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New backbone cyclic substance P analogs

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SUMMARY

Structure–activity relationships of cyclic substance P (SP) analogs were extensively studied in our laboratories utilizing the new concept of backbone cyclization. Employing the C-terminal hexapeptide SP_{6-11}, we examined the influence of chemical changes in peptides containing backbone-to-amino-end cyclization. These changes in the ring have significant influence on activity, and should be carefully designed in order to optimize pharmacological features.

INTRODUCTION

Our approach for conversion of native peptides into peptidomimetics with desired pharmacological features, such as metabolic stability, selectivity and bioavailability, involves gradual rigidification and correlates in an iterative manner synthesis, biological activity, conformational analysis and conformationally based computerized drug design. This approach is based on a recently introduced new concept for imposing conformational constraints on peptides, called 'backbone cyclization' [1,2]. According to this concept, constraint is effected by linking N^{α} and/or C^{α} atoms in the peptide backbone through an appropriate linker [3,4]. We have demonstrated our approach by the synthesis of analogs of several biologically active peptides such as substance P (SP), bradykinin and somatostatin. We present here new backbone cyclic analogs of SP and studies regarding their structure–activity relationships.

SP is a mammalian neurokinin involved in many biological activities, such as transmission of pain stimuli, exocrine gland secretion, intestinal motility, vasodilation, neuronally mediated inflammatory skin reaction and behavioral responses (for a review, see Ref. 5 and references cited therein). The large variety of biological activities exerted by the neurokinins has indicated the existence of several receptors (or receptor subtypes). The three neurokinin receptors are NK-1, NK-2 and NK-3. Even though the neuro-

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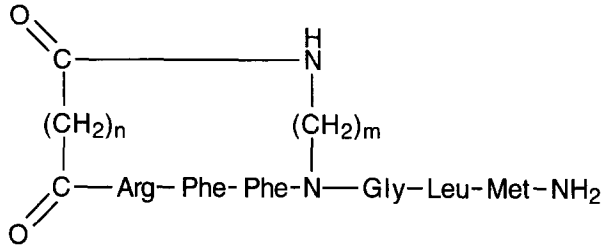


Fig. 1. Backbone-to-amino-end cyclic analogs of WS-septide, incorporating a lactam ring.

kinin receptors are activated preferentially by one specific neurokinin (NK-1 by SP, NK-2 by neurokinin A (NKA) and NK-3 by neurokinin B (NKB)), the selectivity of each neurokinin to its receptor is rather poor [5,6]. Thus, for example, NKB activates both the NK-1 and NK-3 receptors with almost the same EC_{50} (4.2 and 1.3 nM, respectively). In our laboratory the design of selective neurokinin receptor agonists originated with a systematic study, in which each of the peptide bonds in the C-terminal hexapeptide of SP was replaced by an N-methylated peptide bond. These studies revealed that N-methylation of a specific peptide bond resulted in selective loss of biological activity on two out of the three receptor subtypes, while activity on the other receptor remained unchanged. Thus, N-methylation of the Phe^7-Phe^8 or the Phe^8-Gly^9 peptide bond inferred selectivity to the NK-3 and the NK-1 receptor, respectively [7]. Based on structure–activity relationship studies, we prepared the NK-1 selective agonist WS-septide (Ac[Arg^6,Pro^9]-SP_{6-11}) and the highly selective NK-3 agonist senktide (succ[Asp^6,N-Me-Phe^8]SP_{6-11}) [8,9].

We then moved from local to global conformational restraint, introducing a series of six backbone-to-amino-end cyclic analogs of WS-septide, in which Pro^9 was replaced by N^{α}-(ω-aminoalkylene)Gly. A dicarboxylic spacer was coupled to the amino-terminal Arg^6, and cyclization was effected by closing a lactam ring between the ω-amino group of the glycine unit and the spacer (Fig. 1).

The lengths of the alkylene chains, n and m, were designed to give ring sizes from 17 to 22 atoms. A strong dependence of activity on ring

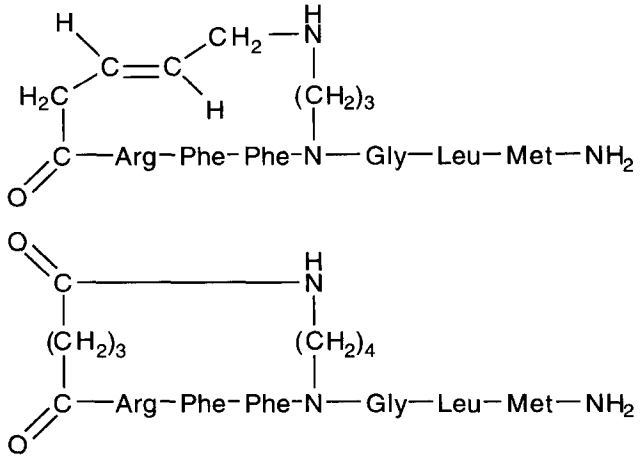
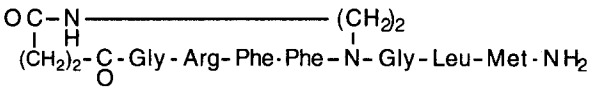
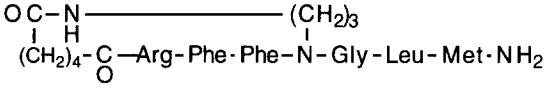
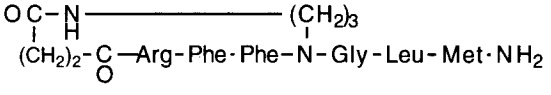
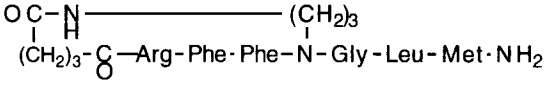
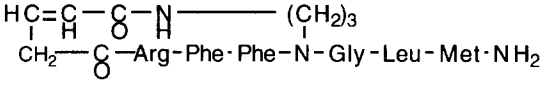
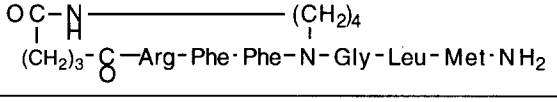


Fig. 2. New backbone-to-amino-end cyclic analogs of WS-septide. SP5 (top) contains a double bond in the ring; in SP6 (bottom) the amide bond closing the ring has moved one position towards the amino-terminus.

TABLE 1
STRUCTURES AND BIOLOGICAL ACTIVITIES OF SIX BACKBONE-TO-AMINO-END CYCLIC ANALOGS

No.	Peptide structure	Biological activity (EC_{50}, nM) towards receptor subtype		
		NK-1	NK-2	NK-3
SP1		>10 000	>50 000	>10 000
SP2		5	>50 000	>10 000
SP3		180	>50 000	>10 000
SP4		11	>50 000	>10 000
SP5		500	>50 000	>10 000
SP6		20	>50 000	>10 000

size was found, with the optimum at 20 atoms (m = 4, n = 3). This analog, called cycloseptide or SP2 [10], activated the NK-1 receptor at 5 nM, and was completely inactive towards NK-2 or NK-3 (Table 1). It was also metabolically stable, and maintained 80% of agonist activity after 2 h of incubation with parotid gland slices [11]. Analogs with 19 (SP3) and 18 (SP4) atoms in the ring showed lower activity (Table 1), but were also NK-1 selective. On the other hand, an analog (SP1) with the same ring size, which contained an additional amide bond within the ring and was hence more constrained than SP2, was totally inactive [11]. These four analogs were studied by NMR in DMSO and water. As expected, the conformations of the pharmacophoric sequence Arg^6-Phe^7-Phe^8 and of the ring resembled each other in the active analogs SP2, SP3 and SP4, but were significantly different in the more constrained, inactive analog SP1 [10,12].

RESULTS

We present here two new analogs of SP2, both having a chemically modified 20-atom ring. Analog SP5 had a trans double bond in the spacer coupled to Arg^6, while in analog SP6 we switched alkylene lengths m and n from 4 and 3 to 3 and 4, respectively (Fig. 2). Both analogs showed agonist activity and NK-1 selectivity. They were prepared by the same methods used to synthesize the former backbone-to-amino-end analogs.

The conformations of all six analogs presented in Table 1 were studied by 2D NMR and molecular dynamics simulations. The NMR and MD experiments on the analogs were performed in DMSO. The most active analog SP2 was also studied in D_2O. Comparison of the chemical shifts and NOEs of the latter molecule showed no significant difference between the two solvents [12]. While large percentages of the cis isomers of SP1,

SP3 and SP4 (34, 18 and 22%, respectively) were found [10,12], only 3% of cis isomer was detected for SP5 under the experimental conditions. SP2 and SP6 were found in the trans isomer only, indicating a unique conformation of the 20-atom rings without additional constraint. MD simulations were carried out in the same solvents as the NMR experiments. NOE restraints were used to determine the conformation, and were compared in each case with free MD to examine the quality of the measurements and simulations.

Although the activity of SP5 was two orders of magnitude lower than that of SP2 (EC_{50} = 500 nM), the influence of the double bond seemed to be less dramatic than that of the additional amide bond in SP1, which caused complete loss of activity. This difference may be attributed either to a greater degree of conformational freedom allowed by the double bond compared to the amide bond, or to the different positions of the two bonds within the ring. An answer to this question cannot be provided at present, and will require additional data.

As expected from the biological results, superposition of SP2 and SP5 shows greater similarity of the ring portion of the two analogs than for SP2 and SP1. Rigidification imposed by the double bond clearly prevents, however, the ring portion from adopting the active conformation of SP2.

SP6 was found to be four times less active than SP2. It had an EC_{50} value of 20 nM, suggesting great resemblance to the three-dimensional structure of SP2, and indeed the low-energy conformations of the ring portions of these two analogs showed very close similarity. Minor conformational differences, revealed by the MD simulated structure, are probably responsible for the somewhat lower activity of SP6.

CONCLUSIONS

The new analogs SP5 and SP6 give additional insight into the conformational requirements of the pharmacophoric sequence Arg^6-Phe^7-Phe^8 in the C-terminal hexapeptide of SP. Careful design of ring size as well as of the chemical nature of the ring is required in order to optimize the desired pharmacological features when new backbone cyclic analogs are derived from linear peptides. The backbone-to-amino-end SP analog series shows clearly that, while the size of the ring seems to be the most crucial feature, slight modifications of specific bonds may alter the activity significantly. The detailed spectral and calculational data will be published elsewhere. Studies of new analogs of SP2 with other modifications of the ring portion are currently in progress.

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