J. Pharm. Pharmacol. 1984, 36: 758–760
Communicated May 18, 1984

© 1984 J. Pharm. Pharmacol.

COMMUNICATIONS

Nmr study of theophylline-ethylenediamine interaction in aqueous solution

ZEEV TASHMA, *Department of Pharmaceutical Chemistry, The Hebrew University School of Pharmacy, P.O. Box 12065, Jerusalem, Israel*

The behaviour of the H(8) nmr signal of theophylline in D_2O solutions containing various amounts of ethylenediamine, indicates that in no case is the latter doubly charged, and so at an ethylenediamine to theophylline molar ratio of 0·5 (corresponding to that found in aminophylline) about half of the theophylline is present in a non-ionic form. At higher ratios an increase in its degree of ionization occurs, as evident from the continued upfield shift of the H(8) signal. The behaviour of the *N*-methyl signals differed from that of H(8). As the proportion of ethylenediamine increased from zero to 1·2 times that of theophylline, these signals first shifted upfield, but then moved back to approximately their initial positions. It is suggested that at low ratios, including that in aminophylline, the solution is composed of monocations of ethylenediamine paired to negatively charged aggregates of theophylline, in which both neutral molecules and ions co-exist. The ions thus act as solubilizing agents for the neutral, and usually slightly soluble, form of theophylline. At high ethylenediamine to theophylline ratios, the latter is fully ionized and the aggregates disintegrate.

Many organic bases form solid complexes with theophylline, which are far more water soluble than the drug itself. While the structure of some of these complexes in the solid state has been investigated (Nishijo et al 1982a, b), their high solubility is poorly understood.

As theophylline is soluble in alkali because of its acidic NH function, sufficient amounts of an amine should also effect its solubility by forming salts with it. This, however, does not fully explain the high solubility of the aminophylline complex (theophylline : ethylenediamine, 2 : 1). While aminophylline is in the solid state, both nitrogen atoms of ethylenediamine are considered to be protonated (e.g. Swinyard 1980); when aminophylline is in aqueous solution our calculations indicate that it must be regarded as a *mono*valent salt of ethylenediamine in which only half of the theophylline is ionized.

In order to calculate the percent to which a salt of a weak acid (AcH) and of a weak base (B) undergoes decomposition in aqueous solution to the free acid and base, $Ac^- + BH^+ \rightleftarrows AcH + B$, the following equation (Vogel 1961) can be used:

$$\frac{x^2}{(1-x)^2} = \frac{K_w}{K_a K_b}$$

In this equation x represents the fraction of the salt decomposed to the free acid and base, K_w stands for the ionic product of water which is 10^{14} at 20 °C, K_a is the acidity constant of the weak acid ($10^{-8.50}$ for theophylline according to Lichtenberg & Bergmann 1971) and K_b is the basicity constant of the weak base $10^{(14-9.93)}$ for the first ionization of ethylenediamine, $10^{(14-6.85)}$ for the second, using its pK_a values as quoted by Perrin 1965).

The values of x (0·16 and 0·87) mean that in about 84% of ethylenediamine only one centre is protonated with a further 13% of the molecules carrying a second positive charge. Thus, in solutions of aminophylline the average positive charge is about one per molecule of ethylenediamine; about 50% of theophylline is therefore present in the neutral form. A concentrated aqueous solution of aminophylline, which is nearly 20% w/v, will thus contain almost 10% of non-ionized theophylline; this far exceeds the solubility of theophylline, which is about 1% w/v.

Towards a better understanding of the solubilization phenomenon, an nmr study was made on theophylline solutions in deuterium oxide containing in different amounts one of the three bases ethylenediamine, aminoethanol or 2-methyl-2-aminopropanol, or sodium hydroxide. Both aminoethanol and 2-methyl-2-aminopropanol are also known to greatly increase the solubility of theophylline when used in a 1 : 1 molar ratio.

Materials and methods

Nmr spectra were measured on a 300 MHz instrument (Bruker WH 300) with sodium 3-trimethylsilyl propionate as an internal standard.

Materials. Theophylline AR (Fluka) was dried for 2 h under vacuum at 100 °C before use. Ethylenediamine, aminoethanol and 2-methyl-2-aminopropanol (Fluka) were kept over KOH and distilled before use. Deuterium oxide was Uvasol 99·7%, Merck. Concentrated NaOH solution, which gives a 1 M solution upon dilution to 500 ml, was purchased from BDH.

Methods. Theophylline (5–6 mg) was accurately weighed in nmr tubes and dissolved in sufficient deuterium oxide to produce 0·8% solutions (Solutions A). About 600 mg of ethylenediamine or aminoethanol, and about

900 mg of 2-methyl-2-aminopropanol were accurately weighed and dissolved in 5 ml deuterium oxide (Solutions B). Aliquots (1 ml) of each of solutions B were further diluted with D_2O to 5 ml (Solutions C).

Measurements were first made on solution A, which was subsequently mixed with calculated amounts of the amine solutions B or C, as required, to give the needed range of concentrations for each amine.

For the titration with sodium hydroxide, a 5 M solution of the base, in water containing about 50% of D_2O, was prepared by diluting the content of the NaOH ampoule with D_2O to a total volume of 100 ml. Titration of theophylline with sodium hydroxide was by stepwise addition of 2 µl quantities of this 5 M solution to 1·5 ml 0·8% solution of theophylline in D_2O. The nmr spectrum was recorded after each addition.

Care was taken to keep the concentration of theophylline almost constant. The maximum increase in volume caused by the added solution was only about 6%, and under these conditions the influence of dilution upon the location of the peaks was negligible. For comparison, a 20% dilution with pure D_2O resulted in a downfield shift of only about 0·01 ppm in the location of the protons of theophylline.

Table 1. Nmr data for 0·8% aqueous solution of theophylline in D_2O containing various amounts of ethylenediamine or sodium hydroxide.

Molar ratio of base to theophylline	Location of the signals in ppm, relative to TSP-Na			
	$CH_3(1)$	$CH_3(3)$	H(8)	NCH_2CH_2N
	Ethylenediamine			
0·00	3·324	3·505	7·955	—
0·15	3·312	3·482	7·885	3·025
0·30	3·300	3·467	7·782	3·023
0·40	3·296	3·464	7·731	3·012
0·50	3·305	3·469	7·695	3·002
0·65	3·316	3·479	7·659	2·991
0·80	3·325	3·488	7·621	2·970
1·00	3·335	3·508	7·580	2·950
1·20	3·340	3·511	7·568	2·884
2·20	3·340	3·510	7·559	2·791
	Sodium hydroxide			
0·0	3·322	3·505	7·955	
0·22	3·301	3·473	7·881	
0·34	3·296	3·464	7·781	
0·45	3·299	3·469	7·710	
0·56	3·313	3·475	7·680	
0·78	3·320	3·485	7·628	
1·12	3·341	3·508	7·565	
1·35	3·343	3·511	7·559	

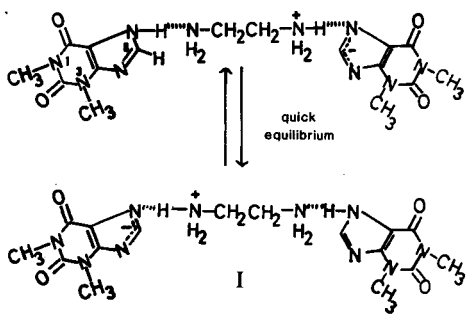
Representative data from the experiments using ethylenediamine and sodium hydroxide are in Table 1. The assignment of the theophylline signals follows that of Lichtenberg & Bergmann (1971) and of Thakkar et al (1971). The results obtained with the other bases were not significantly different and showed the same pattern.

Results and discussion

The H(8) signal shifted consistently to a higher field in a typical sigmoidal fashion as titration proceeded, approaching a plateau only when the molar ratio of the base, including the seemingly dibasic amine ethylenediamine, to theophylline is 1 : 1. This clearly shows that in the reaction between theophylline and ethylenediamine only one centre of the latter is ionized. The same conclusion follows from the behaviour of the methylenic protons of ethylenediamine. Upon increasing its relative concentration from 0·1 to 0·9, the location of the CH_2 signal remained practically the same (3 ppm), thus indicating that the species present at 1 : 1 ratio (monocation) is present also at lower amine : theophylline ratios. At relative concentrations of ethylenediamine greater than 0·9, a gradual shift of the signal to a higher field was observed, in agreement with an increasing proportion of its non-ionized form in the mixture.

The shifts observed for the *N*-methyl signal of theophylline, though much smaller in magnitude than those found for H(8), are more instructive in understanding the real nature of aminophylline in solution.

Table 1 shows an initial upfield shift in the *N*-methyl signals, that continued until an ethylenediamine: theophylline molar ratio of 0·4 was reached. At higher ratios, however, the signals shifted back to a lower field, ending at values lower than those found for pure samples of theophylline. As the molar ratio giving the highest upfield shift is not far from the molar ratio present in aminophylline (0·5), it was first assumed that, at this concentration, a species of type I, in which a single unit charge was divided between two theophylline molecules, predominated, the upfield shift of the *N*-methyl protons being ascribed to the negative charge.



I

However, there seems no reason why the fully ionized theophylline (with signals recorded at amine to theophylline ratios of 1·2 or higher) should exhibit the methyl signals at *lower* fields than the partially ionized form, I. Moreover, it was eventually shown that the same phenomenon occurred also when aminoethanol, 2-methyl-2-aminopropanol or even NaOH was used instead of ethylenediamine, Clearly, in such cases analogues of I cannot be entertained.

As described by Thakkar et al (1971), molecules of theophylline, like those of other xanthines, are self-associated in aqueous solution in a plane-to-plane, or vertical, stacking. In such an arrangement the protons of each molecule are under the shielding region

associated with the carbonyl groups of the adjacent xanthine molecule, and consequently their nmr signals are shifted to higher fields. Disintegration of stacked aggregates of xanthines, including theophylline, caused by dilution, has been decribed by Thakkar et al (1971) to be accompanied by a downfield shift of *N*-methyl signals.

Therefore it is suggested that two opposing factors operate in alkaline solutions of theophylline, namely the inductive effect of the negative charge which causes the upfield shift, and the 'dispersing' effect of the negative charge which leads to a downfield shift. Of these, the second effect is predominant only when about a third of theophylline is ionized. This would suggest that initially theophylline aggregates ionize without disintegration. Only when about one in every three theophylline molecules is ionized do the electrical repulsion forces become strong enough to effect a gradual disintegration of the stacks, an effect that is completed at an amine : theophylline molar ratio of 1·2. When theophylline is partially ionized, neutral molecules are solubilized by the anions which are present in the same stacks.

In conclusion, it seems that the phenomenon of ethylenediamine reacting and crystallizing with two molecules of theophylline to give aminophylline, presumably the double salt, is limited to the solid phase only. In aqueous solution, calculations based on pK_a values suggest that the stoichiometry of the reaction is only 1 : 1, and so a solution of aminophylline contains about 50% of neutral theophylline. Nmr data also failed to show the existence of any specific structure at the ratio found in aminophylline. On the other hand, the nmr data strongly suggest that such partially neutralized solutions of theophylline are composed of mixed aggregates in which both neutral molecules and ions co-exist. This arrangement is probably the key factor in its enhanced solubility in the presence of partial amounts of ethylenediamine, and it seems to be related more to the effect of pH and less to any specific effect of the counter-ion.

REFERENCES

Lichtenberg, D., Bergmann, F. (1971) J. Chem. Soc. (C) 1676–1682

Nishijo, J., Ohno, K., Nishimura, K. (1982a) Chem. Pharm. Bull. 30: 391–397

Nishijo, J., Ohno, K., Nishimura, K. (1982b) Ibid. 30: 2219–2222

Perrin, D. D. (1965) in: Dissociation Constants of Organic Bases in Aqueous Solution, Butterworths, London, p. 27

Swinyard, E. A. (1980) in: A. Osol (Ed) Remington's Pharmaceutical Sciences, 16th edn, Mack Publishing Company, Easton, Pennsylvania, p. 810

Thakkar, A. L., Tensemeyer, L. G., Wilham, W. L. (1971) J. Pharm. Sci. 60: 1267–1269

Vogel, A. I. (1961) in: Quantitative Inorganic Analysis, 3rd edn, Longmans, Green and Co., London, p. 32

J. Pharm. Pharmacol. 1984, 36: 760–762
Communicated April 4, 1984

© 1984 J. Pharm. Pharmacol.

Anti-inflammatory action of drugs that raise adenosine-3′,5′-cyclic monophosphate and putrescine levels in-vivo

S. Mohd Hidir, D. A. Lewis*, *Pharmacological Laboratories, Department of Pharmacy, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET, UK*

Isoprenaline and salbutamol, two β-adrenoceptor agonist drugs, and 3-isobutyl-methyl xanthine and thioacetamide have been shown to be anti-inflammatory. Their mode of action is probably due to an increase in the levels of the three endogenous substances adenosine-3′,5′-cyclic monophosphate and putrescine and spermidine in-vivo.

Putrescine and, to a lesser extent, spermidine are anti-inflammatory against carregeenan-induced oedema in the rat footpad, and putrescine is anti-inflammatory against adjuvant-induced arthritis in the rat (Bird et al 1983). Putrescine, spermidine and spermine are also anti-inflammatory against 5-hydroxytryptamine pad oedema in mice as well as against carrageenan oedema in the rat (Oyanagui 1984). Since the levels of these endogenous oligoamines are controlled by ornithine decarboxylase (ODC), drugs that raise adenosine 3,5′-cyclic monophosphate (cAMP) may be anti-inflammatory since cAMP stimulates ODC activity (Beck et al 1972). cAMP is also anti-inflammatory in its own right (Zurier et al 1973). We have previously reported that theophylline, which raises both cAMP and putrescine levels, is anti-inflammatory (Bird et al 1983), and combinations of theophylline and prostaglandin, E_1 and aminophylline with salbutamol are also anti-inflammatory (Bonta et al 1978; Seo & Saeki 1980). We have now examined two β-adrenoceptor agonists, isoprenaline and salbutamol, for anti-inflammatory activity and related this effect to cAMP and putrecine levels. We have also examined the xanthine derivative 3-isobutyl-1-methyl xanthine for anti-inflammatory activity and examined the blocking action of propranolol on the anti-inflammatory action of salbutamol. Thioacetamide has also been reported to stimulate ODC activity and this compound was investigated for anti-inflammatory activity.

* Correspondence.