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Inferences About the Conformation of Somatostatin at a Biologic Receptor Based on NMR Studies[1]

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Examination of two diastereomeric analogs of somatostatin differing in stereochemistry at the tryptophan residue has revealed a high field resonance in the D-Trp isomer which is assigned to the γ-methylene of Lys[9]. The extent of correlation of this shift with biologic activity for a series of analogs of somatostatin is discussed. From comparison of close analogs, it is suggested that the biologically active conformation of somatostatin at the receptor controlling insulin release is not the major conformation of this hormone in solution. It is suggested that the conformation of somatostatin at this receptor resembles more closely the solution conformation of analogs having tryptophan in the D-configuration. This latter conformation places the Trp[8]–Lys[9] side chains in close proximity, thus shifting the γ-methylene protons of Lys[9] upfield.

INTRODUCTION

In earlier publications (*1*, *2*) we have proposed a biologically active conformation of the peptide hormone somatostatin on the basis of the structure–activity relationships of bis-carba analogs (*1*) and of restricted bicyclic analogs (*2*). This conformation, which differs from a solution conformation proposed by Holladay and Puett (*3*), is typified by the des(Ala[1],Gly[2])desamino[Cys[3]]descarboxy[Cys[14]]dicarba[3,14]-D-Trp[8] analog of somatostain shown in Fig. 1. These earlier studies (*2*) supported the indicated proximity in space of the atoms marked α and α' and the β carbons[2] of Phe[6],Phe[11] marked β in Fig. 1, because appropriate bicyclic analogs possessing a cystine bridge replacing either amino acids 5,12 or 6,11, respectively, possessed high biologic activity. The proposed type II′ β-turn involving amino acids 7,11 was supported also by the greater biologic activity (*4*) of analogs having a D-Trp replacing L-Trp in position 8, a feature which might be expected to stabilize the β-turn (*5*). This conformation implies a proximity of the indole side chain of Trp[8] and of the aliphatic side chain of Lys[9].

We wish to report ^{1}H nmr studies which not only support the proposed conformation (Fig. 1) but also permit, in combination with biologic activities reported herein, speculations concerning the conformation of somatostatin and its biologically active analogs at receptors involved in the inhibition of the release of insulin.

[1] This manuscript is submitted in honor of Professor William S. Johnson on his 65th birthday, and in appreciation of his outstanding achievements as a scientist and teacher.

[2] The somatostatin numbering system is used to describe the analogs of somatostatin throughout this paper.

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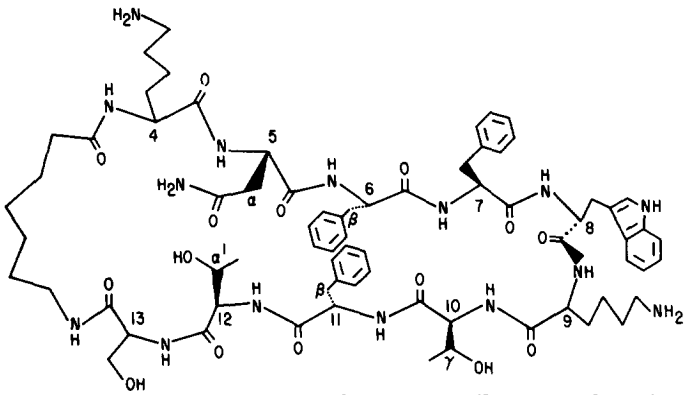

FIG. 1. Proposed conformation of des[Ala[1],Gly[2]]desamino[Cys[3]]descarboxy[Cys[14]]dicarba[3,14] somatostatin. The numbers at each residue refer to the residue number in somatostatin. A sense of the relationships of the side chains is obtained by viewing the side chains of Asn[5], Phe[6], Phe[7], Thr[10], Phe[11], and Thr[12] as being axial while the side chains of Trp[8] and Lys[9] are equatorial. A type II′β turn is proposed for the D-Trp analog while a type I β turn would be present in the L-Trp analog in order to attain the equatorial relationship of the Lys and Trp side chains in both analogs.

RESULTS AND DISCUSSION

Previous studies (*6*, *7*) have been concerned with the shielding (*8–10*) of side chain aliphatic protons in linear dipeptides with one amino acid aromatic and the other aliphatic. These shielding effects have also been interpreted in terms of specific conformations. The extent of this shielding was consistently more pronounced in the case of L,D or D,L dipeptides than in the diastereomeric L,L or D,D dipeptides. The upfield shift was found to be most pronounced for the γ-methylene protons of the aliphatic side chains in such dipeptides (*6*). The increase in the upfield shift of the γ-methylene protons was interpreted in terms of a close proximity on a time average basis of these protons to the aromatic ring. The backbone conformation proposed for such a dipeptide unit (*7*) is the same as that proposed for the D-Trp-L-Lys sequence of the somatostatin analog of Fig. 1.

Nuclear magnetic resonance spectroscopy has now shown that the pmr spectra of the diastereomers 4 and 5 (Table 1) are qualitatively similar. These two compounds differ only in the configuration of Trp in position 8. Although differences are observed throughout the spectra, the most noteworthy difference is the presence of a resonance in compound 5 which we have assigned to the γ-methylene of a lysine residue by double resonance experiments. We have attributed this resonance to the γ-methylene of Lys[9] rather than Lys[4] because a similar resonance was observed by us in the dipeptide D-Trp-L-Lys and also in analogs of compound 5 which lack a lysine residue in position 4 (*11*).

In Table 1, we report the chemical shifts of the Lys[9] γ-methylene resonances and the biologic activity for a series of analogs of somatostatin. The biologically active analogs 4–8, 10, 11, 13, 14, and 16 all show the high field γ-methylene peak. Those biologically active analogs which contain an additional conformational constraint such as a proline residue (compounds 10 and 11) or a bicyclic ring system (compounds 14 and 16) show the upfield shift, while the less active analogs 12 and 17, also having conformational constraints, show a greatly reduced upfield shift. The low activity of these latter two

TABLE 1

INHIBITION OF INSULIN RELEASE (RELATIVE POTENCY) COMPARED TO CHEMICAL SHIFT FOR ANALOGS OF SOMATOSTATIN

Compound	Relative potency of insulin[a]	$\delta(\gamma\text{-CH}_2$ lysine)[b]
1. Lysine	—	1.51
2. D-Trp–L-Lys	—	0.58
3. L-Trp–L-Lys	—	1.3
4. Cyclo(Aha–Lys–Asn–Phe–Phe–Trp–Lys–Thr–Phe–Thr–Ser)	0.26 (0.08–0.6)	>1.0[c]
5. Cyclo(Aha–Lys–Asn–Phe–Phe–D-Trp–Lys–Thr–Phe–Thr–Ser)	0.88 (0.45–1.7)	0.59
6. Cyclo(Aha–Lys–Abu–Phe–Phe–D-Trp–Lys–Thr–Phe–Thr–Ser)	2.2 (1.3–4.2)	0.44, 0.56
7. Cyclo(Aha–Lys–Asn–Phe–Phe–D-Trp–Lys–Thr–Phe–Thr–Gly)	1.4 (0.7–2.8)	0.56, 0.65
8. Cyclo(Aha–Lys–Asn–Phe–Tyr–D-Trp–Lys–Thr–Phe–Thr–Ser)	2.1 (0.9–6.0)	0.62
9. Cyclo(Aha–Lys–Asn–Phe–Tyr(OMe)–D-Trp–Lys–Thr–Phe–Thr–Ser)	0.13 (0.03–0.33)	0.62
10. Cyclo(Aha–Lys–Pro–Phe–Phe–D-Trp–Lys–Thr–Phe–Thr–Ser)	3.2 (1.7–5.7)	0.53
11. Cyclo(Aha–Lys–Asn–Phe–Phe–D-Trp–Lys–Thr–Phe–Thr–Pro)	8.5 (3.4–17)	0.51, 0.67
12. Cyclo(Aha–Lys–Asn–Phe–Phe–D-Trp–Lys–Pro–Phe–Thr–Ser)	0.05 (0.01–0.14)	0.96
13. Cyclo(Aha–Lys–Cys(Acm)–Phe–Phe–D-Trp–Lys–Thr–Phe–Cys(Acm)–Ser)	1.6 (0.4–8.8)	0.42, 0.56
14. Cyclo(Aha–Lys–Cys–Phe–Phe–D-Trp–Lys–Thr–Phe–Cys–Ser)	1.2 (0.45–3.5)	0.63
15. Cyclo(Aha–Lys–Abu–Cys(Acm)–Phe–D-Trp–Lys–Thr–Cys(Acm)–Thr–Ser)	0.14 (0.05–0.96)	0.65
16. Cyclo(Aha–Lys–Abu–Cys–Phe–D-Trp–Lys–Thr–Cys–Thr–Ser)	0.91 (0.46–1.9)	0.37, 0.54
17. Cyclo(Aha–Lys–Abu–Phe–α-Me–Phe–D-Trp–Lys–Thr–Phe–Thr–Ser)	<0.07[d]	0.93, 1.03

[a] Somatostatin = 1.

[b] Observed as either a broad single peak or two broad peaks. δ is measured relative to internal sodium 3-trimethylsilylpropionate-2,2,3,3-d_4.

[c] Hidden by other resonances.

[d] No activity observed at highest dose tested (150 μg/rat).

TABLE 2

CHEMICAL SHIFT (δ) OF γ-CH_2 OF D-Trp-L-Lys. SOLVENT AND pH EFFECTS

Solvent		pD[a] 1.1	1.4	5.8	6.2	12.3
D_2O			0.58	0.56		0.50
CD_3OD[b]		0.89			0.86	
CD_3OD/D_2O	(1:1)	0.66			0.63	
DMSO–D_6[b]		1.02			1.04	

[a] Uncorrected.

[b] An aqueous solution in D_2O at the pD indicated was evaporated to dryness, and the residue was dissolved in the solvent indicated.

analogs is thought to be a result of a change in backbone conformation resulting from the introduction of Pro[10] or α-Me-Phe[7]. This change in backbone conformation apparently effects the preferred side chain conformations of Trp[8]—Lys[9]. The fact that methylation of the tyrosine hydroxyl group of the biologically active compound 8 leads to reduced biological activity, although the close steric relationship of the lysine and tryptophan side chains is retained in the resulting compound 9, suggests that the proximity of the lysine and tryptophan side chains is not the sole determinant of high biologic activity. The most important suggestion to emerge from these studies is that the biologically active conformation of somatostatin on the receptor controlling insulin

release is not the major conformation of this hormone in solution. Thus, since no upfield shift is observed in somatostatin itself, the conformation having the side chains of lysine and tryptophan in close proximity must make a lesser contribution to the overall population. Because 5 is more potent than 4, the conformation of somatostatin at the receptor regulating insulin release is thought to resemble more closely the solution conformation of compound 5 than of compound 4 or of somatostatin. Although higher potency may be a result of a reduced rate of degradation, the higher potency observed *in vitro* for D-Trp[8] analogs (*4*) along with an absence of increased duration in gastric secretion studies by iv administration (*12*) argue against such an interpretation. Because the structure–activity relationships of somatostatin analogs reported here on insulin release tend to parallel those on glucagon release, the proposals contained in this paper are likely to be relevant also to the conformation at the receptor regulating glucagon release.

The proximity of the two side chains in a D,L dipeptide has been postulated (*6*) as being enhanced by the interaction of a polarizable function in the nonaromatic side chain. We note a striking lack of pH dependence for this upfield shift in compound 2 since the lysine amino group should be protonated at pH 5.9 and unprotonated at pH 12.3. As can be seen in Table 2, the magnitude of the upfield shift is substantially reduced, however, by the addition of an organic solvent such as methanol or when the spectra are taken of the compound in DMSO. Thus, this preferred conformation appears to arise to a considerable degree as a result of a hydrophobic interaction in aqueous medium and is not specifically associated with the amino group of lysine. Furthermore, the greater effect in water compared to organic solvents allows that it may play a role under physiological conditions.

EXPERIMENTAL

Materials

The synthesis of compounds **4** and **5** has been published previously (*1*). The synthesis of compounds **6**, **10**, **11**, **12**, **13**, **14**, **15**, and **16** has also been described (*2*). The synthesis of compounds **7**, **8**, **9**, and **17** will be described in detail elsewhere. The methods employed for synthesis, purification, and characterization of these latter four compounds were analogous to those described previously. In the synthesis of the linear precursor of compound **7**, Phe[7] was attached to the solid support; in the synthesis of compound **8**, Phe[6]; compound **9**, Phe[6]; compound **17**, Phe[11]. The new compounds gave satisfactory amino acid analyses, and were homogeneous in at least two thin-layer systems. The nmr spectra were consistent with the assigned structure.

Nuclear Magnetic Resonance Spectroscopy

The nmr spectra were obtained on a Varian SC-300 spectrometer using D_2O as solvent unless otherwise specified. The samples were obtained by lyophilization from aqueous acetic acid and were then dissolved without adjustment of pD unless specified.

Biological Methods

Analogs of somatostatin were compared to somatostatin in their ability to lower the levels of portal vein insulin in male Sprague–Dawley rats anesthetized with urethane, as described previously (*2*).

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REFERENCES

1. D. F. VEBER, R. G. STRACHAN, S. J. BERGSTRAND, F. W. HOLLY, C. F. HOMNICK, R. HIRSCHMANN, M. TORCHIANA, AND R. SAPERSTEIN, *J. Amer. Chem. Soc.* **98**, 2367 (1976).
2. D. F. VEBER, F. W. HOLLY, W. J. PALEVEDA, R. F. NUTT, S. J. BERGSTRAND, M. TORCHIANA, M. S. GLITZER, R. SAPERSTEIN, AND R. HIRSCHMANN, *Proc. Nat. Acad. Sci. USA* **75**, 2636 (1978).
3. L. A. HOLLADAY AND D. PUETT, *Proc. Nat. Acad. Sci. USA* **73**, 1199 (1976). A refinement of their proposed conformation has also been published: L. A. HOLLADAY, J. RIVIER, AND D. PUETT, *Biochemistry* **16**, 4897 (1977).
4. J. RIVIER, M. BROWN, AND W. VALE, *Biochim. Biophys. Res. Commun.* **65**, 746 (1975).
5. R. CHANDRASEKAREN, A. V. LAKSHMINARAYANAN, U. V. PANDYA, AND G. N. RAMACHANDRAN, *Biochim. Biophys. Acta* **303**, 14 (1973).
6. C. M. DEBER AND H. JOSHUA, *Biopolymers* **11**, 2493 (1972), and H. JOSHUA AND C. M. DEBER, "Chemistry and Biology of Peptides" (H. Meienhofer, Ed.), p. 67. Ann Arbor Science, Ann Arbor, Mich., 1972.
7. R. U. LEMIEUX AND M. A. BARTON, *Canad. J. Chem.* **49**, 767 (1971).
8. F. A. BOVEY AND G. V. D. TIERS, *J. Amer. Chem. Soc.* **81**, 2870 (1959).
9. B. HALPERN, D. E. NITECKI, AND B. WEINSTEIN, *Tetrahedron Lett.* 3075 (1967).
10. S. O. LANDE, *Biopolymers* **7**, 879 (1969); see also A. E. TONELLI, A. I. BREWSTER, AND F. A. BOVEY, *Macromolecules* **3**, 412 (1970).
11. Unpublished observation from these laboratories.
12. M. L. TORCHIANA, P. G. COOK, S. R. WEISE, R. SAPERSTEIN, AND D. F. VEBER (submitted for publication).