the human thromboxane A2 receptor (AHL: LQPRLSTRPRRV). In addition, we synthesized a scramble peptide (Scr) composed of the same 17 residues as L-CDR but arranged in a different order (IGVRPEVSLSNLLYPLA).

Labeled products ([^{125}I]substance P: 74 TBq/mmol, ([^{125}I]neurokinin A: 74 TBq/mmol and myo-2-[^{3}H]inositol: 629 GBq/mmol) were from Amersham France. Chinese hamster ovary cells (CHO) stably transfected and expressing the human brain recombinant tachykinin NK_1 receptor for substance P, as well as untransfected control CHO cells, were a generous gift from Dr. L. Pradier (Rhône-Poulenc Rorer, Ivry). Cell culture medium Ham-F-12, fetal calf serum, HEPES, penicillin and streptomycin were from GIBCO (Eregny, France). Unless otherwise stated, other reagents (including epinephrine, used for control experiments) were from Sigma.

Cells were cultured in HAM-F12 medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO_2.

2.2. Measurement of phosphatidyl inositol hydrolysis

Experiments were carried out as already described (Wijkhuisen et al., 1999). Briefly, after labeling with [^{3}H]inositol for 24 h, cells (2×10^5 cells/well) were preincubated for 3 h at 37 °C in the presence of L-CDR, S-CDR, the scramble peptide or three control (Pri7, AHL and CX) unrelated peptides at different concentrations. Substance P or neurokinin A was then added to a final concentration of 10^{-10} M and the incubation was allowed to proceed for 30 min. After cell lysis with 1% Triton X100, [^{3}H]inositol phosphates were separated by chromatography of the aqueous phase recovered after addition of a chloroform/methanol (1:2) mixture. The basal level of inositol phosphate production

was estimated in the absence of substance P. This value was subtracted from all other measurements.

2.3. Measurement of cAMP formation

cAMP formation was measured by enzyme immunoassay using cAMP-acetylcholinesterase as the tracer and a second antibody solid-phase as the separation method (Pradelles et al., 1989). Cells (2×10^5 cells/well) were preincubated for 1 h at room temperature in the presence of L-CDR, S-CDR, the scramble peptide or three control (Pri 7, AHL and CX) unrelated peptides at different concentrations. Substance P was then added to a final concentration of 10^{-10} M and the incubation was allowed to proceed for 30 min. Afterwards, cAMP was extracted by adding ethanol to a final concentration of 70%. After centrifugation, the supernatant was lyophilized and resuspended in the assay buffer (50 mM phosphate, pH 6.2).

Competitive enzyme immunoassay was performed as described elsewhere (Déry et al., 1997). The 96-well microtiter plates were coated with mouse monoclonal antibodies specific for rabbit immunoglobulins. The assay was performed in a total volume of 150 μl. In routine assays, reagents were dispensed as follows: 50 μl of anti-cAMP diluted antiserum, 50 μl of cAMP-acetylcholinesterase conjugate and 50 μl of derivatized standard or biological sample. The derivatization of standard or samples was performed as follows: 50 μl of 4 N KOH, 12.5 μl of acetic anhydride and, after 15-s shaking, 12.5 μl of 4 N KOH were successively added to 250 μl of standard or sample in 50 mM phosphate buffer pH 6.2. In each case, phosphate buffer was treated in the same way and introduced in the assay for the nonspecific and maximal binding (Bo) measurements. After overnight reaction at room temperature, plates were washed before the addition of Ellman's reagent. Absorbance was measured at 414 nm after 0.5–3 h of enzymatic reaction. Nonspecific binding was below 0.1% of the total acetylcholinesterase activity introduced in the assay.

2.4. Binding assays

As described previously (Sagot et al., 2000), CHO cells (2×10^5 cells/well) were washed three times with Krebs buffer (120 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 70 mM NaH_2PO_4, 1.6 mM $CaCl_2$, 0.04 mM bovine serum albumin, 30 mg/ml bacitracin and 6 g/l glucose) before incubation for 90 min at room temperature in the presence of L-CDR, S-CDR, the scramble peptide or three control (Pri7, AHL and CX) unrelated peptides (200 μl) at different concentrations. Since L-CDR and S-CDR were poorly soluble in buffer, they were primarily dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1.41×10^{-2} M, and serial dilutions were made in binding buffer. The scramble peptide and control-unrelated peptides were treated in the same way. Then, [^{125}I]substance P or [^{125}I]neurokinin A (100 μl, 10,000 cpm) was added to a final concentration of 10^{-11} M and the reaction was stopped after 30 min. Cells were washed three times in cold Krebs buffer, lysed with 0.1% Triton X100 and the radioactivity was counted. Nonspecific binding was determined in the presence of 1 μM substance P.

2.5. Autoradiographic studies

Three rats were deeply anesthetized with 60 mg/kg i.p. of pentobarbital (Sanofi, Libourne, France) and perfused intracardially with 500 ml of 0.9% NaCl containing 0.1% sodium nitrite as vasodilator. Brains were rapidly dissected out and frozen in isopentane cooled to 30 °C. Coronal sections (20-μm thick) were cut using a cryostat and then mounted on gelatin-coated slides. Slides, kept at −80 °C until used, were preincubated at room temperature in 50 mM Tris (pH 7.4) and 0.2 g/l bovine serum albumin for 30 min. Then, they were incubated for 2 h in the presence of [^{125}I]neurokinin A at 10^{-10} M in the same buffer solution containing $MnCl_2$ (3 mM) and a cocktail of peptidase inhibitors (20 μg/ml leupeptin, 0.3 μg/ml benzamidine, 0.05 μg/ml bacitracin, 20 μg/ml chymostatin and 0.03 mg/ml phenylmethylsulfonylfluoride). Increasing concentrations of L-CDR, S-CDR, three control-unrelated peptides or cold neurokinin A (10^{-9} to 10^{-6} M) were added to the incubation medium. After four 30-s washes in 50 mM Tris (pH 7.4) and 0.2 g/l bovine serum albumin, slides were dried with cold air and exposed to [^{3}H]Ultrofilm (Amersham, France) for 3–10 days. Autoradiograms were developed in Kodak Microdol for 10 min at 20 °C. They were then digitized using a camera and an IMSTAR image analysis system using the Starwise software.

3. Results

3.1. Effects of CDR peptides on substance P-induced inositol phosphate and cAMP production

The potential biological effects of CDR peptides were assessed in CHO cells expressing the recombinant human tachykinin NK_1 receptor. Since substance P is known to trigger in these cells both the phospholipase C and the adenylyl cyclase transduction pathways, inositol phosphate and cAMP productions were measured. No agonist effect could be detected up to a concentration of 500 μM (not shown) for either complementarity determining region-peptide (nor for control peptides). By contrast, as shown in Fig. 1A and B, both CDR peptides exhibited significant antagonist properties: they dose-dependently inhibited the ability of substance P to increase the intracellular levels of both inositol phosphate and cAMP, with a comparable efficacy (about 30% and 40% inhibition for inositol phosphate and cAMP accumulations, respectively, at a 300-μM concentration). Under identical conditions, no significant effect was observed for four control peptides, including a scramble

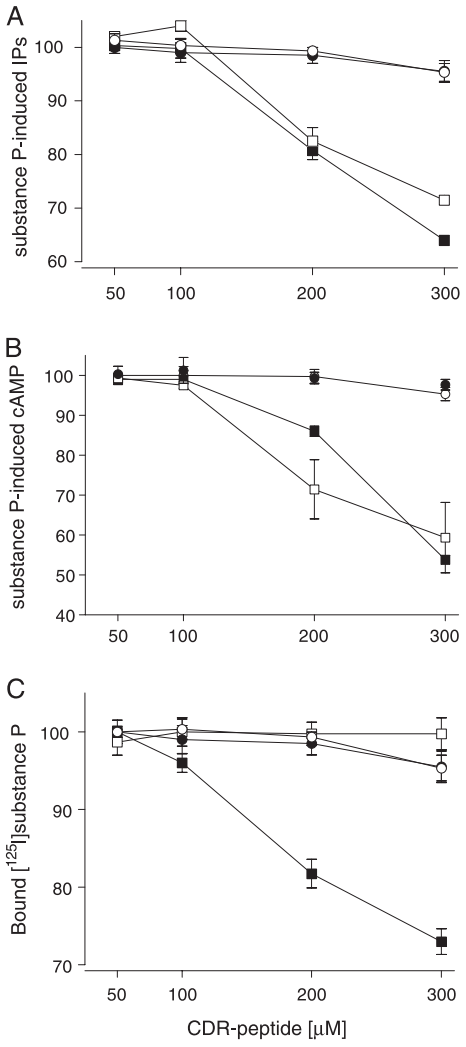

Fig. 1. Comparative effects of CDR-derived peptides on substance P-induced pharmacological activities [panel A: formation of inositol phosphates (IPs) and panel B: cAMP production] and on radiolabeled substance P binding (panel C) in CHO cells expressing the human NK_1 receptor. Pharmacological activities and binding values for substance P were measured in the presence of buffer alone (100%) or increasing concentrations of L-CDR (■), S-CDR (□), the scramble peptide (○) or three control unrelated peptides (●). Since values obtained with control peptides did not significantly differ, they were pooled. Values represent the mean ± S.D. of 6–12 determinations.

peptide. In untransfected control CHO cells, i.e. not expressing the tachykinin NK_1 receptor but constitutively expressing the β-adrenergic receptor, S-CDR and L-CDR peptides (up to a concentration of 300 μM) did not induce any increase in cAMP or inositol phosphate production and remained totally unable to antagonize the cAMP formation triggered by 10^{-10} M epinephrine (data not shown).

3.2. Effects of CDR peptides on substance P binding to the tachykinin NK_1 receptor

Since the preceding results suggested that CDR peptides could compete with substance P for binding to the tachykinin NK_1 receptor, we tested their ability to inhibit the binding of [^{125}I]substance P. Preliminary experiments (not shown) indicated that, under our experimental conditions, iodinated substance P was able to bind cells expressing recombinant receptors (but not untransfected control cells) with a high affinity ($K_D = 2$ nM). Competitive-binding experiments (Fig. 1C) revealed that L-CDR peptide actually displaced [^{125}I]substance P binding, with a potency on the same order as that observed for pharmacological activity (i.e. about 30% inhibition at 300 μM). Surprisingly, however, S-CDR peptide was totally unable to displace substance P from the tachykinin NK_1 receptor, in the same range of concentrations (Fig. 1C). Under identical conditions, no inhibition was observed using the different control peptides. The inability of S-CDR peptide to displace radiolabeled substance P in competition-binding experiments, compared to its ability to exert a clear pharmacological effect at similar concentrations, was reminiscent of the properties described for neurokinin A and other substance P analogs, e.g. septide, which led to the previous suggestion of the occurrence of a "neurokinin A-septide" site on the tachykinin NK_1 receptor (Wijkhuisen et al., 1999). For this reason, we investigated the effects of the two CDR peptides on the pharmacological and binding properties of neurokinin A.

3.3. Effects of CDR peptides on neurokinin A-induced inositol-phosphate production

Since neurokinin A is known to activate adenylylcyclase poorly upon binding the tachykinin NK_1 receptor, the effects of CDR peptides were assessed only on the inositol phosphate transduction pathway triggered by this neurokinin. As seen in Fig. 2A, both L-CDR and S-CDR peptides antagonized inositol phosphate formation induced by neurokinin A, albeit with different efficacies (S-CDR peptide being slightly but significantly more potent than L-CDR: 60% versus 40% inhibition at 300 μM, respectively). Under identical conditions, no effect was observed using the four control peptides. In untransfected control CHO cells, no biological effect could be detected either for neurokinin A or for CDR peptides (data not shown).

3.4. Effects of CDR peptides on neurokinin A binding to the tachykinin NK_1 receptor

Taken together, the preceding results seemed compatible with the hypothesis that L-CDR peptide interacts with a site (or sites) recognized by both substance P and neurokinin A, whereas S-CDR peptide binds preferentially to a "neurokinin A site", more specifically devoted to the binding of this tachykinin. If true, S-CDR peptide, like L-CDR, might efficiently compete with neurokinin A for binding the tachykinin NK_1 receptor. Competition-binding experiments were thus carried out using [^{125}I]neurokinin A and the two CDR peptides. As shown in Fig. 2B, L-CDR peptide

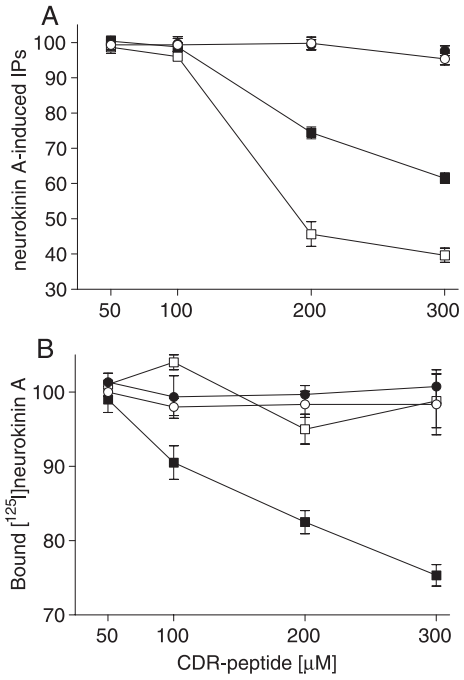

Fig. 2. Comparative effects of CDR-derived peptides on neurokinin A-induced formation of inositol phosphates (IPs) (panel A) and on radiolabeled neurokinin A binding (panel B) in CHO cells expressing the human NK_1 receptor. Inositol phosphate production and binding values for neurokinin A were measured in the presence of buffer alone (100%) or increasing concentrations of L-CDR (■), S-CDR (□), the scramble peptide (○) or three control-unrelated peptides (●). Since values obtained with control peptides did not significantly differ, they were pooled. Values represent the mean ± S.D. of 9–12 determinations.

actually dose-dependently inhibited the binding of neurokinin A to the receptor, with a potency on the same order of magnitude as that observed for the inhibition of the biologic response (Fig. 2A). In contrast, S-CDR peptide was totally unable to compete with the binding of [^{125}I]neurokinin A, at concentrations for which a clear pharmacological antagonism towards neurokinin A was measured (Fig. 2A): this result invalidates the hypothesis that S-CDR peptide directly recognized a "neurokinin A site".

However, in order to check this last result, we decided to investigate the possible effects of both CDR peptides on the binding of neurokinin A, using another technique on another material. Autoradiography experiments using [^{125}I]neurokinin A (10^{-10} M) were thus performed on rat brain coronal sections. As expected, an intense labeling (Fig. 3A) was observed in regions already known to contain high levels of tachykinin NK_1 receptors (Helke et al., 1990). This labeling was shown to be specific since it completely disappeared after a preincubation in the presence of 10^{-6} M unlabeled neurokinin A (not shown). As seen in Fig. 3B, the addition of L-CDR peptide (300 μM) to the preincubation medium largely decreased the [^{125}I]neurokinin A labeling. A quantitative analysis performed in the striatum (Table 1) indicated an estimated 75% reduction in labeling, a value higher than that measured in binding experiments performed on CHO

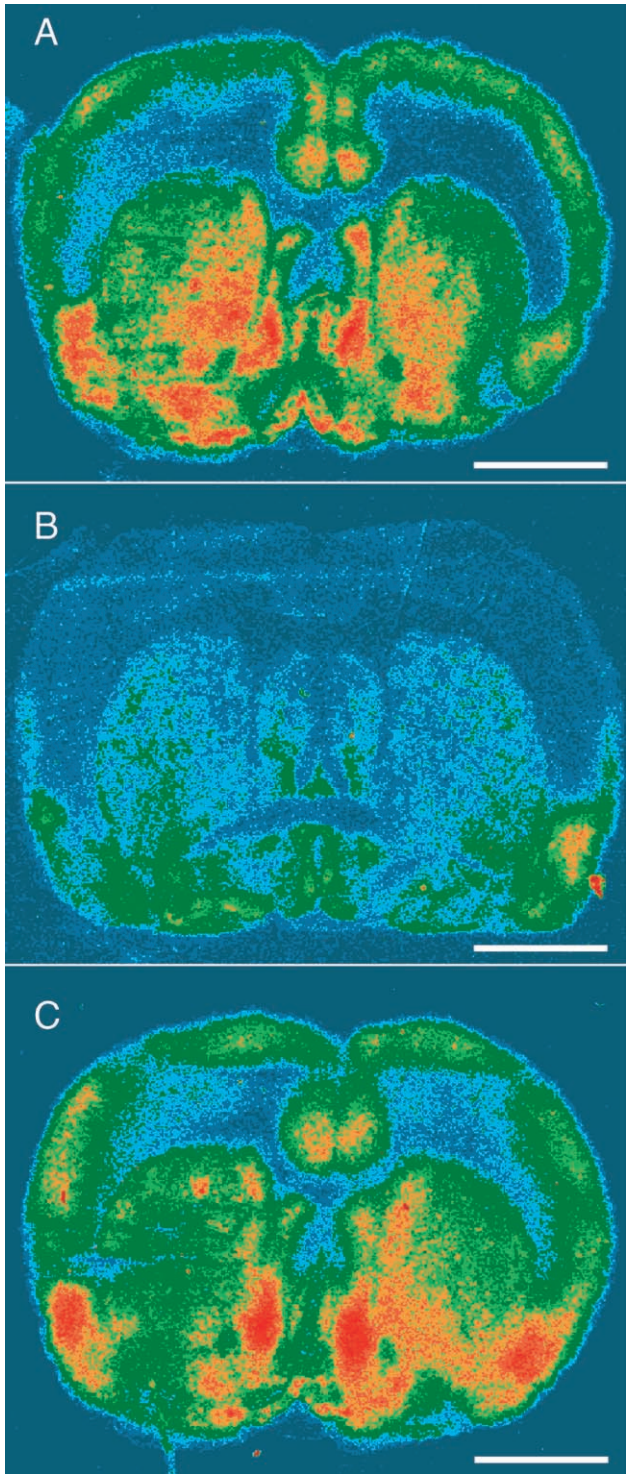

Fig. 3. Comparative effects of CDR-derived peptides on [^{125}I]neurokinin A autoradiographic labeling in the rat brain. Three consecutive coronal rat brain sections were preincubated in the presence of buffer alone (A), 300 μM L-CDR peptide (B) or 300 μM S-CDR peptide (C) before addition of 0.1 nM [^{125}I]neurokinin A. Increasing optical densities on digitized autoradiograms are indicated by a color scale from cold colors (blue and green) to warm colors (yellow and red). Scale bar: 2 mm.

Table 1
Quantitative analysis of the effect of CDR-derived peptides on [^{125}I]neurokinin A autoradiographic labeling in the rat striatum

Conditions of preincubation	Optical density of autoradiograms (incubations with [^{125}I]neurokinin A)	
	Arbitrary units	% of control
Buffer	0.231 ± 0.040	
L-CDR (300 μM)	0.059 ± 0.023	25
S-CDR (300 μM)	0.220 ± 0.075	95
Control peptides (300 μM)	0.218 ± 0.043	94

Quantifications were performed by optical density measurements of autoradiograms (like those presented in Fig. 3) obtained after preincubation in the presence of buffer alone, CDR-derived peptides, or three control-unrelated peptides, before addition of 0.1 nM [^{125}I]neurokinin A. Since values obtained with control peptides did not significantly differ, they were pooled. Values are means ± S.D. of three to nine determinations.

cells expressing the human tachykinin NK_1 receptor (using the same concentration of peptide against [^{125}I]neurokinin A at 10^{-11} M, Fig. 2B): this suggests that L-CDR peptide recognizes the murine tachykinin NK_1 receptor with a better affinity than that it exhibits for the human receptor. Importantly, as shown in Fig. 3C and quantitatively reported in Table 1, S-CDR peptide (300 μM) was totally unable to displace [^{125}I]neurokinin A under the same experimental conditions, confirming the results of binding experiments on CHO cells (Fig. 2A). No effect was observed either using control peptides (Table 1).

4. Discussion

We report here that two closely related peptides, designed following an antibody-based pharmacological approach, can both antagonize in vitro the biological activities of the neuropeptides substance P and neurokinin A, two endogenous ligands of the tachykinin NK_1 receptor. Interestingly, however, these biological effects occur through distinct mechanisms, since one peptide (L-CDR peptide) could significantly prevent the binding of the two agonists on the tachykinin NK_1 receptor, while the other one (S-CDR peptide) was totally inefficient at the same concentrations, as revealed both by binding experiments and autoradiography analysis. First of all, it must be stressed that the pharmacological effects of CDR peptides described here are observed at high concentrations, strongly suggesting that the affinities of the peptides for the tachykinin NK_1 receptor (which could be accurately determined only from homologous-binding analysis) are probably very low. This was not unexpected though and these bioactivities are similar to those of different peptides derived from the structure of pharmacologically active anti-receptor antibodies described in other systems (Monfardini et al., 1995; Monnet et al., 1999), probably due to the fact that free peptides cannot readily adopt in solution the conformation displayed in the native antibody molecule. What is important however is that the S-CDR and L-CDR peptides clearly displayed significant pharmacological activity against substance P and/or neurokinin A at concentrations (even high) which did, or did not, prevent substance P and/or neurokinin A binding to the tachykinin NK_1 receptor: the discussion is then based on the differences in the concentrations required to antagonize the biological response of agonists on the one hand and to compete with agonist binding on the other hand.

From a pharmacological point of view, the results presented here extend the feasibility of using monoclonal anti-receptor antibodies to develop novel antagonists in the tachykininergic system, in agreement with previous studies of other endogenous messengers or viruses (Taub et al., 1992; Pride et al., 1992; Levi et al., 1993). However, even though control experiments (performed using transfected as well as untransfected CHO cells) argue for a specific interaction of both S-CDR and L-CDR peptides with the NK_1 receptor under our experimental conditions, it is out of question to envisage the direct utilization of these peptides for in vitro or in vivo studies, due to the high concentrations required for activity. Instead, S-CDR and L-CDR peptides, like the few other CDR-derived peptides described until now, should only be viewed as first-generation bioactive compounds which could serve as a template for the design of antagonists with better pharmacological properties. Different approaches successfully used in recent years to enhance the affinity of antibody CDR-derived peptides (Von Feldt et al., 1992; Peterson and Greene, 1994), could thus be considered, e.g. development of conformationally constrained peptides (Saragovi et al., 1991), dimeric peptides (Williams et al., 1991), and organic non-peptide mimetics designed from computer modeling of cyclized CDRs (Saragovi et al., 1991).

Our data also point to the importance of the hydropathic pattern in the binding of receptor ligands (see McGuigan, 1994 for a review) since both CDR peptides were hydropathically similar to substance P, the tachykinin NK_1 receptor natural ligand. In this connexion, these results could thus indicate that a general principle for developing antagonists for an endogenous peptide mediator would be to design synthetic peptides hydropathically similar to the mediator, as suggested by the pioneering studies of Clarke and Blalock (1990) and Weigent et al. (1994). An important additional finding is that the size of the bioactive peptides also appears to be a factor that markedly governs their fine pharmacological and/or binding properties, probably also as a consequence of the slightly distinct conformations that they adopt in solution according to their length: indeed, only the L-CDR peptide (17 amino acids long) but not the S-CDR (11 amino acids long) was capable of preventing substance P and neurokinin A binding to the tachykinin NK_1 receptor, in the range of the concentrations tested, whereas both CDR peptides were efficient, at the same concentrations, in antagonizing substance P and neurokinin A bioactivities. The importance of the peptide size for hydropathically similar peptides was also recently demonstrated by Villain et al. (2000), who showed that a difference

of four amino acids was sufficient to change a calmodulin-binding peptide with calcium-agonist property into a calcium-antagonist peptide.

Finally, within the framework of a dynamic model for G protein-coupled receptors (Hoare et al., 2001), proposing that ligands may first interact with the receptor through low-affinity primary contact sites [possibly corresponding in the receptor to domains hydropathically opposed to ligand peptide sequences (McGuigan, 1994; Wijkhuisen et al., 1999) before being guided towards a secondary high-affinity binding site, our results actually suggest the occurrence of multiple primary contact sites for antagonists on the tachykinin NK_1 receptor, some of which are possibly different from those for agonists. Indeed, the S-CDR peptide appears to affect exclusively the structural conformational change leading to G-protein activation upon agonist (substance P or neurokinin A) binding: it behaves as a classical noncompetitive antagonist, at least in the concentration range tested, and could thus recognize contact site(s) distinct from that (those) for substance P and neurokinin A. By contrast, L-CDR peptide, which prevents both substance P and neurokinin A binding, could compete with these endogenous agonists for the same contact and/or binding site(s), although the possibility remains that L-CDR peptide interacts with yet another site, distinct from those recognized by substance P, neurokinin A and S-CDR peptide. The precise delineation of these multiple contact and binding sites for agonists and antagonists will, of course, require not only photolabeling and mutagenesis experiments but also biophysical techniques and kinetic studies, capable of accounting for the dynamics of ligand–receptor interaction. We hope that the peptides described here will be of some help in identifying these sites, which also could represent a first step in the development of novel pharmacologically active compounds.

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