NMR INVESTIGATION OF HISTAMINE-PHOSPHOLIPID INTERACTION

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Summary

Dispersions of histamine and phosphatidyl-L-serine, phosphatidyl-ethanolamine, and phosphatidylcholine were investigated at acidic and alkaline pD values using nmr. A specific interaction between histamine and phosphatidyl-L-serine was observed in the acidic pD range and involved only the imidazole protons of histamine. No interactions between histamine and the other phospholipids were observed.

That histamine initiates its activity by interaction with the cell membrane appears to be firmly established in the case of smooth muscle (1). Uptake of histamine by other tissues presumably also requires interaction of histamine with cell membrane components. Since phospholipids are among the major elements of cell membranes, it was of interest to determine if histamine could interact in a specific manner with phospholipids and to examine the nature of the interaction. Similar interactions in various sytems of biological interest have been widely investigated using nmr spectroscopy (2-5); we have used this technique to study the interaction of histamine with three major phospholipids and have found a specific interaction between phosphatidyl-L-serine and histamine involving the imidazole protons of the latter.

Experimental Section

Spectra were obtained on a Varian T-60 spectrometer (probe temperature 35°, sweep time 250 sec., sweep width 500 Hz). A Beckman Expandomatic pH meter was used for pH and pD measurements, and a Heat-Systems-Ultrasonic Inc. W140D sonicator was employed

for preparing phospholipid dispersions. All pH measurements were corrected to pD values by the method of Glasoe and Long (6). Chemicals used were phosphatidyl-L-serine (92% pure-Nutritional Biochemicals Corp.), phosphatidylethanolamine (97% pure-β, γ-dipalmito-L-α-kephalin Fluka), phosphatidylcholine (97% pure-β, γ-dipalmito-α-lecithin Fluka), D_2O and $CDCl_3$ (both of 99.8% isotopic purity, Wilmad Glass Co.), histamine•2HCl (Calbiochem) and anhydrous Na_2CO_3.

Preparations of Solutions and Dispersions

Phosphatidyl-L-serine (20-100 mg) was dispersed in 0.8 ml D_2O and sonicated at a setting of 3 for 10 min (7). To this dispersion histamine•2HCl (10 mg) was added to give a solution of 0.06775 M histamine and 2.5-12.5 mg% phosphatidyl-L-serine. For spectra run in acidic media, half of this solution was used directly. Spectra in alkaline media were obtained on solutions titrated to the desired pH with anhydrous Na_2CO_3.

Phosphatidylcholine solutions were obtained by dissolving the desired quantity of compound in a minimum amount of $CDCl_3$, evaporating in vacuo to leave a thin oil-film of the phospholipid (7), adding D_2O (0.8 ml), and sonicating the mixture as described for phosphatidyl-L-serine. This technique was unnecessary with phosphatidyl-L-serine or phosphatidylethanolamine as identical spectra were obtained whether they were first dissolved in $CDCl_3$ or not. Phosphatidylcholine dispersions, however, did not provide acceptable spectra unless the phospholipid was first dissolved in $CDCl_3$.

Alkaline solutions of phosphatidylethanolamine were prepared in the same manner as described for phosphatidyl-L-serine. Acidic solutions were impossible to obtain due to the inability of the phosphatidylethanolamine to remain in highly dispersed form in the presence of histamine•2HCl.

To determine the composition of the precipitate formed when

histamine•2HCl was added to dispersed phosphatidylethanolamine in attempting to make an acid solution (pD 3.5), the precipitate was centrifuged, and a spectrum of the supernate showed only the presence of histamine. The pellet was resuspended and washed twice with a solution of DCl-D_2O (pD 2.5). After the final wash, D_2O was added, the pellet resuspended, and Na_2CO_3 added to titrate the suspension to neutrality, dissolving the pellet in the process. All solvent was evaporated in vacuo, leaving a white solid which was treated with a 1:1 mixture of CD_3OD and d_6-acetone (both of 99.8% isotopic purity; Wilmad Glass Co.) to dissolve the organic portion of the solid. The spectrum of this solution indicated that only phosphatidylethanolamine was present. The remaining portion of the solid was dissolved in D_2O and produced a weak spectrum of phosphatidylethanolamine only.

Results and Discussion

Change in line width at half peak height ($\Delta\gamma_{\frac{1}{2}}$) is related to the spin-spin relaxation rate by the expression $1/T_2$ (sec^{-1}) = $\pi\Delta\gamma_{\frac{1}{2}}$ (8). Figure 1 indicates change in spin-spin relaxation ($1/T_2$) as a function of phosphatidyl-L-serine concentration for the two imidazole protons of histamine, but no change was seen for the methylene peaks. This change was seen only in the spectra run at pD 3.5-4.5; no changes were observed at pD 9.5-10.5.

In contrast, no interactions of this type were observed between histamine and phosphatidylcholine at either pD 3.5-4.5 or pD 9.5-10.5, or between histamine and phosphatidylethanolamine at pD 9.5-10.5 as indicated by changes in $1/T_2$ value for the histamine peaks.

For observation of this type interaction the spin-spin relaxation rate for both bound and free forms of histamine must be slower than the exchange rate between bound and unbound histamine. This

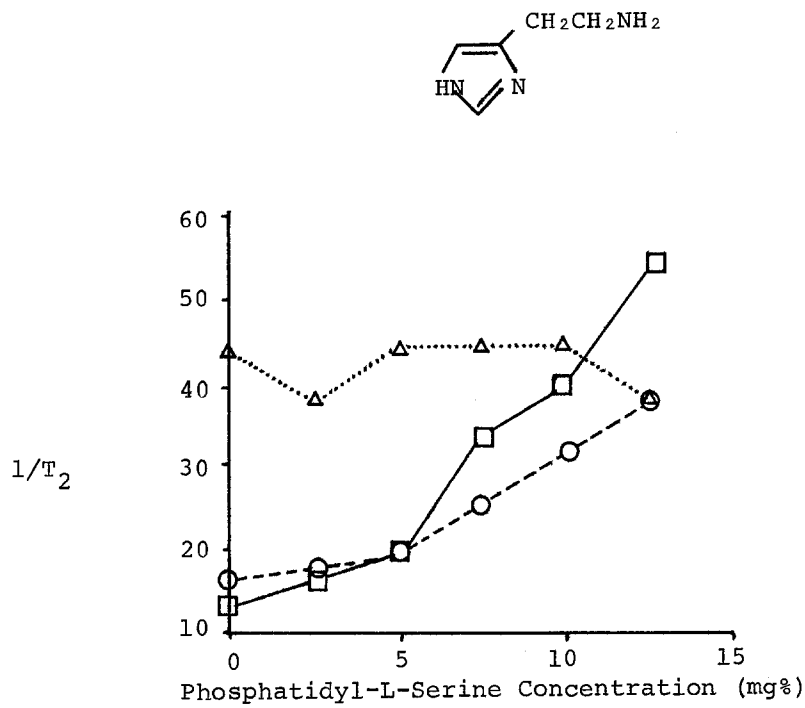

Figure 1: Relaxation rates ($1/T_2$) of histamine protons (6,775 x 10^{-2} M) as a function of phosphatidyl-L-serine concentration: (-□-) aromatic peak at 9.0 δ; (--0--) aromatic peak at 7.9 δ; (----Δ----) methylene peak at 3.8 δ.

allows contribution to the spectrum from both forms, thus observation of the broadened peak, in contrast to the case where one or both of the free and bound forms have a more rapid relaxation rate than the rate of exchange between forms (8).

A number of non-specific sources of line broadening were excluded as possible origins of the observed effects. Presence of paramagnetic impurities in the solutions could alter the observed $1/T_2$ values; however, no change in $1/T_2$ of the solvent (HOD) signal was noted throughout the experiment indicating lack of or constant contribution of this factor (8). Change in viscosity of the dispersion with increasing phospholipid concentration might change the histamine $1/T_2$ values observed. Again the constant solvent line width, coupled with the observation that phosphatidylcholine

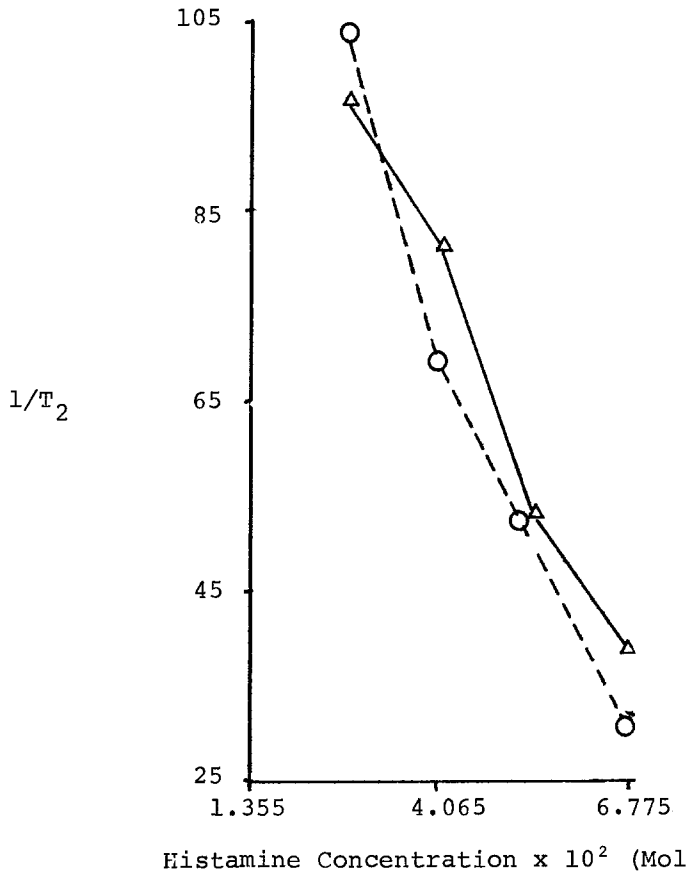

Figure 2: Relaxation rates ($1/T_2$) of histamine aromatic protons as a function of histamine concentration of constant (10 mg%) phosphatidyl-L-serine concentration: (— Δ —) aromatic peak at 9.0 δ; (--0--) aromatic peak at 7.9 δ.

dispersions appeared even more viscous than those of phosphatidyl-L-serine without showing line width changes, indicate that viscosity of the dispersions may be ruled out. Variation in the internuclear distances within the histamine molecule, which in a non-rigid system might affect the relaxation rate (from the relation $1/T_2 = C\tau_c \sum \frac{1}{\langle r_{ij} \rangle^6}$ (8) are non-existent in the aromatic portion of the histamine molecule. The protons which were observed to have line width changes were the aromatic protons, thus rendering changes in internuclear distances impossible. Finally, the possibility of histamine-histamine interactions can be ruled out since

increasing concentrations of histamine at a constant phosphatidyl-L-serine concentration resulted in decreasing $1/T_2$ values for the histamine aromatic protons (Figure 2). If histamine-histamine interactions were the source of the observed line width changes, the likelihood of such interactions would increase with increasing concentrations of histamine, causing increases in line width rather than the decreases actually observed.

Hauser et al (9) reported precipitation of procaine or tetracaine with phosphatidylserine and concluded that the precipitate was a complex of the anesthetic and lipid molecules. Their examination of the nmr spectra of non-precipitating mixtures further revealed an interaction between phosphatidylserine and the anesthetics. In contrast, our results indicated no interaction between histamine and phosphatidylethanolamine, and the precipitate which formed from solutions containing these two substances consisted only of phosphatidylethanolamine. Thus, precipitation of phosphatidylethanolamine in acidic media in the presence of histamine appears to be due simply to an alteration in the composition of the solution in such a manner that it is unable to accommodate the phosphatidylethanolamine dispersion.

The specific interaction between the imidazole protons of histamine and phosphatidyl-L-serine should be considered in light of recent ideas concerning biologically active forms of histamine. Ganellin (10) has pointed out the possible importance of the imidazole ring in generating histaminic activity and has further suggested that if agonist conformations of histamine are able to discern between H_1 and H_2 receptors, then the differentiating factor is rotation of the imidazole ring. Thus, our finding that only the aromatic imidazole protons of histamine are involved in interaction with phosphatidyl-L-serine is consistent with the concept

of a special role for the imidazole ring in generation of histaminic activity.

Acknowledgments

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