Biophysical Chemistry 14 (1981) 217–220
Elsevier/North-Holland Biomedical Press

EFFECT OF MORPHINE ANALOGUES ON THE CONFORMATIONAL STATE OF POLYRIBOADENYLIC ACID IN SOLUTION:

AN NMR STUDY

Ida Barni COMPARINI

Institute of Forensic Medicine, University of Siena, Via Laterina 8, 53100 Siena, Italy

Elena GAGGELLI and Gianni VALENSIN *

Institute of General Chemistry, University of Siena, Pian dei Mantellini 44, 53100 Siena, Italy

Received 18 February 1981
Revised manuscript received 2 June 1981

The interaction of morphine analogues with polyadenylic acid in aqueous solution at pH 7 has been investigated. The NMR spectra measured at several temperatures were shown to give evidence of a lowering of the transition temperature from the helix to the random-coil structure. The measured chemical shifts and relaxation times were interpreted in terms of an ionic interaction between the $^{+}N-CH_3$ group of the drug and the phosphate residue of the polynucleotide.

1. Introduction

The narcotic drugs have common structural features, their action is strictly stereospecific and structurally related competitive antagonists are able to counteract the narcotic action; it is therefore generally believed that the pharmacological effect stems from the interaction with specific receptors located in the central nervous system.

As a consequence, many efforts [1–6] have been dedicated to identifying specific sites for the action of morphine opiates and to isolating and characterizing the drug receptors, but the results obtained thus far have not been conclusive.

The relevance of studying the interaction between opiates and nucleic acids is underlined by the following evidence:

(i) brain nuclei and mitochondria can accumulate morphine in vitro [7];

(ii) chronic morphine treatment increases the chromatin template activity in tolerant mice [8];

(iii) binding of morphine to calf thymus DNA was demonstrated with chromatographic techniques [9].

In this report we have investigated the interaction between morphine analogues and polyriboadenylic acid (polyA) as a model for the binding of the drug to DNA. In fact, the conformation of polyA in aqueous solution is subject to many factors, including pH and temperature; a sharp transition from a single-strand helix to a random-coil structure is observed by raising the temperature at pH 6 [10,11]. Since this transition can be suitably detected by changes in the NMR spectrum [12,13], we have performed experiments on monitoring changes in the NMR parameters of polyA to evaluate the effects of opiates on the helix stability.

2. Materials and methods

The potassium salt of polyA, from Sigma Chemical Co., was used without further purification. A polyA concentration of 8.72 mM (P) was used for all measurements. The concentration was

* To whom correspondence should be addressed.

0301-4622/81/0000–0000/$02.50

determined using a molar residue extinction coefficient of 1.01×10^4 at pH 7.0, 257 nm. Heroin and morphine hydrochlorides were purified and crystallized in the Institute of Forensic Medicine, University of Siena. All the solutions were in D_2O 99.75% (Merck) and contained 10 mM 3-(Trimethylsilyl)propanesulfonic acid sodium salt as internal standard for the measurements of chemical shift. The pH was adjusted with DCl or NaOD [14]. Heroin and morphine were added to 0.5 ml of the polyA solution as microamounts to minimize the dilution effect.

Proton NMR spectra were obtained in the Fourier Transform mode at 90 MHz using a Bruker WH 90 spectrometer equipped with a Nicolet BNC-12 computer. The temperature was controlled to ± 1°C with a standard Bruker unit for temperature control. The intensity was measured by dividing the amplitude of the peak by the amplitude of the water peak, which was constant throughout the experiments. The spin-lattice relaxation times T_1 were determined with the 180°-τ-90°-t pulse sequence with t at least 6-times the longest measured T_1. A two-parameter nonlinear regression analysis was used to obtain T_1 from the single exponential decay functions, using the peak resonance intensities.

3. Results and discussion

NMR spectra were registered at temperatures in the range 301–353 K for polyA in aqueous solution at pH 7.0 in the absence and presence of different amounts of heroin and morphine. The spectra obtained at 301, 313 and 353 K are reported in fig. 1. It is apparent that the peaks in the polyA spectrum are broadened and diminished in intensity below 313 K. Upfield shifts are observed for the H-8, H-2 and H-1′ protons arising from base stacking as a consequence of lowering the temperature. These findings were interpreted [12,13] in terms of an organized structure having stacked bases (single-strand helix) in polyA in aqueous solution in agreement with other indications from ultraviolet spectroscopy [15,16]. The change in intensity suggests that 'structured' regions are coexisting with 'disorganized' zones and that the two conformations are characterized by very different degrees of motional freedom of the base protons. Using the intensity of the water peak as unit and measuring the intensities of either the base or the ribose protons, the 'melting' of the helix structure occurs in the range 295–325 K. It is consequent that polyA in aqueous solution at pH 7 does provide a suitable model for studying the effect of drugs on the helix structure in nucleic acids.

The spectra reported in fig. 1 for polyA with 1 mM heroin and 1.2 mM morphine demonstrate

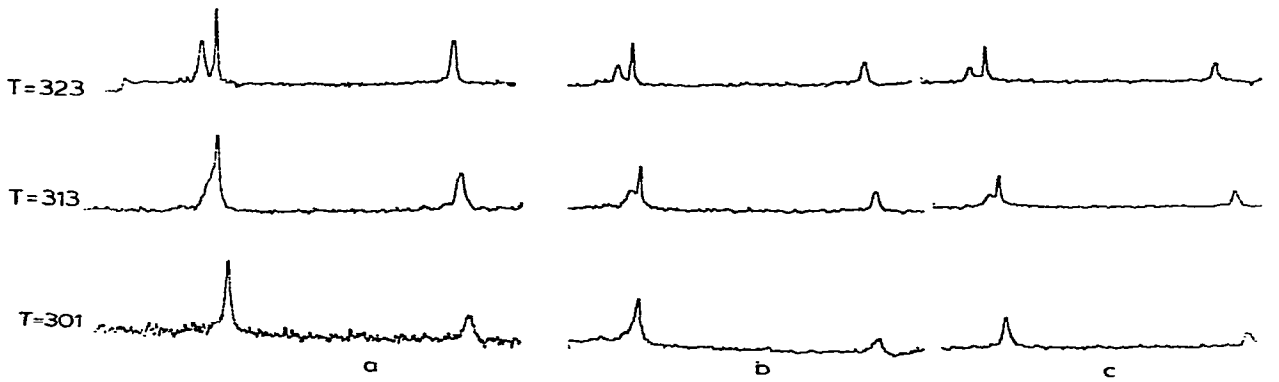

Fig. 1. Low-field region of the 90 MHz ^{1}H-NMR spectrum of polyA in D_2O at pD=7.0 without (a) and with 1 mM heroin (b) or 1 mM morphine (c). The concentration of polyA was 8.72×10^{-3} M (P).

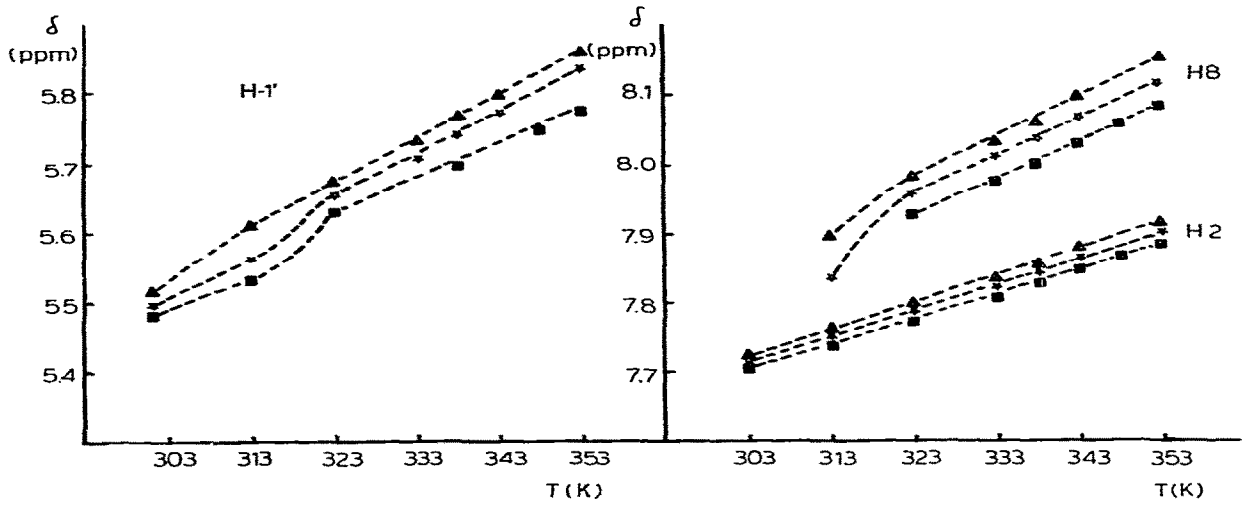

Fig. 2. Temperature dependence of the chemical shift for the H-1' (left) and H-8 and H-2 (right) protons in polyA solutions in D_2O at $pD = 7.0$. (■) polyA; (★) polyA + heroin or morphine (1 mM); (▲) polyA + heroin or morphine (10 mM). The concentration of polyA was 8.72×10^{-3} M (P).

that the drugs shift the helix-coil equilibrium to the right, since the onset of spectra typical of the 'disorganized' structure occurs at lower temperatures. To elucidate this interaction we have performed measurements of the chemical shift and of the spin-lattice relaxation time T_1.

The effect of the drug upon the chemical shift is reported in fig. 2 for both the H-1' (left) and H-8 and H-2 peaks (right). Heroin or morphine was added up to a final concentration of 1 or 10 mM. A small downfield shift was observed in every case for all the three protons, but no difference was shown by the two opiates. The observed shift was concentration dependent, suggesting the involvement of a drug-induced effect. Since the formation of stacked adducts is expected to cause upfield shifts of about 0.2 ppm [17,18], the measured downfield shifts are pointing to a small rearrangement of the electronic environments rather than to a disruption of pairwise-stacked entities.

The chemical shift data of fig. 2 allow also further considerations about the effect of morphine opiates upon the helix-coil transition. Namely, it is obvious that the separation of the H-8 and H-2 peaks occurs at lower temperatures but it is also evident from fig. 2 that a dependence on the drug concentration does exist. At 313 K the peak separation is larger in the presence of 10 mM opiate than in 1 mM opiate. Moreover, it is worth noting that the temperature dependence of the chemical shift of the H-1' peak for polyA in aqueous solution undergoes an inflection point near 318 K, which has to be related to the helix-coil transition. As the H-2 peak does not display the same trend, it may be suggested that the 'structuring' process involves the deoxyribose rings more than the adenine moieties. The inflection point is shifted to lower temperatures in the presence of drug molecules, again demonstrating the weakening of the helix structure.

Table 1 summarizes the measured T_1 values at 353 K. The recovery of the longitudinal magnetizations was found to be characterized by a single exponential. The relaxation mechanism is provided by the intra- and intermolecular ^1H-^1H dipolar interaction [19] modulated by local internal motions rather than by rotational motion of the whole polyA molecule ($\tau_R \simeq 10^{-5}-10^{-6}$ s) or by the rate of the helix-coil conformational change ($\tau_{ex} \simeq 10^{-6}-10^{-7}$ s) [20]. The internal motion correlation time was found to be in the range 0.3–0.5 ns at room temperature, as determined by ^1H, ^{31}P

Table 1

Proton spin-lattice relaxation times [a] for polyA at 353 K and pD=7.0

Proton	T_1 (s)		
	PolyA	PolyA + 1 mM heroin	PolyA + 1 mM morphine
H-8	0.43 ± 0.01	0.29 ± 0.01	0.31 ± 0.02
H-2	1.21 ± 0.03	0.87 ± 0.02	0.88 ± 0.02
H-1′	0.58 ± 0.01	0.40 ± 0.01	0.38 ± 0.01

[a] The ± figures denote approximate 95% confidence limits (≈two standard deviations) as determined by computer analysis. The concentration of polyA ws 8.72 mM (P).

and ^{13}C spin-lattice relaxation times [19,21]. As a consequence, the equal T_1 shortening observed for H-8 and H-2 in the presence of either heroin or morphine can arise from a longer $\tau_c(\omega_o\tau_c < 1)$, as well as from a change in the interaction responsible for the relaxation. In the case of polyA both factors could be effective. The H-2 and H-8 protons are relatively isolated (especially H-2) and their relaxation could be quickened by the approaching of protons from the drug molecule: alternatively, the presence of the drug can reduce the effectiveness of internal motions in modulating the dipolar interaction.

As the intercalation of aromatic moieties into the nucleic acid helix is expected to result in big changes in the chemical shift of the protons involved and specific binding is expected to produce different effects on the T_1 of protons in different parts of the molecule, the NMR behavior shown by polyA in the presence of opiates is consistent with a steric hindrance on the base protons from the drug molecules. These consideration and the similar effects given by heroin and morphine suggest that the $N-CH_3$ group of the opiate, which is almost fully protonated at pH 7.0 (pK = 8.7), is responsible for the drug action through ionic interaction with the phosphate groups of the polynucleotide. This mode of action is like that of many metal ions [10,11] which are also known to cause 'melting' of the helix structures.

References

[1] E.J. Simon, J.M. Hiller and I. Edelman, Proc. Natl. Acad. Sci. U.S.A. 70 (1973) 1947.
[2] L. Terenius, Acta Pharmac. Tox. 32 (1973) 317.
[3] C.B. Pert and S.H. Snyder, Science 179 (1973) 1011.
[4] D.L. Wong and J.S. Horng, Life Sci. 13 (1973) 1543.
[5] L.I. Lowney, K. Schulz, P.J. Lowery and A. Goldstein, Science 183 (1974) 748.
[6] H.H. Loh, T.M. Cho, Y.C. Wu and E.L. Way, Life Sci. 14 (1974) 2231.
[7] S. Navon and A. Lajtha, Brain Res. 24 (1970) 534.
[8] N.M. Lee, I.K. Ho and H.H. Loh, Biochem. Pharmacol. 24 (1975) 1983.
[9] N.M. Lee and H.H. Loh, Biochem. Pharmacol. 24 (1975) 1249.
[10] Y.A. Shin, J.M. Heim and G.L. Eichhorn, Bioinorg. Chem. 1 (1972) 149.
[11] D. Pörschke, Biophys. Chem. 4 (1976) 383.
[12] C.C. McDonald, W.D. Phillips and S. Penman, Science 144 (1964) 1234.
[13] J.P. McTague, V. Ross and J.H. Gibbs, Biopolymers 2 (1964) 163.
[14] P.K. Glasoe and F.A. Long, J. Phys. Chem. 64 (1960) 188.
[15] K.E. Van Holde, J. Brahms and A.M. Michelson, J. Mol. Biol. 12 (1965) 726.
[16] J. Brahms, A.M. Michelson and K.E. Van Holde, J. Mol. Biol. 15 (1966) 467.
[17] P.O.P. Ts'o, N.S. Kondo, P.M. Schweizer and D.P. Hollis, Biochemistry 8 (1969) 997.
[18] T.R. Krugh, E.S. Mooberry and Y.C. Chen Chiao, Biochemistry 16 (1977) 740.
[19] K. Akasaka, Biopolymers 13 (1974) 2273.
[20] D. Pörschke, Eur. J. Biochem. 39 (1973) 117.
[21] P.H. Bolton and T.L. James, J. Phys. Chem. 83 (1979) 3359.