SYNTHESIS AND BIOLOGICAL PROPERTIES OF THE [5-(N^5-DIMETHYL)-GLUTAMINE]-ANALOGUE OF THE C-TERMINAL HEPTAPEPTIDE OF SUBSTANCE P

Active-site studies

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1. Introduction

Substance P (SP), an undecapeptide amide, is the hypotensive, spasmogenic agent detected [1], the sialogogic peptide noticed in [2], and the moto-neuron-depolarizing peptide studied [3]. Complete amino acid sequence of SP was determined [4,5] (fig.1a). Its primary structure was confirmed by synthesis either by the solid-phase method [4,6,7] or conventional solution techniques [8–10].

Studies on the relationship between chainlength and activity revealed that the C-terminal heptapeptide of SP exhibited higher contractile activity on the isolated guinea pig ileum than that of synthetic SP [7,9]. Thus, in an effort to identify sites involved in the receptor recognition and activation of SP, the N-glutamine residue of the C-terminal heptapeptide of this hormone has been modified by replacing its carboxamide hydrogen atoms by methyl groups (fig.1b). This modification of the $-NH_2$ portion of the carboxamide group renders it less hydrophilic and possibly less available to the receptor due to steric hindrance.

a H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
1 2 3 4 5 6 7 8 9 10 11

b H-Glu $N(CH_3)_2$ -Gln-Phe-Phe-Gly-Leu-Met-NH_2
5 6 7 8 9 10 11

Fig.1.(a) Amino acid sequence of substance P (SP). (b) [5-N^5-dimethyl)-glutamine] C-terminal heptapeptide of SP; numbers indicate sequence positions of individual residues. Amino acids, with the exception of glycine, are of the L-configuration.

2. Experimental

The synthetic route chosen is based on the coupling of the appropriate pentapeptide with the C-terminal H–Leu–Met–NH_2 part of the molecule. The pentapeptide derivative, boc-(N^5-dimethyl)Gln–Gln–Phe–Phe–Gly–OBzl, was constructed stepwise starting from glycine benzylester *p*-toluenesulphonate [11] and using the *N-tert* butyloxycarbonyl (boc) group for the α-amino protection and DCC, 1-hydroxybenzotriazole [12] for activation. boc-(N^5-dimethyl)-L-glutamine m.p. 124–125°C (from ethyl acetate), $[\alpha]_D^{20}$ + 2.2°C (*c* 1, EtOH) was prepared from N^5-dimethyl-L-glutamine [13] using *S*-boc-4,6-dimethyl-2-mercapto-pyrimidine [14] as the acylating agent.

The obtained pentapeptide benzyl ester was catalytically hydrogenated to yield the corresponding acid, boc-(N^5-dimethyl)Gln–Gln–Phe–Phe–Gly–OH, m.p. 183–185°C, $[\alpha]_D^{20}$ −23.9°C (*c* 0.5, DMF), which was then condensed with H–Leu–Met–NH_2 [15] by the DCC, 1-hydroxybenzotriazole method [12]. The resulting heptapeptide derivative, boc-(N^5-dimethyl)Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2, m.p. 218–222°C, $[\alpha]_D^{20}$ −35.9°C (*c* 0.5, DMF), was deprotected with CF_3COOH, desalted on a ion-exchange resin (Dowex-1X8) using ethanol as the eluent and purified by gel-filtration on Sephadex G-15 with 50% acetic acid to give the desired analogue. Amino acid analysis gave the following molar ratios: Glu, 2.03; Phe, 2.05; Gly, 1.00; Leu, 1.02; Met, 0.91; NH_3, 1.98. It had m.p. 234–238°C (dec.) and $[\alpha]_D^{20}$ −56°C (*c* 0.18, DMF). Calc. for

$C_{43}H_{64}N_{10}O_9S$: C, 57.57; H, 7.19; N, 15.61%. Found: C, 57.79; H, 6.98; N, 15.74. Thin-layer chromatography of the analogue showed one spot R_F 0.40 in *n*-BuOH–AcOH–H_2O (4:1:1), R_F 0.75 in *n*-BuOH–AcOH–H_2O–pyridine (30:6:24:20) and R_F 0.32 in *n*-BuOH–AcOH–H_2O (4:1:5, upper phase). Thin-layer chromatograms were done on silica gel plates with sample loads of 20–50 μg, and were revealed by reaction with ninhydrin and chlorine–tolidine reagent.

3. Results and discussion

The analogue was tested on the isolated guinea pig ileum and has an agonistic activity 25% of the activity of SP. When the analogue was tested at 5×10^{-7} M (maximal contraction) then the contraction of the ileum produced by SP was inhibited by ~30%. Thus the analogue has a weak agonistic and also a weak antagonistic activity.

These results may suggest that the hydrogens of the N^5-amide portion of the N-terminal glutaminyl side chain are not themselves essential from a conformational standpoint for the biologically active model of the hormone. Considering that pGlu–Gln–Phe–Phe–Gly–Leu–Met–NH_2 [16] is more active than substance P, while our [5-(N^5-dimethyl)-glutamine]-analogue has a weak activity, it is rather tempting to assume that the carbonyl group of the carboxamide of glutamine at position 1 of the C-terminal heptapeptide of substance P (position 5 for substance P) which is sterically hindered in the methyl-substituted heptapeptide, might be an essential 'element' for biological response on the isolated guinea pig ileum.

Finally, it should be mentioned that specific competitive inhibitors of SP would be extremely useful, but none is yet available. Although it is known for SP that its continued presence or of another tachykinin in the bath reduces the response of the isolated smooth muscle to subsequent application [17,18], it seems likely that a proper modification of the carboxamide portion of the [5-(N^5-dimethyl)-glutamine]-analogue may afford a more potent antagonist.

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