European Journal of Pharmacology 592 (2008) 1–6

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

The role of the N-terminal and mid-region residues of substance P in regulating functional selectivity at the tachykinin NK_1 receptor☆

Shane A. Perrine [a,b,1], Debbie J. Beard [c], John K. Young [c], Mark A. Simmons [a,b,*]

[a] Department of Physiology & Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, OH, United States
[b] Program in Pharmacology, Kent State University, Kent, OH, United States
[c] Department of Chemistry, Mississippi State University, Mississippi State, MS, United States

ARTICLE INFO

Article history:
Received 12 February 2008
Received in revised form 18 June 2008
Accepted 22 June 2008
Available online 2 July 2008

Keywords:
Substance P
Tachykinin
NK_1 receptor
G protein coupled receptor
Nuclear magnetic resonance
Receptor binding
Calcium responses
Desensitization

ABSTRACT

Previous studies have shown that tachykinin peptide ligands of the tachykinin NK_1 receptor exhibit functional selectivity with respect to signal activation and desensitization. The differences are most dramatic between the naturally occurring peptides substance P (RPKPQQFFGLM-NH_2) and ranatachykinin C (HNPASFIGLM-NH_2). To understand the structural features of the peptides that underlie these differences, four peptide analogs have been designed and tested. The analogs were designed to assess the major structural differences between substance P and ranatachykinin C, including the role of the N-terminal Arg and the substitution of the mid-region Gln with Ala and Ser (Q5 replaced with A and/or Q6 replaced with S). Receptor binding, receptor activation of intracellular calcium fluxes, and receptor desensitization of the rat tachykinin NK_1 receptor were quantified for each ligand. All of the peptides bound to the rat tachykinin NK_1 receptor with high affinity, produced robust calcium signal activation, and led to agonist-induced receptor desensitization. It was found that deletion of the N-terminal Arg of substance P or replacement of either or both Q5 and Q6 altered the functional selectivity of substance P based on the relationship of receptor binding to receptor activation and activation to desensitization. When considered in light of our previously published nuclear magnetic resonance structure data, the data presented herein suggest that the one, five and six positions of the substance P backbone are key structural residues that govern the relative degree of tachykinin peptide-mediated receptor signaling and desensitization.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

The importance of tachykinin structure–activity relationships in determining peptide ligand–receptor interactions has been known for more than 30 years (Bury and Mashford, 1976; Couture et al., 1979; Duplaa et al., 1991; Perrine et al., 2000). Ligand structure and receptor pharmacology (structure–activity) relationships for the family of tachykinin peptides and their receptors have been well-studied; however, the importance of ligand structure on receptor regulation events, such as desensitization and internalization has not been as extensively characterized. Systematic amino acid substitution studies have increased our understanding of structure–activity relationships and the importance of single amino acids on peptide ligand–receptor interactions. For example, the peptide agonist substance P, which selectively binds to tachykinin NK_1 receptors (Regoli et al., 1989), is greatly affected by replacement of Phe^7, Leu^{10}, or Met^{11}, either of which decreases receptor binding, while substitution of Gly^9 by Ala or D-Gly has no significant effect (Couture et al., 1979; Duplaa et al., 1991). These studies show the importance of single amino acid changes in altering receptor binding and initial activation, but they do not take into account the role of ligand structure in regulation of receptor function by desensitization.

Previous studies from our lab show that a distinct functional difference exists between substance P and a related tachykinin isolated from bullfrog, ranatachykinin C (Perrine et al., 2000; 2003; Simmons, 2006). The two peptides vary in their relative abilities to produce receptor activation and desensitization. These differences are observed with respect to the actions of these peptides to inhibit M-type K^+ current in single neurons which endogenously express a receptor for substance P or to elevate intracellular calcium in Chinese hamster ovary cells stably transfected with the rat tachykinin NK_1 receptor. These results have led us to conclude that signal activation and desensitization of the tachykinin NK_1 receptor are functionally distinct phenomena that can be differentially activated depending on the peptide agonist. The peptide structural features that underlie these differences are not known. To understand the structure–activity

☆ This work was supported in part by a Research Challenge Grant from the Ohio Board of Regents to MAS.

* Corresponding author. Department of Integrative Medical Sciences, Northeastern Ohio Universities College of Medicine, 4209 State Route 44, P.O. Box 95, Rootstown, OH 44272-0095, United States.
E-mail address: simmons@neoucom.edu (M.A. Simmons).
[1] Current address: Department of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine, Detroit, MI, United States.

0014-2999/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2008.06.097

relationships that determine the difference between ligand-mediated signaling and ligand-induced desensitization at the tachykinin NK_1 receptor, we designed a series of peptide analogs using substance P and ranatachykinin as templates.

In this study we have investigated the relationship between ligand binding, signal activation, and receptor desensitization. We have looked at the ability of the tachykinin analogs to compete with [^{125}I]-substance P for binding sites at the rat tachykinin NK_1 receptor expressed in Chinese hamster ovary cells. Additionally, we have compared the calcium signaling properties of the analogs with their abilities to cause receptor desensitization. The major findings show that the one, five and six amino acid positions of substance P all influence its ability to induce signal activation relative to desensitization.

2. Materials and methods

2.1. Materials

Common chemicals and supplies were purchased from Sigma (St Louis, MO) or Fisher Scientific (Pittsburgh, PA), unless stated otherwise. SP2-11, Q5A-SP, Q6S-SP, and Q5AQ6S-SP were synthesized by and purchased from Sigma-Genosys (The Woodlands, TX).

2.2. Peptide design rationale

The tachykinin family of peptides is distinguished by the presence of the conserved, amidated, carboxy terminal sequence of Phe-Xaa-Gly-Leu-Met-NH_2 (Fig. 1), where Xaa is a variable amino acid. In substance P, Xaa = Phe, and in ranatachykinin C, Xaa = Ile, a relatively conservative change, particularly in view of the results of quantitative structure–activity studies of substance P showing that size or bulk is the most important functional determinant of the residue in this position (Norinder et al., 1997). In contrast, in the first six positions there is considerable diversity that might contribute to differences in the effects of these two peptides.

The major structural differences between substance P and ranatachykinin C are at the N-terminus and in the mid-region of the peptides. The two most significant differences are the lack of an Arg at position 1 of ranatachykinin C and the replacement of the Gln at positions 5 and 6 of substance P with Ala and Ser, respectively, in ranatachykinin C. On the basis of these structural and the aforementioned pharmacological differences, four peptide analogs of substance P have been designed to determine the importance of these two regions in the responses observed. SP2-11 was synthesized to test the importance of the Arg at position 1; Q5A-SP and Q6S-SP to test the importance of the two mid-region residues separately, and Q5AQ6S-SP to test the combined replacement of these middle residues. This series of peptide analogs, whose sequences are shown in Fig. 1, has been used to investigate the relationship between peptide structure and receptor pharmacology at the rat tachykinin NK_1 receptor.

2.3. Receptor binding: competition radioligand binding

Chinese hamster ovary cells stably transfected with cDNA encoding the rat tachykinin NK_1 receptor were used to determine the binding properties of the peptides. Bolton-Hunter [^{125}I]Lys^3-substance P (NEN, Boston, MA) at a concentration of 50 pM and increasing amounts, 10^{-11} to 10^{-5} M, of unlabeled peptide analogs, were used in the binding assays. The assay conditions used here are common competition radioligand binding conditions and have been used previously in our lab (Bennett and Simmons, 2001). Briefly, cells expressing the rat tachykinin NK_1 receptor were incubated with radiolabeled substance P and unlabeled peptide for 2 h at 4 °C, washed to remove any unbound ligand, collected and the radiation counted using a Packard Cobra II series auto-gamma counter (Packard, Meriden, CT).

Specific binding was determined and the data were normalized as the ratio of bound to free (B/B_0) for each unlabeled peptide, where B is specifically bound [^{125}I]Lys^3-substance P in the absence of unlabeled peptide and B_0 is specifically bound [^{125}I]Lys^3-substance P in the presence of 100 pM unlabeled peptide. Data are plotted as the competitor concentration vs. fraction specific binding. Each data point represents the mean ± standard error of the mean (S.E.M.) ($n \geq 4$).

	POSITION										
	1	2	3	4	5	6	7	8	9	10	11
SP	= Arg-	Pro-	Lys-	Pro-	Gln-	Gln-	Phe-	Phe-	Gly-	Leu-	Met-NH_2
SP2-11	=		Pro-	Lys-	Pro-	Gln-	Gln-	Phe-	Phe-	Gly-	Leu-	Met-NH_2
Q5A-SP	= Arg-	Pro-	Lys-	Pro-	Ala-	Gln-	Phe-	Phe-	Gly-	Leu-	Met-NH_2
Q6S-SP	= Arg-	Pro-	Lys-	Pro-	Gln-	Ser-	Phe-	Phe-	Gly-	Leu-	Met-NH_2
Q5AQ6S-SP	= Arg-	Pro-	Lys-	Pro-	Ala-	Ser-	Phe-	Phe-	Gly-	Leu-	Met-NH_2
RTKC	=		His-	Asn-	Pro-	Ala-	Ser-	Phe-	Ile-	Gly-	Leu-	Met-NH_2

Fig. 1. Amino acid sequences of select tachykinin peptides. Sequences of substance P, ranatachykinin C and the analogs designed for this project are shown.

2.4. Receptor activation: calcium signaling assay

Transfected cells were maintained in α-MEM containing 10% FBS and 0.8 mg/mL G418. Cells were grown to 80 to 90% confluency on 6-well plates and incubated at 37 °C and 5% CO_2 for 3–4 days prior to use. On the day of the experiment, cells were rinsed 3× with extracellular solution, containing 1 mM $MgCl_2$, 5 mM KCl, 115 mM NaCl, 10 mM HEPES, 10 mM glucose, 2.3 mM $CaCl_2$, and 2.5 mM probenecid, pH 7.4, prior to being placed in a fluorescent plate-reader (FLUOstar Galaxy, BMG Lab Technologies, Durham, NC) at 37 °C. The fluorescence is measured in an area of 2.0 mm^2 in the center of each well. The number of cells within this area was 2000–3000. Extracellular solution supplemented with 1 μM Fluo 3-AM, a calcium indicator dye (Molecular Probes, Eugene, OR), and 0.1% Pluronic F-127 (Texas Fluorescence Labs, Austin, TX) was added to the wells and uptake of the dye was allowed to proceed for 30 min. The cells were rinsed (3×) with extracellular solution just before measurement of intracellular calcium. The fluorescence emission at 520 nm following excitation at 485 nm was measured and used as an index of intracellular calcium levels.

After loading the cells, graded concentrations ranging from 10^{-11} to 10^{-5} M of each peptide were manually applied to the wells to establish the concentration–response relationship. The calcium ionophore, ionomycin (10 μM), was applied at the end of each experiment to normalize the agonist-induced responses between samples. Data are expressed as a fraction of the maximal response (E_{max}) for each agonist (mean ± S.E.M.) and include an n of 4 or more.

2.5. Receptor desensitization

Desensitization was assessed by comparing the amplitude of the response to substance P after preincubation in the presence of various concentrations of the analogs to produce desensitization. Each well of cells was exposed to a single concentration of analog. To observe desensitization, cells were first exposed to a single analog concentration within the range of 10^{-11}–10^{-5} M for 5 min at 37 °C. The cells were then rapidly washed three times with extracellular solution. This was followed by application of a fixed concentration of substance P (100 nM) to obtain the "Desensitized response." Ionomycin (10 μM) was applied after the second application to measure the maximum response from that well of cells. To obtain the "Non-desensitized response," separate wells of cells were not exposed to analog, but were incubated at 37 °C for 5 min, rapidly washed three times, and then exposed to substance P (100 nM). The fractional desensitization was calculated as 1-(Desensitized response/Non-desensitized response). Data are plotted as the mean ± S.E.M. and have a sample size of 4–8.

2.6. Data analysis

All data were analyzed using Prism 4 (Graphpad Software v4.03) and graphed using Sigmaplot. Concentration–response curves were fit to the

Download English Version:

https://daneshyari.com/en/article/2534853

Download Persian Version:

https://daneshyari.com/article/2534853

Daneshyari.com