Biochemical Pharmacology, Vol. 26, pp. 2393–2395. Pergamon Press, 1977. Printed in Great Britain.

STUDIES ON INTRAVENOUS PHOSPHATIDYL CHOLINE PREPARATIONS; THEIR EFFECTS ON LIPOLYTIC ENZYMES IN THE NORMAL RABBIT

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(*Received* 11 *February* 1977; *accepted* 6 *May* 1977)

Groups of normal rabbits were injected intravenously with phosphatidyl cholines from soya (EPL) and egg yolk (EYL) as hydrosols or as solutions in sodium deoxycholate. Lipolytic enzymes in serum, aorta and liver, were examined after 15 min. The hydrosols stimulated phospholipase and lipase in the liver, and decreased aortic lipase activities. The effect of solution in deoxycholate was to magnify the changes. EPL solution was more active in increasing liver lipase than EYL solution. The increase in lipase activity of the liver occurred even in the presence of M NaCl, suggesting that the phospholipids could stimulate "liver lipase" activity. These results are consistent with the view that "liver lipase" is both a triglyceride lipase and phospholipase.

The original observation of Schrade, Böhle and Biegler [1] that soya lecithin, when injected intravenously in man, had a rapid clearing effect against triglycerides has stimulated much interest in the use of this preparation as a therapeutic agent [2]. Polyunsaturated phosphatidyl choline from soya (EPL) forms an opaque suspension with saline or buffer but the addition of sodium deoxycholate in high concentration has a potent solubilizing effect and it is possible to obtain a clear solution of EPL. It is this preparation which is commercially available as Lipostabil, (Nattermann, Cologne).

Since bile acid salts are known to have an effect on enzyme activity *in vitro* [3], it is important to determine whether the clearing effect of Lipostabil can, in part, be attributed to the bile salts. The role of the polyunsaturated fatty acid component of EPL is uncertain, since other sources of phosphatidyl choline have not so far been investigated.

The object of the present experiments was to compare EPL with and without bile salts, and also to investigate egg yolk phosphatidyl choline, by means of acute experiments in normal rabbits.

MATERIALS AND METHODS

Experimental design. Preparations containing soya lecithin (EPL) and egg yolk lecithin (EYL) were obtained from Nattermann, Cologne.

Adult rabbits of New Zealand White breed (weighing approx. 3 kg) were used in the fed (non-fasted) state. A sample of blood was taken from the ear vein and then the following solutions injected, each in a volume of 2 ml;

(I) saline;

(II) EPL solution (Nattermann, Cologne, containing 200 mg EPL and 80 mg sodium deoxycholate);

(III) EPL suspension (equiv. 200 mg EPL without sodium deoxycholate). This suspension was made as follows:— An ice cold mixture of 10 g EPL plus 20 ml H_2O was sonicated for 3 min at low energy to avoid heating. 30 ml H_2O were added and the sonication procedure was repeated for 3–5 min. The suspension was filled up with ice cold H_2O to give 100 ml and again sonicated for 3–5 min at low energy to give a milky suspension;

(IV) EYL suspension (equiv. to 200 mg EYL without sodium deoxycholate), made in a similar manner to that described above;

(V) EYL solution (equiv. to 200 mg EYL and 80 mg sodium deoxycholate);

(VI) sodium deoxycholate (80 mg in saline).

After 15 min, a further specimen of blood was taken from the ear and the animal killed by i.v. nembutal. Liver and aorta were removed and kept at −20° before treatment.

Acetone butanol powders. These were obtained as described previously by Waligora *et al.* [4] (1975).

Assay of enzyme activities. Phospholipase, lipase and cholesterol esterase* were measured as described by Waligora *et al.* [4] (1975). For groups (V) and (VI), only liver lipase was estimated.

RESULTS

Serum. As shown in Table 1, EPL solution but not EPL suspension or EYL suspension decreased phospholipase and increased lipase activity. There was no effect on cholesterol esterase activity.

Aorta. As shown in Table 2, EPL solution, EPL suspension and EYL suspension had no effect on phospholipase but decreased lipase activity. EPL solution and suspension but not EYL suspension decreased cholesterol esterase activity.

Liver. As shown in Table 3, EPL solution and suspension, and EYL suspension increased phospholipase and lipase activities. Cholesterol esterase was

* Phospholipase A—phosphatide acyl-hydrolase (EC 3.1.1.4), lipase-glycerol-ester hydrolase (EC 3.1.1.3), cholesterol esterase-sterol-ester hydrolase (EC 3.1.1.13).

Table 1. Effect of EPL solution, EPL suspension, and EYL suspension on serum lipolytic enzymes in normal rabbits

Group	Injection	No.	Phospholipase	Lipase neq/mg/hr	Cholesterol esterase
I	Saline	9	24.4 ± 3.9	18.0 ± 4.5	11.1 ± 3.8
II	EPL soln.	10	7.7 ± 4.3	27.3 ± 7.4	8.9 ± 2.3
III	EPL susp.	9	22.7 ± 6.8	24.4 ± 11.7	10.2 ± 2.9
IV	EYL susp.	9	31.7 ± 12.5	24.9 ± 11.9	14.5 ± 2.8
Statistical Analysis					
I v II			***	**	NS
I v III			NS	NS	NS
I v IV			NS	NS	NS

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$.

Table 2. Effect of EPL solution, EPL suspension, EYL suspension on lipolytic enzymes in aorta

	Group	Injection	No.	Phospholipase	Lipase neq/mg/hr	Cholesterol esterase
	I	Saline	10	156 ± 32.5	151 ± 41.8	96.4 ± 18.0
	II	EPL soln.	10	203 ± 59.8	52.4 ± 20.1	55.2 ± 9.6
Aorta	III	EPL susp.	9	155 ± 25.6	87.8 ± 31.4	74.1 ± 20.8
	IV	EYL susp.	9	214 ± 57.1	97.4 ± 10.5	93.0 ± 24.5
Statistical Analysis						
I v II				NS	***	***
I v III				NS	**	*
I v IV				NS	**	NS

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$.

Table 3. Effect of EPL solution, EPL suspension, EYL solution and EYL suspension on lipolytic enzymes in liver

Group	Injection	No.	Phospholipase	Lipase neq/mg/hr	Cholesterol esterase
I	Saline	10	26.0 + 4.5	26.5 ± 5.1	20.8 ± 3.4
II	EPL soln.	10	58.2 ± 11.7	50.4 ± 22.4	22.8 ± 4.6
III	EPL susp.	9	38.8 ± 7.7	33.9 ± 3.8	17.6 ± 2.9
IV	EYL susp.	9	35.9 ± 2.4	33.2 ± 10.6	25.7 ± 8.3
V	EYL soln.	8		35.2 ± 7.2	
VI	Sodium Deoxycholate	8		27.6 ± 3.8	
Statistical Analysis					
I v II			**	**	NS
I v III			***	**	NS
I v IV			***	*	NS
I v V				**	
I v VI				NS	
II v III			***	**	NS
II v IV			***	***	NS
II v V				NS	
II v VI				**	
III v IV				NS	
IV v V				NS	

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$.

Table 4. Effect of M NaCl on lipase activity in rabbit liver

Injection	Lipase activity (neq/mg/hr) without M NaCl	with M NaCl
Saline	22.1	52.0
	23.0	25.4
EPL solution	43.0	35.0
	36.0	56.7
EPL hydrosol	46.3	79.5
	35.9	47.6

unchanged. In addition, EYL solution but not sodium deoxycholate increased lipase activity.

The order of activation of lipase being EPL soln. > EYL soln. > EPL suspension > EYL suspension. When the incubation was carried out in the presence of M NaCl, there was either no change or enhancement of lipase activity (Table 4).

DISCUSSION

In normal animals, EPL solution increases lipase activity in serum and liver but decreases activity in the aorta [4]. It has now been shown that these effects are due to the EPL itself, and not deoxycholate used as a detergent for solubilising EPL. However, the activity of EPL is enhanced by solution in deoxycholate although the latter is inactive alone. Any explanation for this enhancement can only be speculative, but is probably due to the greater availability of EPL in the miscellar dispersion produced by deoxycholate.

The lipolytic properties of EPL are not unique for this particular phosphatidyl choline since egg yolk lecithin also shows activity, albeit somewhat less. Presumably all phosphatidyl cholines will enhance lipolytic activity, the magnitude of which, will depend on its structure, especially the degree of unsaturation of the fatty acid components.

The effect of lipase activity in serum is similar to that observed with heparin [5]. The latter enhances lipolytic activities against a variety of substrates including triglycerides, and phospholipids. Heparin stimulates the release of two lipases [6, 7], "liproprotein lipase" which is inhibited by M NaCl and "liver lipase" which is not suppressed. Our results favour the view that the chief lipase enhanced is "liver lipase" since lipase activity was not inhibited by M NaCl. Recent work from Ehnholm *et al.* [8] indicates that purified "liver lipase" also has phospholipase activity. This is consistent with the properties of EPL which increases phospholipase in liver and serum.

With these above facts established it is now possible to suggest a possible mode of action of EPL as a "clearing" agent. Presumably EPL increases phospholipase activity because it is a substrate for such enzymes. This includes "Liver lipase" which is a phospholipase. This latter enzyme is also a triglyceride lipase, and substrates such as chylomicrons or very low density lipoproteins which contain chiefly triglycerides will also be lipolysed.

Acknowledgement—We are grateful to Nattermann & Co. of Cologne for a grant in support of this work.

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