Synthesis, in Vitro Opiate Activity, and Intramolecular Tyrosine-Tryptophan Distances of [4-Tryptophan]enkephalin Analogues. A Reassessment of Conformational Models of Enkephalin in Solution

Peter W. Schiller* and Johanne St.-Hilaire

Laboratory of Chemical Biology and Peptide Research, Clinical Research Institute of Montreal, Montreal, Quebec H2W1R7, Canada. *Received September 18, 1979*

A series of [Trp^4]enkephalin analogues designed to prevent the formation of various intramolecular hydrogen bonds was synthesized by the Merrifield method. In vitro opiate activities of [Trp(N$^\alpha$Me)^4,Met^5]enkephalin, [Trp^4,Leu(N$^\alpha$Me)^5]enkephalin, [Trp^4,Pro^5]enkephalin and [Tyr(OMe)^1,Trp^4,Met^5]enkephalin and of their corresponding [Phe^4]analogues were determined with the rat brain receptor assay and the guinea pig ileum assay. The discrepancies between potencies observed in the two assay systems provided further evidence for the existence of several subclasses of opiate receptors. The significant activity obtained with the N$^\alpha$-methylated analogues indicates that hydrogen bonds involving the backbone amino groups in positions 4 or 5 are not required in the biologically active conformation. A conformational study by fluorescence techniques was performed under monomeric conditions in dilute aqueous solution (10^{-5} M). Average intramolecular Tyr–Trp distances were determined by evaluation of singlet–singlet resonance energy transfer on the basis of the Förster equation. Distances ranged from 8.8 to 10.7 Å and are thus comparable to the Tyr–Trp distance observed in [Trp^4,Met^5]enkephalin (9.3 Å). These findings indicate that folded conformations of enkephalin in H_2O may be brought about by solvent interactions and need not be stabilized by intramolecular 4 → 1 or 5 → 2 hydrogen bonds between amino and carbonyl groups of the peptide backbone or between the tyrosine hydroxyl and a backbone carbonyl group. The results are discussed in relation to various proposed solution conformations of enkephalin.

Opiate agonists and the recently discovered opioid peptides [Met^5]enkephalin (H·Tyr-Gly-Gly-Phe-Met·OH) and [Leu^5]enkephalin (H·Tyr-Gly-Gly-Phe-Leu·OH) compete for identical receptor sites and elicit the same pharmacological response. An explanation for this observation may be found in structural similarities between the two classes of compounds. It is thus of considerable interest to determine critical chemical functions in the peptide which might play a role identical with that of corresponding moieties in the rigid framework of morphine and its derivatives. Furthermore, it is relevant to establish the spatial disposition of these critical chemical functions in the peptide by conformational analysis. Early structure–activity studies[1] revealed that the phenol ring and the α-amino group of tyrosine in position 1 of enkephalin may correspond to the phenol ring and the tertiary nitrogen of morphine, even though it has recently been demonstrated[2] that the conformation of the tyramine moiety in the receptor-bound conformation of the peptide is different from that present in the rigid morphine molecule. It has also been shown[3] that residues 4 and 5 must be present for significant activity, and it appears that their correct spatial orientation relative to the tyrosine residue is crucial for an optimal interaction with opiate receptors. According to one proposal,[4] the aromatic ring of the phenylalanine residue in position 4 would perform the same function as the phenylethyl substituent on carbon-19 of the potent morphine derivative 7α-[1-(*R*)-hydroxy-1-methyl-3-phenylpropyl]-6,14-*endo*-ethenotetrahydrooripavine (PEO). The correct spatial disposition of the two aromatic rings in the peptide would be brought about by a 1–4 βI bend which positions the phenol moiety approximately 10 Å apart from the phenyl ring, as it is the case in PEO. Alternatively, the meta and the para positions of phenylalanine in position 4 have been ascribed a role corresponding to that of carbon-5 and -6 of morphine.[5] A unique pharmacophoric conformation of enkephalin satisfying the steric requirements of the latter proposal is characterized by a very short phenol–phenyl intramolecular distance (~5 Å).

Numerous proposals for the conformation of enkephalin in unsolvated form, in the crystalline state, in solution, and in the receptor-bound form have been made and reviewed in a recent article.[6] Proposed hydrogen-bonding schemes include the 1–4[4] and 2–5 βI bend[7-10] and the 1–4 βII′ bend stabilized by a hydrogen bond between the phenolic hydroxyl of Tyr^1 and the carbonyl group of Gly^3 or Phe^4.[11] A β bend with two antiparallel hydrogen bonds between the amino group of Tyr and the carbonyl oxygen of Phe and between the amino nitrogen of Phe and the carbonyl group of Tyr was observed in the crystal structure.[12] Experimental evidence for the 2–5 βI bend was mainly obtained from NMR experiments carried out in H_2O or Me_2SO at high concentrations (10^{-1}–10^{-3} M). However, the interpretation of the latter experiments may be complicated by the occurrence of self-aggregation[9,13] at concentrations above 10^{-4} M. Fluorescence studies with [Trp^4,Met^5]enkephalin in aqueous solution provided no evidence for self-aggregation in the concentration range from 10^{-5} to 5×10^{-7} M, and singlet–singlet energy-transfer experiments carried out at these low concentrations re-

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Table I. Opiate Receptor Affinities and in Vitro Activities of Enkephalin Analogues[a]

no.	analogue	rel receptor affinity, % ([Met⁵]enkephalin = 100%) X = Phe	rel receptor affinity, % ([Met⁵]enkephalin = 100%) X = Trp	rel act. (guinea pig ileum), % ([Met⁵]enkephalin = 100%) X = Phe	rel act. (guinea pig ileum), % ([Met⁵]enkephalin = 100%) X = Trp
I	H·Tyr-Gly-Gly-X-Met·OH	100	129 ± 30	100	27 ± 2
II	H·Tyr-Gly-Gly-X-Leu·OH	38 ± 6	32 ± 7	24 ± 8	10 ± 2
III	H·Tyr-Gly-Gly-X(N$^{\alpha}$Me)·Met·OH	18 ± 4	46 ± 6	18 ± 1	39 ± 18
IV	H·Tyr-Gly-Gly-X-Leu(N$^{\alpha}$Me)·OH	44 ± 6	31 ± 7	70 ± 27	4 ± 0.7
V	H·Tyr-Gly-Gly-X-Pro·OH	6 ± 1	5 ± 0	1 ± 0.2	1 ± 0.1
VI	H·Tyr(OMe)¹·Gly-Gly-X-Met·OH	2 ± 0.4	<1	2 ± 0.4	0.5 ± 0.1

[a] Mean of three determinations plus or minus SEM.

Table II. Fluorescence Quantum Yields and Intramolecular Tyr-Trp Distances in [Tryptophan]enkephalin Analogues

no.	analogue[a]	ϕ_A	ϕ_D^0	E	R_0, Å	r, Å[b]
I	H·Tyr-Gly-Gly-Trp-Met·OH	0.058	0.027	0.70 ± 0.015	10.7	9.3 ± 0.2
II	H·Tyr-Gly-Gly-Trp-Leu·OH	0.057	0.027	0.69 ± 0.01	10.7	9.4 ± 0.1
III	H·Tyr-Gly-Gly-Trp(N$^{\alpha}$Me)-Met·OH	0.050	0.031	0.57 ± 0.04	10.9	10.4 ± 0.3
IV	H·Tyr-Gly-Gly-Trp-Leu(N$^{\alpha}$Me)·OH	0.054	0.029	0.77 ± 0.01	10.8	8.8 ± 0.1
V	H·Tyr-Gly-Gly-Trp-Pro·OH	0.057	0.031	0.64 ± 0.02	10.9	9.9 ± 0.2
VI	H·Tyr(OMe)-Gly-Gly-Trp-Met·OH	0.058	0.038	0.73 ± 0.04	12.6	10.7 ± 0.4

[a] Peptide concentration = 1.0×10^{-5} M; solvent = H_2O. [b] Experimental errors reflect uncertainty in E.

vealed the existence of folded conformations with an average intramolecular Tyr–Trp distance of the order of 10 Å.[1,14] This distance is considerably shorter than the Tyr–Trp distance to be expected in a fully extended conformation (~15 Å) and it is close to that observed between the aromatic rings in PEO and in β-bend models of enkephalin. However, as an alternative to hydrogen bonds, folded conformations of enkephalin could also be stabilized by solvent interactions.

In the present paper, we describe a conformational study by energy-transfer measurements with [4-tryptophan]-enkephalin analogues designed to prevent the formation of the various proposed hydrogen bonds. These compounds include [Trp(N$^{\alpha}$Me)⁴,Met⁵]enkephalin, [Trp⁴,Leu(N$^{\alpha}$Me)⁵]enkephalin, [Trp⁴,Pro⁵]enkephalin, and [Tyr(OMe)¹,Trp⁴,Met⁵]enkephalin.

Results and Discussion

Relative opiate receptor affinities of the [4-tryptophan]enkephalin analogues were determined at 0 °C in order to prevent enzymatic degradation[15] and are listed in Table I. For reasons of comparison, the corresponding [4-phenylalanine]enkephalin analogues are also included. N-Methylation in position 4 of [Met⁵]enkephalin reduces the affinity to one-fifth. In contrast to this result, the same modification in [D-Ala²,Met(0)⁵-ol]enkephalin led to an increase in affinity under somewhat different assay conditions.[16] Interestingly, the corresponding tryptophan analogue, [Trp(N$^{\alpha}$Me)⁴,Met⁵]enkephalin, retained almost half the affinity of [Met⁵]enkephalin (Table I). N-Methylation in position 5 of [Leu⁵]enkephalin and of [Trp⁴,Leu⁵]enkephalin had no influence on affinity. However, low affinity is observed with the analogues containing proline in position 5 in accordance with the weak binding reported for [D-Ala²,Pro⁵]enkephalin.[17] In analogy to the result previously obtained[1] with [Tyr(OMe)¹,Met⁵]enkephalin, [Tyr(OMe)¹,Trp⁴,Met⁵]enkephalin also displayed very low affinity. In general, a good correlation is observed between the affinities of [4-phenylalanine]enkephalin analogues and their corresponding [4-tryptophan]enkephalin analogues.

In the guinea pig ileum bioassay (Table I), the 4-tryptophan analogues of [Met⁵]enkephalin and [Leu⁵]enkephalin display about one-third the activity of the corresponding compounds with phenylalanine in position 4 (compounds I and II). In analogy to the results of the binding studies, [Phe(N$^{\alpha}$Me)⁴,Met⁵]enkephalin shows a fivefold decrease in potency relative to [Met⁵]enkephalin, while the activity of [Trp(N$^{\alpha}$Me)⁴,Met⁵]enkephalin is comparable to that of its parent compound [Trp⁴,Met⁵]-enkephalin. N-Methylation of the leucine residue in position 5 has a divergent effect on the potencies of [Leu⁵]enkephalin (threefold increase) and [Trp⁴,Leu⁵]-enkephalin (2.5-fold decrease). This is in contrast to the binding experiments where the same modification had no effect on affinity in either case. In agreement with the weak affinities obtained in the binding assay, low potencies were determined for the analogues containing *O*-methyltyrosine in position 1 or proline in position 5.

The results obtained in the guinea pig ileum assay reveal significant differences in potency between [Phe⁴]enkephalin analogues and corresponding [Trp⁴]enkephalin analogues (compounds I–IV) in contrast to the parallel behavior which is observed in the binding assay. These discrepancies may be due to the existence of several different subclasses of opiate receptors[18] which display different affinities for a given analogue and whose distribution varies from one assay system to another. The low activity of the [Pro⁵]enkephalin analogues probably indicates that the proline side chain interacts poorly with the receptor either due to its specific structure or because of misalignment. The potency of [Pro⁵]enkephalin analogues is greatly enhanced by introduction of a D-amino acid in position 2 and/or of an ethylamide group into the COOH terminus of the peptide.[19] The latter modification may

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possibly involve improved binding to the receptor through interaction with a new binding site. The observation that analogues with N-methylation in positions 4 and 5 (compounds III and IV) retain significant activity suggests that 4 → 1 and 5 → 2 hydrogen bonds are not required for stabilization of the pharmacophoric conformation.

In order to deal with the peptide analogues in their monomeric form, spectroscopic measurements were carried out in aqueous solution at a concentration of 10^{-5} M.[14] Fluorescence quantum yields and the efficiencies of transfer of excitation energy from the phenol ring (donor) to the indole moiety (acceptor) were determined for the calculation of the intramolecular distances between the two aromatic side chains in positions 1 and 4 of the enkephalin analogues on the basis of the Förster equation.[20] The average intramolecular Tyr–Trp distance, r, of compounds III–VI all lie within the range from 8.8 to 10.7 Å (Table II) and are thus close to the distance determined for [Trp^4,Met^5]enkephalin (r = 9.3 Å). It therefore appears that only relatively subtle conformational differences exist between the latter peptide and compounds III–VI. Since the intramolecular distance constitutes a single conformational parameter, the possible existence of structures with significantly different backbone conformations but with similar Tyr–Trp distances has to be taken into consideration. However, the nearly constant values of the fluorescence quantum yields of tyrosine (ϕ_D^0) and tryptophan (ϕ_A) observed throughout the series of peptides (Table II) provide an argument against the latter possibility. The fluorescence of both tyrosine and tryptophan residues in peptides is quenched by peptide bonds, and this quenching has strict spatial (conformational) requirements.[21,22] Furthermore, tryptophan fluorescence intensity is reduced by positively charged amino groups in a distance-dependent fashion, and such quenching could occur in conformations with the α-amino group in proximity of the indole ring. On the other hand, the tryptophan fluorescence quantum yield could also be enhanced in a conformation which positions the indole ring in a less polar environment. Thus, a 60% increase in quantum yield (ϕ_A = 0.088) and a blue shift (2 nm) of the fluorescence emission maximum have been observed[14] with the analogue [D-Trp^4,Met^5]enkephalin as compared to [L-Trp^4,Met^5]enkephalin (ϕ_A = 0.058), which is indicative of a slightly hydrophobic environment of the indole ring in the former compound. A tryptophan fluorescence quantum yield of similar magnitude (ϕ_A = 0.086) and the same blue shift have recently been described for the C-terminal 7-peptide of cholecystokinin.[23] For these reasons, grossly different backbone conformations would most probably show considerable variation in tyrosine and tryptophan fluorescence quantum yields. Since no such variation is observed, the possibility of significantly different backbone conformations within this series of peptides is unlikely. Finally, the determined constant wavelength of the tryptophan fluorescence maximum at 350 nm indicates a predominantly aqueous environment of the indole ring in all analogues as it is the case with [Trp^4,Met^5]enkephalin.[1]

The average intramolecular distances observed in this series of peptides indicate that folded conformations can be maintained in aqueous solution without stabilization by hydrogen bonds between the amino group of Phe^4 and the carbonyl oxygen of Tyr^1 or between the amino group of the residue in position 5 and the carbonyl moiety of Gly^2. The folded structure observed with [Tyr(OMe)^1,Trp^4,Met^5]enkephalin also excludes the necessity of a hydrogen bond between the tyrosyl hydroxyl and a carbonyl group of the backbone in either position 3 or 4.[11] Previous measurements of the tyrosine fluorescence quantum yield of [Met^5]enkephalin had also indicated that conformations with the phenolic hydroxyl forming a hydrogen bond to a carbonyl group are not predominant in aqueous solution.[24] This result was subsequently confirmed by absorption data.[25] In a more recent fluorescence study,[26] the 10% reduction in the tyrosine fluorescence quantum yield of [Met^5]enkephalin relative to tyrosinamide was taken as evidence for the existence of this type of hydrogen bond in aqueous solution. Since formation of a hydrogen bond between a tyrosyl hydroxyl and a carbonyl group quenches tyrosine fluorescence completely,[22] it might be inferred from the above result that 10% of the conformers contain such a hydrogen bond. However, the same degree of quenching has also been observed[27] in the shorter peptides H·Tyr-Gly·OH and H·Tyr-Gly-Gly·OH and is most likely caused by a number of other factors rather than by intramolecular hydrogen bonding. In any case, intramolecular hydrogen bonds may be unlikely to occur in small peptides because of competition of water molecules and the folded structures we observed may be maintained through other types of intramolecular interactions and/or as a result of solvent interaction. Two antiparallel hydrogen bonds between Tyr^1 and Phe^4 were detected in the crystal structure of [Leu^5]enkephalin.[12] However, it is questionable whether the latter structure is of relevance to the solution conformation, since the crystals used for X-ray analysis contained only one water molecule per enkephalin molecule. Furthermore, it remains unclear whether crystal packing forces can significantly affect the conformation of small peptides.

Whether a single, preferred conformation of enkephalin or an equilibrium of different conformers exists in solution remains an unresolved problem. In the latter case, the measured intramolecular Tyr–Trp distances would represent an average. Arguments both for a unique preferred conformation[7,10] and for an equilibrium situation[13,25] have been presented in the literature.

Experimental Section

Peptide Synthesis. A Beckman Model 990 peptide synthesizer was employed for the synthesis of peptide analogues by the solid-phase technique.[28] The *tert*-butyloxycarbonyl group was used for protection of the α-amino group of all amino acids. *tert*-Butyloxycarbonyl-N^{α}-methylphenylalanine and *tert*-butyloxycarbonyl-N^{α}-methylleucine were synthesized according to the method of Olsen.[29] N^{α}-Methyltryptophan was prepared by following a synthetic route described by Peter et al.,[30] and its *tert*-butyloxycarbonyl derivative was obtained using the method of Schwyzer et al.[31] The preparation of *tert*-butyloxy-

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carbonyl-*O*-methyltyrosine has been described elsewhere.[1] All other *tert*-butyloxycarbonyl amino acids were obtained from Bachem, Inc., Torrance, Calif. The 2,6-dichlorobenzyl group was used for the protection of the phenolic hydroxyl of tyrosine. Reaction of the cesium salts of the protected C-terminal amino acids with chloromethyl resin[32] (Bio-Beads S-X1, 200–400 mesh, 1.25 mequiv of Cl/g) resulted in yields of 0.5–1.0 mmol of *tert*-butyloxycarbonyl amino acid per gram of resin. Peptide chains were assembled by coupling of the *tert*-butyloxycarbonyl amino acids according to a protocol described elsewhere.[1] Deprotection of the α-amino group at each step was achieved by treatment with 25% F_3Ac in $CHCl_3$. In the case of tryptophan-containing peptides, F_3Ac–$CHCl_3$ was replaced by 1 N HCl in AcOH containing 2% mercaptoethanol as deprotecting agent in order to exclude oxidation of the indole moiety. Cleavage and deprotection of the completed peptide chain were achieved by treatment with HF for 1 h at 0 °C with 20 mL of HF and in the presence of 1 mL of anisole and 1 mL of ethyl methyl sulfide as scavengers per gram of resin. An additional 200 mg of skatole per gram of resin was added for the protection of tryptophan-containing peptides. After removal of the HF and following several washings with ethyl acetate, peptides were extracted from the resin with 90% acetic acid and lyophilized.

All peptides were purified by a combination of ion-exchange chromatography and partition chromatography. Crude products dissolved in 1 N acetic acid containing 0.1 N ammonium acetate were deposited on a SP-Sephadex C-25 column which was then eluted with a linear gradient of 0.1–0.4 N ammonium acetate in 1 N AcOH. Further purification was achieved by partition chromatography on a Sephadex G-25 column with the system 1-butanol–acetic acid–H_2O (4:1:5). Final products were obtained as lyophilisates, and their homogeneity was verified by ascending thin-layer chromatography on precoated plates (silica gel G, 250 μm, Analtech, Inc.) in the following systems: (a) 1-butanol–acetic acid–H_2O (BAW) (4:1:5, organic phase), (b) butanol–pyridine–acetic acid–H_2O (BPAW) (15:10:3:12), and (c) *sec*-butyl alcohol–3% ammonium hydroxide (SH) (100:44). A single major spot was detected for all peptides with the exception of the methionine-containing analogues, which showed a second faint spot with a smaller R_f due to the presence of a small amount (<5%) of methionine sulfoxide peptide. Peptides were hydrolyzed (110 °C, 24 h) in 6 N HCl in the presence of 5% thioglycolic acid,[33] and amino acid analyses of the hydrolysates were carried out on a Beckman Model 121C amino acid analyzer equipped with a system AA computing integrator. Runs with standard solutions of the N^{α}-methylated amino acids gave low color factors. The elution times for N^{α}-methylphenylalanine, N^{α}-methyltryptophan, and N^{α}-methylleucine were 38.1, 59.7, and 27.8 min, respectively. The overlap of the N^{α}-methylphenylalanine peak with the methionine peak was circumvented by analysis of an oxidized sample. N^{α}-Methylleucine could not be analyzed because its elution time coincided with that of glycine; however, this had a minimal effect on the determination of glycine because of the very low color factor of N^{α}-methylleucine.

H-Tyr-Gly-Gly-Phe(N^{α}Me)-Met-OH: Amino acid analysis gave Tyr, 1.00; Gly, 2.03; Phe(N^{α}Me), 0.87; Met, 0.94. TLC R_f 0.48 (BAW), 0.64 (BPAW), 0.32 (SH); yield 13%; peptide content 98%.

H-Tyr-Gly-Gly-Trp(N^{α}Me)-Met-OH: Amino acid analysis gave Tyr, 1.00; Gly, 2.03; Trp(N^{α}Me), 0.95; Met, 0.94. TLC R_f 0.52 (BAW), 0.70 (BPAW), 0.29 (SH); yield 6%; peptide content 97%.

H-Tyr-Gly-Gly-Phe-Leu(N^{α}Me)-OH: Amino acid analysis gave Tyr, 0.90; Gly, 2.00; Phe, 0.91. TLC R_f 0.49 (BAW), 0.67 (BPAW), 0.32 (SH); yield 15%, peptide content 91%.

H-Tyr-Gly-Gly-Trp-Leu(N^{α}Me)-OH: Amino acid analysis gave Tyr, 1.02; Gly, 2.00; Trp, 0.96. TLC R_f 0.53 (BAW), 0.69 (BPAW), 0.33 (SH); yield 7%; peptide content 92%.

H-Tyr-Gly-Gly-Phe-Pro-OH: Amino acid analysis gave Tyr, 1.04; Gly, 2.19; Phe, 1.00; Pro, 0.92. TLC R_f 0.40 (BAW), 0.61 (BPAW), 0.29 (SH); yield 52%; peptide content 83%.

H-Tyr-Gly-Gly-Trp-Pro-OH: Amino acid analysis gave Tyr, 1.04; Gly, 2.00; Trp, 1.01; Pro, 0.87. TLC R_f 0.42 (BAW), 0.61 (BPAW), 0.28 (SH); yield 30%; peptide content 86%.

H-Tyr(OMe)-Gly-Gly-Trp-Met-OH: Amino acid analysis gave Tyr, 1.08; Gly, 2.00; Trp, 0.96; Met, 0.94. TLC R_f 0.48 (BAW), 0.62 (BPAW), 0.29 (SH); yield 11%; peptide content 87%.

The syntheses of [Met^5]enkephalin, [Trp^4,Met^5]enkephalin, [Leu^5]enkephalin, [Trp^4,Leu^5]enkephalin, and [Tyr-(OMe)^1,Met^5]enkephalin have been described elsewhere.[1,14]

In Vitro Opiate Activities. Relative opiate receptor affinities were obtained by displacement of [^{3}H]naloxone from rat brain membrane preparations essentially as described by Pasternak et al.[34] In order to minimize enzymatic peptide hydrolysis, incubations were performed at 0 °C. Details of the binding assay have been reported in a previous publication.[14]

The determination of narcotic agonist activities was performed with an assay based on inhibition of electrically evoked contractions of the guinea pig ileum. Single pulses of 4-ms duration were delivered with voltages ranging from 3 to 6 V and isometric contractions were recorded. Further details of the guinea pig ileum assay have been described elsewhere.[35]

Spectroscopic Measurements and Energy-Transfer Experiments. Optical densities were determined with a Beckman Model 25 spectrophotometer. A Hitachi Perkin-Elmer fluorescence spectrophotometer MPF-3L was employed for recording fluorescence emission spectra (uncorrected), whereby a constant temperature of 25 °C was maintained through thermostatting of the cell block. Dilute solutions of peptides (1×10^{-5} M) in H_2O were made up for the measurement of all fluorescence parameters. Literature values[36] of 0.14 and 0.13, respectively, were taken as fluorescence quantum yields, ϕ_{AA}, for L-tyrosine and L-tryptophan in H_2O, which served as standards for determining the quantum yields of tyrosine and tryptophan fluorescence in the peptide analogues at respective excitation wavelengths of 275 and 293 nm. Relative fluorescence intensities were obtained through integration of the spectral envelope, and the quantum yield of the peptide fluorophore, ϕ_{PP}, was calculated with eq 1, where I_{PP} and I_{AA} are

$$\phi_{PP} = \phi_{AA}(I_{PP}A_{AA}/I_{AA}A_{PP}) \quad (1)$$

the fluorescence intensities and A_{PP} and A_{AA} the absorbances of the peptide fluorophore and the standard, respectively.

According to Förster,[20] the efficiency, E, of excitation energy transfer from a donor (tyrosine) to an acceptor (tryptophan) is related to the intramolecular donor–acceptor distance, r, as shown in eq 2, where the Förster critical distance, R_0 (cm), is determined

$$r = (E^{-1} - 1)^{1/6}R_0 \quad (2)$$

experimentally on the basis of eq 3, where κ^2 = dipole–dipole

$$R_0 = [(8.79 \times 10^{-25})(\kappa^2/n^4)\phi_D{}^0 J_{AD}]^{1/6} \quad (3)$$

orientation factor, n = refractive index, $\phi_D{}^0$ = donor fluorescence quantum yield in the absence of transfer, and $J_{AD} = \int_0^{\infty} F_D(\lambda)\epsilon_A(\lambda)\lambda^4 d\lambda$ = spectral overlap integral between the molar decadic absorption coefficient of the acceptor (ϵ_A) and the spectral distribution of the donor fluorescence normalized to unity, F_D, modified by the wavelength factor λ^4. The suitability of energy-transfer experiments for measuring intramolecular distances in biopolymers has been demonstrated extensively,[37,38] and particular aspects of the method related to conformational studies of peptides have been discussed.[39]

Transfer efficiencies were determined from donor fluorescence quenching according to eq 4, where ϕ_D is the donor fluorescence

$$E = 1 - (\phi_D/\phi_D{}^0) \quad (4)$$

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quantum yield in the presence of transfer. ϕ_D^0 was measured with the corresponding [Phe^4]enkephalin analogues. In the computation of R_0, a value of $^2/_3$ was used for κ^2 on the basis of arguments which have recently been outlined in detail.[14] For the Tyr-Trp donor-acceptor pair a value of $J_{AD} = 4.8 \times 10^{-16}$ M^{-1} cm^6 was adopted from the literature,[40] while in the case of the Tyr-(OMe)-Trp pair a performed computation based on the corrected fluorescence emission spectrum of [Tyr(OMe)^1,Met^5]enkephalin and the absorption spectrum of tryptophan resulted in a value of 9.1×10^{-16} M^{-1} cm^6 for the overlap integral.

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Serotonin Receptor Affinities of Psychoactive Phenalkylamine Analogues

Richard A. Glennon,* Stephen M. Liebowitz, and George M. Anderson III

Department of Pharmaceutical Chemistry, School of Pharmacy, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298. *Received September 5, 1979*

Employing a rat fundus model, the serotonin (5-HT) receptor affinities of 45 phenalkylamine analogues were determined. Phenethylamine and phenylisopropylamine possess relatively low receptor affinities; in general, mono-, di-, and trimethoxylation enhance affinity. Of the disubstituted compounds, methoxyl groups at the 2 and 5 positions are optimal for imparting a high affinity. 4-Methylation, 4-ethylation and 4-bromination also enhance receptor affinity, while N,N-dimethylation of the terminal amine decreases affinity. α-Methylation of phenethylamines has little effect on affinity when racemates are examined. Introduction of a benzylic keto group can either increase or decrease affinity, depending upon the presence of other aromatic substituents. The most behaviorally active compounds were found to possess the highest 5-HT receptor affinities, while less active compounds were found to possess lower affinities.

The involvement of serotonergic systems may play a role in the mechanism of action of various hallucinogenic/psychotomimetic agents such as LSD and derivatives of tryptamine and phenalkylamine. Suggestions to this effect are supported by microiontophoretic studies, brain homogenate binding assays, and various biochemical investigations; much of this evidence has been reviewed by Brimblecombe and Pinder[1] and, more recently, by Freedman.[2] Other neurotransmitter systems, e.g., dopaminergic, might also be involved, to varying degrees, in the mechanism of action of these agents;[3,4] however, there is a need to investigate the serotonergic component of these mechanisms in greater detail. Vane initially reported that several phenethylamine and phenylisopropylamine analogues interact in an agonistic manner with 5-HT receptors of the rat stomach fundus preparation and other investigators have since examined the agonistic effects of a number of such compounds using a variety of isolated tissue preparations.[5] In a recent publication from this laboratory, we reported that the more potent hallucinogenic tryptamine and phenalkylamine analogues possess a relatively high affinity for the 5-HT receptors of the isolated rat fundus preparation while the less behaviorally active derivatives possess a lower affinity.[6] In a subsequent publication, we reported the results of a more extensive SAR investigation of a series of tryptamine analogues.[7] We now delineate the results of a detailed examination of the 5-HT receptor affinities of a series of phenalkylamine analogues.

Chemistry. While biological data have been previously reported for the 2,4-dimethoxy compound **16**, no melting point or microanalytical data could be found in the literature. Compound **16** was prepared by the catalytic hydrogenation of the 2-aminopropiophenone **43** to a diastereomeric mixture of 2-aminopropanols **43a**; continued reduction of the latter in the presence of $HClO_4$ gave the desired product. Alternatively, condensation of 2,4-dimethoxybenzaldehyde (**16a**) with $EtNO_2$ afforded the nitropropene **16b**, which could be reduced to **16** with $LiAlH_4$.

Elbs persulfate oxidation of 2-hydroxy-3-methoxybenzaldehyde, followed by methylation of the resultant hydroquinone, gave 2,3,5-trimethoxybenzaldehyde. The aldehyde was condensed with $EtNO_2$ and the nitropropene reduced with $LiAlH_4$ to yield the desired 2,3,5-trimethoxy compound **28**.

Compound **33** was prepared in a manner similar to that reported by Shulgin,[8] i.e., $LiAlH_4$ reduction of 1-(3,4,5-trimethoxyphenyl)-2-nitropropene (**33a**). However, in an initial attempt to prepare **33a**, a benzene solution of 3,4,5-trimethoxybenzaldehyde was allowed to react with $EtNO_2$ and NH_4OAc under refluxing conditions; in addition to **33a**, a white crystalline product, **33b**, was isolated. Compound **33b** was assigned the following structure based on NMR, ^{13}C NMR, and mass spectra. Additional support for the presence of an imino group is derived from the acid-catalyzed hydrolysis of **33b** to the parent 3,4,5-trimethoxybenzaldehyde and the corresponding amine **33c**. It is speculated that the desired nitropropene **33a** was formed and then underwent Michael attack to yield **33b**. A survey of the literature reveals that a similar reaction

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