A similar structural analogy may be drawn between the standard hypotensive drugs hydralazine and catapres and the synthesized compounds **1** and **2**. Both hydralazine and catapres have the common group -N=CNH- as against >NCNH- in compounds **1** and **2**. Apparently the substitution -N= by a bridgehead nitrogen in our compounds did not retard or alter the bioactivity.

Experimental Section

All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were taken in $CDCl_3$ on a JEOL MH-100 spectrometer using Me_4Si as internal standard. Mass spectra were recorded on LKB Model 9000. Ir spectra were obtained in KBr on a Beckman IR-12.

Synthesis of the Nitroso Intermediates. General Method. 3-Nitroso-2-phenylimidazo[1,2-*a*]pyridine and 5-nitroso-6-phenylimidazo[2,1-*b*]thiazole were obtained by a combination of methods described by Almirante et al.[5,6] and LaRocca et al.[7] ω-Bromoacetophenone was condensed respectively with 2-aminopyridine and 2-aminothiazole. The condensation products were suspended in 10% NaOH solution, extracted with $CHCl_3$, and evaporated to dryness to obtain the respective free bases. These free bases were finally dissolved in acetic acid and nitrosated with sodium nitrite solution. 3-Nitroso-2-phenylimidazo[1,2-*a*]pyridine after one recrystallization from ethanol had mp 165–167 °C and 5-nitroso-6-phenylimidazo[2,1-*b*]thiazole after one recrystallization from acetone had mp 175–177 °C.

Pyridino[1,2-*a*]imidazo[5,4-*b*]indole (**1**). A mixture of 9.0 g of analytically pure 3-nitroso-2-phenylimidazo[1,2-*a*]pyridine (0.04 mol) and 10 ml of 97% triethyl phosphite (0.05 mol) in 50 ml of anhydrous toluene was refluxed for 15–30 min with stirring and under a constant flow of dry nitrogen gas. The temperature of the oil bath was kept between 110 and 120 °C. After cooling, the solvent and excess triethyl phosphite were removed by vacuum distillation at 0.2 Torr. The temperature of the oil bath was kept under 120 °C also during the distillation. The residue which was a thick oily liquid was kept overnight at 0 °C during which time it solidified. The solid was washed on a glass filter with cold CCl_4. The residue was taken in a small quantity of $CHCl_3$ and was eluted over a column of activated alumina (80–325 mesh) presoaked with CCl_4. $CHCl_3$ was used as eluent and the first colored zone was collected and evaporated to dryness. The residue after one recrystallization from 2-propanol had mp 78–80 °C; crude yield ~4 g; ir 3410, 3080, and 2580 cm^{-1}. Anal. ($C_{13}H_9N_3$) C, H, N.

Thiazolo[3,2-*a*]imidazo[5,4-*b*]indole (**2**). About 8.5 g of analytically pure 5-nitroso-6-phenylimidazo[2,1-*b*]thiazole (0.04 mol) was taken with 10 ml of triethyl phosphite and 50 ml of toluene and was refluxed for 3 h with stirring and under constant flow of nitrogen. The rest of the procedure was the same as above. The product after one recrystallization from 2-propanol had mp 102–104 °C; crude yield ~3 g; ir 3120, 3080, and 1560 cm^{-1}. Anal. ($C_{11}H_7N_3S$) C, H, N, S.

3-Amino-2-phenylimidazo[1,2-*a*]pyridine (**3**). The procedure was the same as for compound **1** except that the reflux was continued for 3 h. The product had mp 212–214 °C after recrystallization from ethanol; crude yield ~3 g; ir 3340, 3080, and 1560 cm^{-1}; mmp (with a synthesized sample of 3-amino-2-phenylimidazo[1,2-*a*]pyridine) 212–214 °C. Anal. ($C_{13}H_{11}N_3$) C, H, N.

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Communications to the Editor

A Circular Dichroism Study of the Interaction of Sodium and Potassium Ions with Methionine-Enkephalin[1]

Sir:

Several laboratories have shown that a group of peptides found in brain tissue[2-4] and the pituitary gland[5,6] possesses binding characteristics and biological activity similar to that of morphine.[2-12] One of these substances is the pentapeptide, Tyr-Gly-Gly-Phe-Met, known as methionine-enkephalin (Met-enkephalin).[12] As the stereospecific binding of Met-enkephalin to rat-brain homogenate is known to be reduced in the presence of Na^+,[6,9] we have investigated the possibility that the reduced affinity might in part be related to a sodium-induced conformational change of the ligand due to complexation. In this communication we report on the interaction of Na^+ and K^+ with Met-enkephalin using circular dichroism (CD) as a means of detecting conformational changes.

The use of CD to assess oligopeptide conformations in solution is based mainly on empirical comparisons of standard curves obtained from the model peptide, poly-L-lysine.[13] We have extended the use of this model using an indirect approach[14] based on the assumption that the aromatic side chain and carbonyl contributions to the CD spectrum of Met-enkephalin are additive[15] and that aromatic contributions are relatively constant[16] in different conformational states of the peptide chain. Under such conditions conformational changes of the peptide chain can be detected by difference curves.[17]

The CD spectrum of Met-enkephalin in water is characterized by a group of ^1L_b aromatic transitions of weak positive ellipticities from 290 to 254 nm (Figure 1). An inflection at 225 nm and a peak at 218 nm (Figure 2) fall in the diagnostic area of the tyrosine and phenylalanine residues.[18] An additional band is detected at 212 nm and has been tentatively assigned to the terminal carboxylate on the basis of its position and the increasing ellipticity

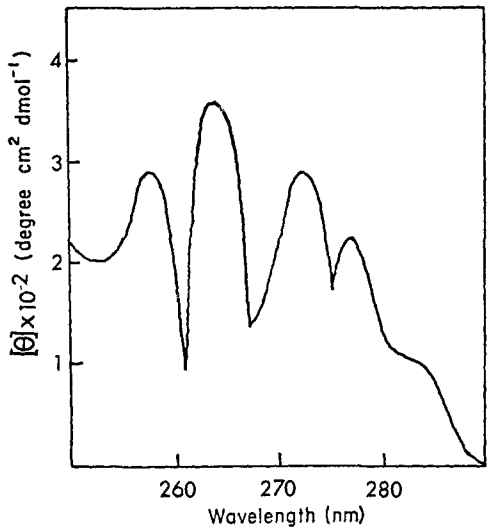

Figure 1. Circular dichroism spectrum of Met-enkephalin (1×10^{-3} g/ml, 1.79×10^{-3} M) in water at 25 °C.

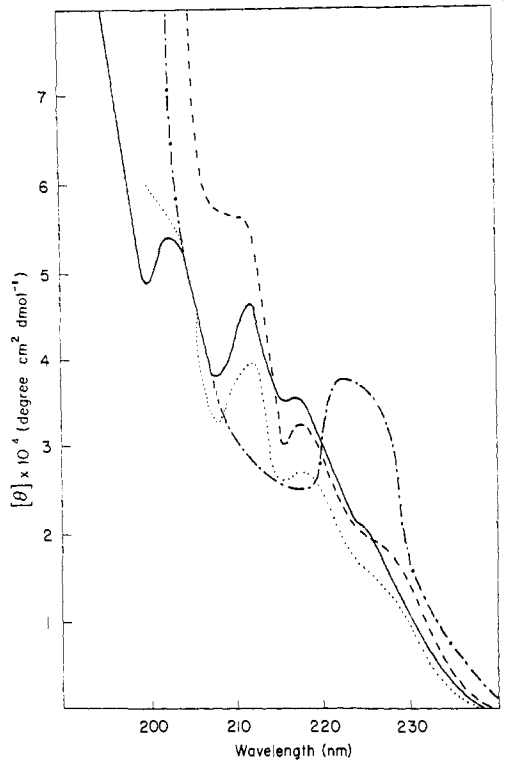

Figure 2. Circular dichroism spectra of Met-enkephalin (7.2×10^{-5} g/ml, 1.28×10^{-4} M) in water (—), in 100 mM NaCl (· · ·), and in 100 mM KCl (- - -) at 25 °C. Additive contribution of *N*-acetyl-L-tyrosinamide and *N*-acetyl-L-phenylalaninamide (-·-) in water.

under the effect of protonation.[19] A band at 202 nm has also been observed, superimposed on the positive background expected for a small peptide with a high content of aromatic side-chain chromophore.[20]

Comparison of the CD curve obtained from the additive contributions of *N*-acetyl-L-phenylalaninamide and *N*-acetyl-L-tyrosinamide[21] and the experimental curve of Met-enkephalin in water (Figure 2) affords evidence (on the basis of lower ellipticity at 225 nm[22] for the latter curve) that the pentapeptide may retain some organization in water. This receives support from the sign of the ^{1}Lb transition; *N*-acetyl-L-phenylalaninamide in dioxane shows positive ellipticity for the ^{1}Lb transition, although in water

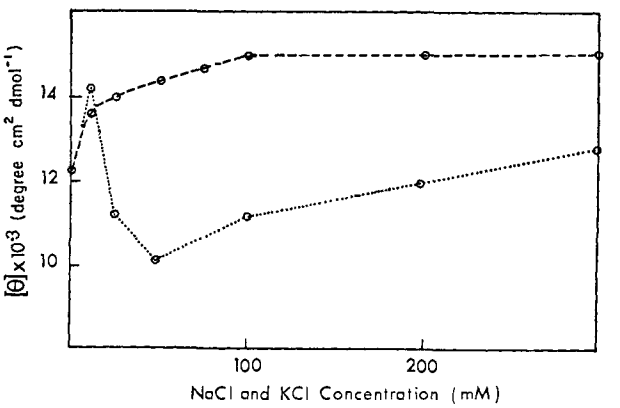

Figure 3. Molar ellipticity at 230 nm (25 °C) of Met-enkephalin (1×10^{-4} g/ml, 1.79×10^{-4} M) vs. NaCl (· · ·) and KCl (- - -) concentration.

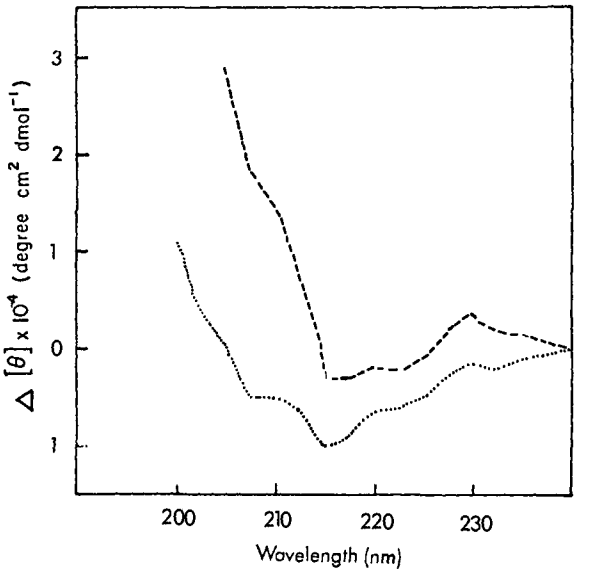

Figure 4. Circular dichroism difference curve produced by Met-enkephalin (7.2×10^{-5} g/ml, 1.28×10^{-4} M) in 100 mM NaCl (· · ·) and 100 nM KCl (- - -) referred to water to 25 °C.

it is negative.[21] As the sign of the ^{1}Lb transition of Met-enkephalin in water is positive, this could mean that the aromatic side chains are held partially in a hydrophobic surrounding.

A striking difference in the interaction of sodium ion with the pentapeptide compared to potassium is exhibited by the CD curves of Met-enkephalin in the presence of NaCl or KCl. These ions affect the intensities of the bands rather than the positions. As no salt effect is detected for the ^{1}Lb transition,[23] this supports the assumption concerning the constancy of the aromatic contribution. Accordingly, the modifications of the CD spectra can be attributed to a conformational change of the peptide network as the result of metal–peptide complexation involving chelation of the carbonyls by metal ions.[24] Monitoring of the ellipticity at 230 nm with respect to electrolyte concentration (Figure 3) reveals the complex stoichiometry[24] and emphasizes the dramatically different behavior of NaCl from that of KCl. Difference curves (Figure 4) show qualitative agreement with a conformational change to a "β-like" structure.[25,26]

As the dissociation constants (pK_a's) 3.55 (COOH), 7.2 (NH_3^+), and 10.4 (phenolic OH) are in the normal range, these groups do not appear to be shielded or involved in electrostatic interaction with other moieties in the enkephalin molecule.

It can be noted that while the interaction of Na^+ with Met-enkephalin appears to be weak, the large excess of Na^+ relative to the peptide suggests that under physiologic conditions a substantial fraction of this ligand might be in the coordinated form. Moreover, the difference in conformational behavior of Met-enkephalin in the presence of Na^+ and K^+ suggests that this interaction is of a specific nature under physiologic conditions and not attributable to a general salt effect. Since Na^+ and K^+ induce different conformational changes, it is possible that this may in part be related to the specific effect of Na^+ in reducing receptor binding of Met-enkephalin to rat brain homogenate.[6,9]

In light of these results it is conceivable that the reduction in potency of Met-ankephalin caused by Na^+ is mediated in part through a conformational change of this peptide via the formation of a coordination complex possessing reduced receptor affinity. There is considerable precedent for such complexation among cyclic peptides[24] and it is thus not unreasonable to expect a similar type of coordination for Met-enkephalin.

This study also draws attention to the possibility that endogenous Met-enkephalin in brain homogenate preparations might give rise to greater binding of naloxone in the presence of Na^+ as a consequence of reduced receptor affinity of the Na^+-coordinated peptide. Under such conditions agonist ligands would appear to possess reduced affinity.

Finally, the present study illustrates the complexity of the opiate receptor system, as the "sodium response ratio"[27] may reflect conformational changes in opiate receptors, conformational changes of enkephalin, or both.

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