Br. J. Pharmac. (1980), **69**, 11–12

LYSOPHOSPHATIDYLSERINE-INDUCED RELEASE OF INTRACELLULAR AMINES IN MICE

E. BIGON, A. BRUNI, L. MIETTO* & G. TOFFANO*

Institute of Pharmacology, University of Padova, Largo Meneghetti 2, 35100 Padova
and Fidia Research Laboratories*, Abano Terme, Italy

In the presence of mouse plasma, lysophosphatidylserine stimulates histamine secretion from isolated mast cells. The extensive modification of carbohydrate metabolism produced by lysophosphatidylserine in mice was largely prevented by the antihistaminic drug, pyrilamine. However, to prevent completely the change in carbohydrate metabolism induced by lysosphosphatidylserine the administration of an antihistamine and an adrenoceptor antagonist was required. It is concluded that the effect of lysophosphatidylserine in mice is due to release of intracellular amines. Histamine and catecholamines are involved.

Introduction The pharmacological action of phosphatidylserine in mice results from its metabolic conversion to lysophosphatidylserine (Bigon, Boarato, Bruni, Leon & Toffano, 1979a,b). When the purified lysoderivative is tested in mice at low doses (1 to 2 mg/kg) impressive modifications occur in blood and brain glucose levels. Under the same conditions lysophosphatidylcholine and lysophosphatidylethanolamine are ineffective. The observation that this compound produces histamine release from isolated mast cells exposed to concanavalin A (Martin & Lagunoff, 1979) prompted further interest in lysophosphatidylserine. Since this phospholipid is ineffective in the absence of enhancing agents, the relevance of this observation to the effect *in vivo* has not been clear. This communication presents evidence indicating a correlation between the effects *in vivo* and *in vitro*.

Methods Purified lysophosphatidylserine (Bigon *et al.*, 1979b) was dissolved in 0.05 M Tris HCl buffer (pH 7.8) and injected intravenously into male albino mice. Methods for the rapid removal of the brain and quantification of carbohydrate metabolites have already been described (Bigon *et al.*, 1979a). Rat peritoneal mast cells were collected and used either directly or after their purification in concentrated serum albumin solution (Lagunoff, 1972). Such purification did not alter the results. Histamine release was assayed on the guinea-pig isolated ileum. Blood was collected in the presence of heparin 100 iu/ml, centrifuged and used immediately. Mouse plasma was partially purified by filtration and washing through Amicon CF-25 membranes followed by 30 min centrifugation at 50000 rev/min (195000 *g*) at 0°C to remove a small sediment and the floating layer of chilomicra. The resulting preparation was fractionated with ammonium sulphate to obtain the fraction between 25 and 40% saturation.

Results The ineffectiveness of lysophosphatidylserine in releasing histamine from rat peritoneal mast cells was again confirmed (Table 1). In contrast, this phospholipid was highly effective in the presence of mouse, but not rat or guinea-pig, plasma. All plasma obtained in the presence of heparin and used alone without delay did not affect the basal histamine release. The effectiveness of lysophosphatidylserine

Table 1 The effect of lysophosphatidylserine on histamine secretion

Addition	*% histamine release*
None	2.0 ± 0.6
Lysophosphatidylserine (1 μmol/l)	3.2 ± 0.4
Mouse plasma (12 mg protein)	2.1 ± 0.5
Lysophosphatidylserine plus mouse plasma	72.3 ± 6.6*
Lysophosphatidylserine plus rat plasma	4.7 ± 0.7
Lysophosphatidylserine plus guinea-pig plasma	4.5 ± 0.3

Unwashed rat peritoneal mast cells containing 5 μg histamine were incubated 5 min at 37°C in 1 ml of balanced saline solution (Martin & Lagunoff, 1979) with or without lysophosphatidylserine. After this time 1 mmol/l $CaCl_2$ was added and the incubation followed for an additional 15 min. Plasma was added together with $CaCl_2$ where indicated. The results are expressed as % (±s.e. mean) of total cell histamine obtained by subjecting the mast cells to 2 min sonication ($n = 5$).*$P < 0.01$.

0007-1188/80/050011-02 $01.00

was still manifest at 0.01μmol/l. This concentration is compatible with the dose found effective *in vivo*. On purification, the specific activity of mouse plasma increased 100 to 400 fold. Some purified preparations showed activity in the absence of lysophosphatidylserine but were inhibited by the addition of unfractionated plasma. This indicates that the component responsible for histamine release is maintained in an inactive form unless lysophosphatidylserine is added. After 210 min centrifugation at 198000 *g* the activity of the purified preparations was concentrated in the sediment. The activity of plasma preparations was destroyed by heating 20 min at 60°C. These experiments show that the appropriate agent which will release histamine in the presence of lysophosphatidylserine is amongst the plasma components. Therefore the *in vivo* phospholipid effect was tested in mice pretreated with 10 mg/kg pyrilamine. Under these conditions the reported increase of 63% in blood glucose and of 280% in brain glucose levels, 30 min after 2.5 mg/kg lysophosphatidylserine administration (Bigon *et al.*, 1979b), were found to be reduced to increases of only 50% and 145%, respectively ($P < 0.01$). When the adrenoceptor blocking agent, dihydroergotoxine (5 mg/kg), was given with pyrilamine, the lysophosphatidylserine effect diminished so that the increases were only of 11% and 27%, respectively ($P < 0.01$). Atropine and methysergide did not modify the phospholipid effect.

Discussion These results demonstrate that histamine and catecholamines mediate the pharmacological effects of lysophosphatidylserine in mice. Histamine secretion apparently results from the combined effect of lysophosphatidylserine and plasma proteins. Since the plasma activity is recovered in a sediment after high-speed centrifugation it is likely that components bound to a plasma macroglobulin are involved. The ineffectiveness of rat and guinea-pig plasma is in accord with parallel experiments showing that lysophosphatidylserine affects carbohydrate metabolism much less in these animals than in mice. Catecholamine release may be secondary to a histamine effect on the adrenals since phosphatidylserine administration has been shown to decrease the catecholamine content in the adrenals of mice (Bruni, Toffano, Leon & Boarato 1976). Alternatively the influence of lysophosphatidylserine is not confined to histamine secretory cells. Because of its high biological activity it appears likely that the generation of lysophosphatidylserine from endogenous phosphatidylserine is under strict control in the tissues of sensitive animals. Correspondingly only trace amounts of lysophosphatidylserine are detected in cells and in subcellular organelles (Rouser, Nelson, Fleischer & Simon, 1968). However, since phosphatidylserine is a normal constituent of the cell membrane and phospholipase activity is widely distributed, it is possible that small amounts of lysophosphatidylserine are occasionally produced serving as *in vivo* messengers in the activation of differentiated cells. Cycles of phosphatidylserine deacylation and reacylation may serve to control the lysophosphatidylserine effect.

References

BIGON, E., BOARATO, E., BRUNI, A., LEON, A. & TOFFANO, G. (1979a). Pharmacological effects of phosphatidylserine liposomes: regulation of glycolysis and energy level in brain. *Br. J. Pharmac.*, **66**, 167–174.

BIGON, E., BOARATO, E., BRUNI, A., LEON, A. & TOFFANO, G. (1979b). Pharmacological effects of phosphatidylserine liposomes: the role of lysophosphatidylserine. *Br. J. Pharmac.*, **67**, 611–616.

BRUNI, A., TOFFANO, G., LEON, A. & BOARATO, E. (1976). Pharmacological effects of phosphatidylserine liposomes. *Nature, Lond.*, **260**, 331–333.

LAGUNOFF, D. (1972). The mechanism of histamine release from mast cells. *Biochem. Pharmac.*, **21**, 1889–1896.

MARTIN, T.W. & LAGUNOFF, D. (1979). Interactions of lysophospholipids and mast cells. *Nature, Lond.*, **279**, 250–252.

ROUSER, G., NELSON, G.J., FLEISCHER, S. & SIMON, G. (1968). Lipid composition of animal cell membranes, organelles and organs. In *Biological Membranes Physical Fact and Function*. ed. Chapman, D., pp. 5–69. New York and London: Academic Press.

(*Received February 7, 1980.*)