exchange capacity[28]. However, cellulose is the fibre most similar to alginates, the properties of which appear to differ from those of cellulose mainly because of the carboxyl groups, whereas the hydroxyl group determines moisture, absorbancy and other cellulose qualities[29].

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Effect of substitution of glycine by D- or L-alanine on the activity of the C-terminal hexapeptide analogue of substance P on isolated guinea-pig ileum

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Summary. Two new C-terminal hexapeptide analogues of substance P having D- or L-alanine in position 9 were synthetized. Their contracting activities on isolated guinea-pig ileum were considerably lower than that of < Glu-SP$^{7-11}$.

Substance P (SP), a naturally occurring undecapeptide was discovered in the brain and equine gut by von Euler and Gaddum[1] in 1931. However it was not purified until 1970 when Chang et al.[2] isolated substance P from the hypothalamus and determined the amino acid sequence of this peptide[3] (table 1, **I**).

The C-terminal hexapeptide fragment of substance P - SP$^{6-11}$ (**II**) and its pyroglutamyl-analogue (< Glu-SP$^{7-11}$, **III** in table 1) show the full activity of the native undecapeptide in most biological tests[4,5]. The same holds true for non-mammalian tachykinins such as eledoisin or physalaemin[6]. All these tachykinins contain a glycine residue in position 9 which may account for their biological activity[7].

The purpose of this study was to find out to what extent a substitution of glycine in position 9 by other amino acids could affect the biological activity of the C-terminal hexapeptide analogue of SP on the isolated guinea-pig ileum.

Material and methods. 1. Peptides. We synthetized 2 new peptides, **IV** and **V**, whose chemical structure is presented in table 1, by a 3 + 3 fragment coupling method (fig. 1).

The method involved the same protecting groups, coupling procedures and purification as was described for **III**[8]. The physico-chemical properties of the peptides **IV** and **V** are summarized in table 2.

2. Bioassay. The investigations were performed on guinea-pig ileum according to Yau[9]. 3-month-old guinea-pigs of either sex were anaesthetized with chloroform. A 3-3.5-cm-long segment of ileum was removed, washed and suspensed in 50 ml of Krebs solution at 37 °C. A 95% O_2-5% CO_2 mixture was bubbled through the bath. The peptides were dissolved in dextran-water solution and diluted with Krebs

Table 1. Amino acid sequences of substance P, C-terminal hexapeptide fragment and its analogues

Number	Peptide
I	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
II	Gln-Phe-Phe-Gly-Leu-Met-NH_2
III	< Glu-Phe-Phe-Gly-Leu-Met-NH_2
IV	< Glu-Phe-Phe-D-Ala-Leu-Met-NH_2
V	< Glu-Phe-Phe-L-Ala-Leu-Met-NH_2

Abbreviation: < Glu = pyroglutamyl.

Table 2. Physico-chemical properties of the peptides used for bioassay

Peptide	M.p. (°C)	$[\alpha]_D^{24}$ c = 1, DMF	Chromatography R_f^I	R_f^{II}	R_f^{III}
< Glu-(D-Ala^9)-SP$^{7-11}$	235–240	−31°	0.87	0.90	0.94
< Glu-(L-Ala^9)-SP$^{7-11}$	225–230	−24°	0.85	0.87	0.81
< Glu-SP$^{7-11}$	233–235	−38°	0.89	0.91	0.81

TLC on silica gel plates 60 F_{254} (Merck 5729). R_f-values in the following systems: I – EtOAc, Py, AcOH, H_2O (20:20:6:11), II – n-BuOH, Py, AcOH, H_2O (4:1:1:2), III – n-BuOH, AcOH, H_2O (4:1:1). Abbreviations: EtOAc = ethyl acetate, Py = pyridine, AcOH = acetic acid, n-BuOH = n-butyl alcohol.

solution to the desired concentration. A volume of 100 µl was added to the incubation medium. The ileum was always washed with a fresh Krebs solution after each testing. Contraction of the ileum was recorded on a smoked drum using a light lever. Increasing concentrations of each peptide were introduced to the medium as long as the contraction of the ileum became maximal, i.e. failed to rise further with higher doses. The concentration of the peptide which caused half of the maximal spasm was taken as EC_{50}.

Results and discussion. As seen from table 3 substitution of glycine in position 9 of < Glu-SP$^{7-11}$ by D- or L-alanine decreased the activity of the hexapeptide on the isolated guinea-pig ileum.

The peptide sterical condition might be one of the reasons for the presence of a glycine residue in the peptide chain; only the presence of a glycine residue allows the natural peptides to adopt structures (e.g. β^{II} turn) resembling the structures of synthetic analogues with D-chirality in the glycine position. This may be the case with luliberin, where the replacement of glycine by a D-amino acid residue considerably increased the biological activity of the analogues[10]. In our study the substitution of the glycine residue by L- or D-alanine decreased the activity of the analogues which excludes the possibility that glycine is important as a special conformation formation element.

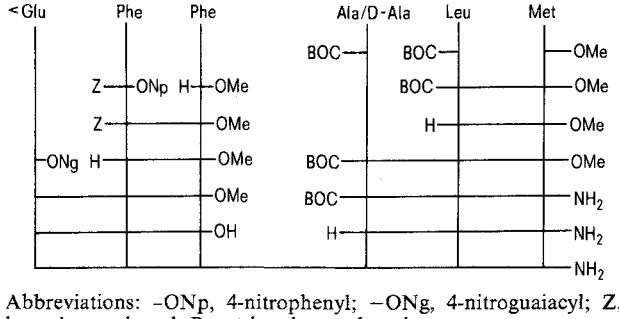
An alternate possibility is that the presence of the glycine residue provides a higher flexibility of the peptide chain, compared with its flexibility with another amino acid in the same position. The lower activity of < Glu-L-Ala^9-SP$^{7-11}$ and < Glu-D-Ala^9-SP$^{7-11}$ compared to the < Glu-SP$^{7-11}$ may be explained by a lower flexibility of their peptide chains. Therefore, our results support the suggestion of Ruegen et al.[11] that substance P and its analogues interact with the receptor through a 'zipper' mechanism[12]. The practically equal biological activity of the 2 analogues gives an additional support for this mechanism. Only in the dynamic ('zipper') mechanism of peptide-receptor interaction can the different steric hindrances resulting from a difference in chirality of the alanine residues be averaged.

Table 3. Contracting activities of C-terminal hexapeptide analogues of substance P on isolated guinea-pig ileum

Peptide	Mean EC_{50} (M)	n
< Glu-(D-Ala^9)-SP$^{7-11}$	2.15×10^{-6}	4
< Glu-(L-Ala^9)-SP$^{7-11}$	1.22×10^{-6}	3
< Glu-SP$^{7-11}$	4.60×10^{-9}	3

n, number of determinations.

Scheme of synthesis



Abbreviations: -ONp, 4-nitrophenyl; -ONg, 4-nitroguaiacyl; Z, benzyloxycarbonyl; Boc, t-butyloxycarbonyl.

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