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The solubilization of some local anaesthetic esters of *p*-aminobenzoic acid by lysophosphatidylcholine

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The solubilization by lysophosphatidylcholine (LPC) of three n-alkyl esters of *p*-aminobenzoic acid has been studied. These esters have a local anaesthetic action. Quantitative studies show that the amount of compound solubilized is proportional to the LPC concentration and that solubilization increases in the order ethyl, n-propyl and n-butyl ester. 100MHz nmr studies indicate that the local anaesthetic esters are solubilized in the hydrocarbon interior of the LPC.

The solubilizing effects of lysophosphatidylcholine (LPC) have been studied by several workers. Rousseau & Pascal (1938) observed that LPC had a solvent effect on certain portions of the streptococcus cell, and Hasegawa & Nakamoto (1939) showed that LPC dissolved live but not dead pneumococci. Webster (1957) showed that LPC had a clearing action on aqueous homogenates of whole rat brain and that it also solubilizes homogenates of kidney and liver and muscle tissue.

Because of its surface-active properties, LPC can increase the solution rate of a drug by decreasing the interfacial energy barrier between the drug and solution medium, thereby increasing wetting of the drug. It can also increase the apparent solubility and rate of solution by micellar solubilization. The solution rate of salicylic acid powder and aspirin from the tablet form has been shown to be markedly increased by LPC. It has also been shown to increase both the rate of solution and apparent solubility of water insoluble drugs at physiological concentrations (Bates, Lin & Gibaldi, 1967; Lin, Menig & Lachman, 1968).

A high concentration of LPC has been observed in the duodenal region of the small intestine (Borgstrom, 1957) and micellar absorption of fatty acids from the gut may be facilitated by LPC (Lennox, Lough & Garton, 1968). LPC has been shown to assist in the solubilization of fatty acids by bile salts (Scott & Lough, 1969), stearic acid being preferentially solubilized. The presence of LPC in the small intestine, together with its solubilizing properties has led Bates & others (1967) to the suggestion that LPC may play a role in the absorption of insoluble drugs from the small intestine.

Thus LPC has been shown to have a solubilizing action on complex biological systems, but its actions in these instances may be very different from its actions on the pure components of these systems. Studies on simple systems have been more limited. Robinson & Saunders (1959) have examined the solubilizing action of LPC on cholesterol, tri-olein and monostearin. All three compounds were solubilized to a large extent, monostearin being the most solubilized, forming a viscous gel with LPC. Kellaway & Saunders (1969) showed that LPC haemolysis of red blood cells was inhibited by PC, progesterone cholesterol and tri-olein. LPC has been shown by Saunders (1957) to interact with phosphatidylcholine to form viscous sols which are resistant to precipitation by salts.

In this paper studies of the solubilization of three n-alkyl esters of *p*-aminobenzoic acid by LPC are reported. These compounds have a local anaesthetic action.

MATERIALS AND METHODS

Materials

Preparation of LPC. Phospholipids were extracted from fresh egg yolks according to the method described by Singleton, Gray & others (1965). Phosphatidylcholine was extracted from the egg yolk extract by column chromatography using alumina and LPC was prepared from this eluate by Saunders' modification of the method used by Hanahan, Rodbell & Turner (1954). This method utilizes phospholipase A from viper venom (Saunders, 1957). The purity of the LPC was checked by thin layer chromatography. N and P determinations gave N; 2·72, P; 5·8%, corresponding to a molecular weight of 525. Analysis by gas-liquid chromatography of the fatty acids obtained on hydrolysis of samples of the LPC gave: saturated (palmitic and stearic) 92·1%; mono unsaturated (oleic) 6·1%, others (chiefly linoleic) 1·8%.

The ethyl, n-propyl and n-butyl esters of *p*-aminominobenzoic acid were laboratory reagent grade, re-crystallized from diethyl ether or ethanol before use.

D_2O (99·7%) was obtained from Koch-Light Laboratories Limited.

Methods

Quantitative measurements. An LPC sol of known concentration (approximately 40 mM) was made up in water. This sol was then diluted to give 3 ml samples of approximate concentration 5, 10, 20, 30 and 40 mM. An excess, 50 mg, of ester was added and the mixture shaken at room temperature (23–25°) for up to 72 h. The mixture was then centrifuged at 27 000 *g* for 30 min to sediment any non-solubilized solid. An aliquot of the supernatant was removed, weighed and diluted with a known volume of 95% ethanol to give an ultraviolet absorbance in the range 0·5–1·5. The amount of solubilized material was estimated at the wavelength of maximum absorbance (λ max). A Unicam SP3000 automatic spectrophotometer was used in the analysis. Measurements were made on the solubilization by LPC of the esters in purified water (pH 5·5), and also at pH 4–4·5 and 8–8·5. The pH was altered by the addition of a small amount of 0·1M NaOH or HCl, the effect on the concentration of the LPC sols being small. The solubility of each ester was determined by shaking a small portion of the solid in water for 48 h, centrifuging off the undissolved solid and measuring the absorbance in the same way as for the solubilized material.

Nmr measurements

Nmr spectra were obtained with the PCMU Varian HA 100, 100 MHz nmr spectrometer using a field sweep. The temperature was ambient and a sweep time of 500 s was used. TMS was used as an external reference. The spectra of LPC sols in D_2O and sols containing varying amounts of ester were examined.

RESULTS

Quantitative measurements

Samples of solubilized material were examined after various periods of shaking and it was found that a maximum solubilization occurred after 24 h. All mixtures were

sampled after shaking for 48 h. The LPC sols containing solubilized ester were quite stable, showing no tendency to become cloudy or precipitate even when the amount of solubilized material was at, or near, the maximum. Solubilization of the esters caused an increase in viscosity of the LPC sols. This effect was more pronounced the longer the n-alkyl chain attached to the ester group on the local anaesthetic.

Fig. 1 shows the total concentration of ethyl *p*-aminobenzoate dispersed plotted against LPC concentration. For each pH value a straight line was obtained, passing through a point on the vertical axis corresponding to the solubility of the compound, at zero LPC concentration. Similar graphs were obtained from all three compounds examined.

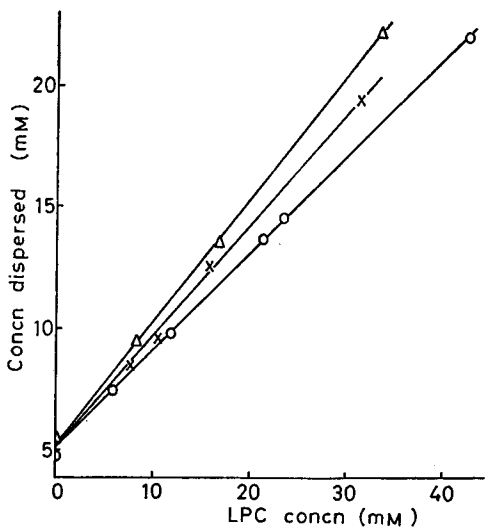

FIG. 1. Plot of LPC concentration against total concentration of ethyl *p*-aminobenzoate dispersed.

Table 1 shows the number of moles of ester solubilized per mole of LPC. In these results the solubility in water has been subtracted from the total amount dispersed to give the amount solubilized per mole of LPC.

Nmr measurements

The 100 MHz spectrum of a 40 mM LPC sol is shown in Fig. 2, with assignments, and is in reasonable agreement with that observed by Chapman & Morrison (1966).

Table 1. *Moles of local anaesthetic ester solubilized per mole of LPC in the range of LPC concentration* 0–40 mM

pH	Ethyl *p*-aminobenzoate	n-Propyl *p*-aminobenzoate	n-Butyl *p*-aminobenzoate
4–4·5	0·501	0·567	0·472
5·5	0·406	0·427	0·569
8–8·5	0·462	0·496	0·427
Solubility in water mM	4·84	2·22	1·03

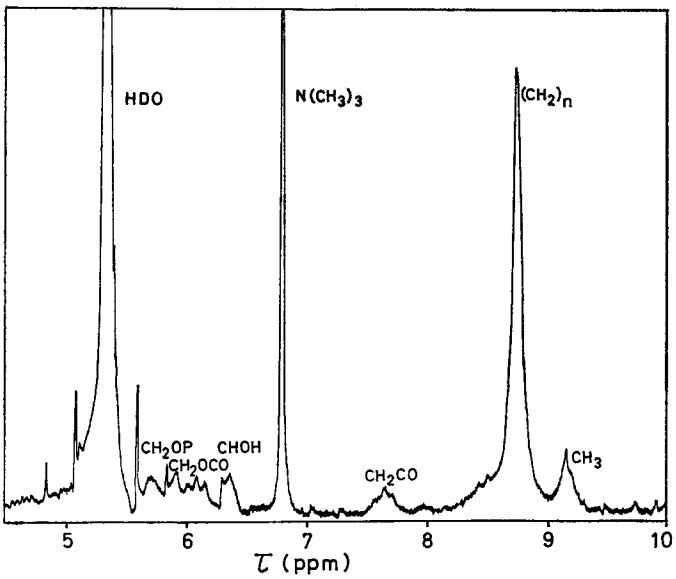

FIG. 2. 100 MHz spectrum of a 40 mM LPC sol.

There are two major peaks, one due to the hydrocarbon chains at τ 8·75, and one due to the three CH_3 groups on the choline part of the molecule at τ 6·8. The spectrum also shows a peak at about τ 6·3 due to >CHOH. This shows the compound is all α-acyl LPC as if the β-compound were present there would be a peak at τ 4·8 due to >CHOCOR (Chapman & Morrison 1966). The spectrum also shows a low degree of unsaturation in the hydrocarbon chains due to a lack of absorption at τ 4·7 caused by –CH=CH–. Spectra run at 20 mM LPC concentration and 200 mM LPC concentration showed no difference in chemical shift and no different peaks from Fig. 2. The nmr spectrum of 20 mM LPC sol, however, was rather noisy and several of the peaks were poorly defined.

It was found that the esters, when solubilized by LPC sols, caused considerable broadening of the peak at τ 8·75 due to the hydrocarbon chain on the LPC molecule, and the peak due to the terminal methyl group almost disappeared. The peak at τ 6·8 due to the CH_3 groups on the choline part of the molecule was not affected. As the concentration of local anaesthetic was increased, this broadening of the hydrocarbon chain peak became more pronounced and peaks appeared due to the ester but were too small to observe broadening. No change in the chemical shift was produced by addition of ester. The spectrum of a 40 mM LPC sol with 12 mM n-propyl *p*-aminobenzoate added (0·308 mol of ester per mol of LPC) is shown in Fig. 3.

Fig. 4 shows the effect of the esters on the width at half height of the peak due to the LPC hydrocarbon chains. For a given molar ratio of ester to LPC, the longer the n-alkyl chain in the ester, the greater the line broadening.

The addition of ester to LPC sols produced a broadening of the small peaks due to the LPC ester groups. Comparison of Figs 2 and 3 showed that the addition of 12 mM n-propyl p-aminobenzoate to a 40 mM LPC sol had broadened the signal from CH_2OCO so that it merged into the signal from CHOP, and the CH_2CO signal was not visible. The signal due to CHOH was not affected.

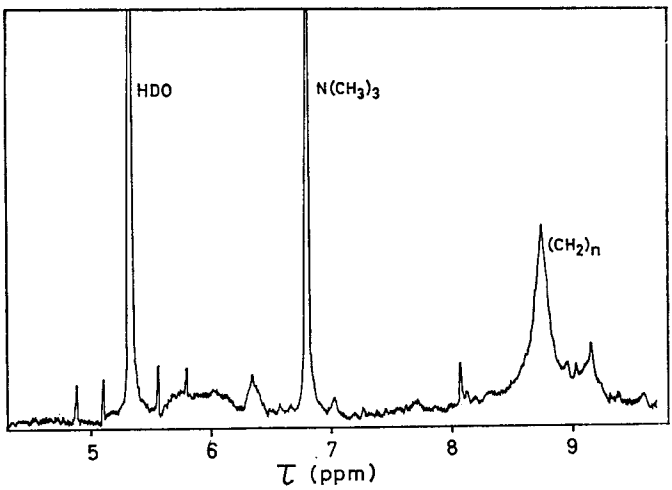

FIG. 3. Spectrum of a 40 mM LPC sol with 12 mM n-propyl *p*-aminobenzoate added.

DISCUSSION

The solubility in water of the three esters examined decreased as the length of the n-alkyl chain was increased. The amount solubilized, however, increased with increasing length of the n-alkyl chain (at pH 5·5) due to an increase in the affinity of the hydrophobic part of the molecule for the hydrocarbon interior of the LPC micelle.

For the ethyl and n-propyl esters, increasing the pH from 5·5 to 8–8·5, or decreasing the pH to 4–4·5 increased the amount solubilized. These changes in pH produced a decrease in solubilization of the n-butyl ester, possibly due to the formation of large aggregates containing LPC and n-butyl ester.

The solubility of the esters in water varied very little with the pH changes investigated. The pKa of the most soluble, ethyl *p*-aminobenzoate is reported by Richardson & Meakin (1974) as 2·57, which would give a low degree of ionization even at pH 4–4·5.

The micellar weight of LPC was measured using the light scattering technique, and the following results obtained (Hunt, 1972): 0·1 acetate buffer (pH 4·75) $1{\cdot}41 \times 10^5$,

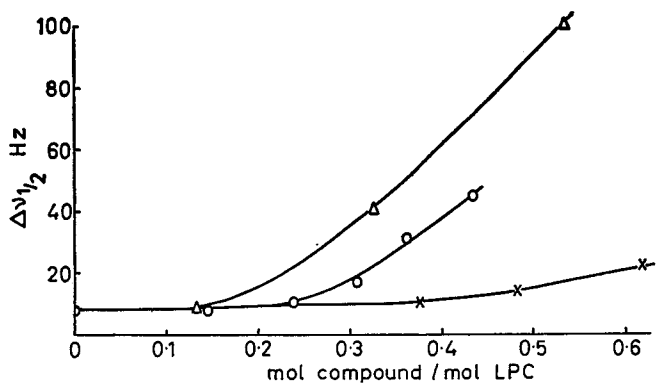

FIG. 4. Plot of width at half height ($\Delta\nu_{\frac{1}{2}}$) of the nmr peak due to the hydrocarbon chains of 40 mM LPC sols against ratio of ester to LPC.

purified water (pH 5·5) $1{\cdot}37 \times 10^5$, 0·1M phosphate buffer (pH 7·40) $1{\cdot}24 \times 10^5$. These results do not explain the changes in solubilization of the esters with pH.

The three alkyl esters of *p*-aminobenzoic acid, when solubilized by LPC sols, produced a broadening of the nmr peak arising from the methylene groups comprising the LPC hydrocarbon chain. This was due to a restriction of molecular motion in the hydrocarbon chains of the LPC, resulting in a faster relaxation rate. The fact that the peak from the hydrocarbon chain was considerably broadened, but that from the methyl groups on the choline part of the LPC molecule was not, indicated that the ester was solubilized in the hydrophobic interior of the micelles. The nmr peaks due to CH_2CO and CH_2OCO were also broadened, indicating a restriction of molecular motion in the ester linkage of the LPC.

Fig. 4 shows that the longer the hydrocarbon chain attached to the ester, the greater the line broadening effect. The quantitative measurements showed that the longer this hydrocarbon chain, the greater was the maximum amount of local anaesthetic that could be solubilized by LPC. Thus it seems that the longer the alkyl chain on the local anaesthetic molecule the stronger is its association with LPC.

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