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Mast cell – pituitary interaction: modulation by serine phospholipids

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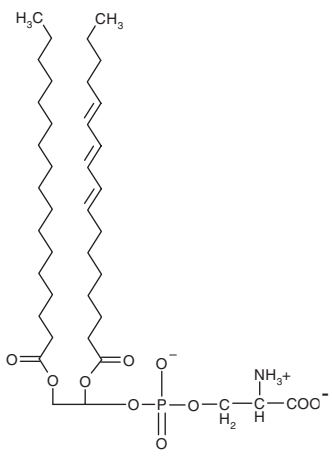
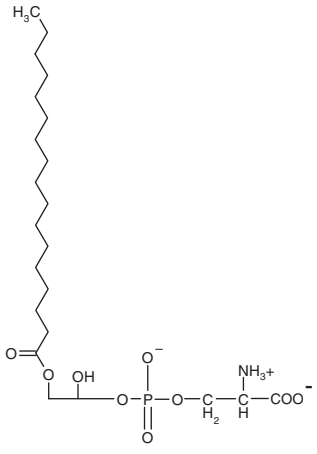
The influence of mast cell activation on the secretion of prolactin has been studied in rats receiving lysophosphatidylserine, a natural occurring phospholipid with secretagogue activity in these cells. After the i.v. injection of lysophosphatidylserine (10 mg/kg) a plasma prolactin peak correlates with an increased blood histamine level. Following the secretory event, which is inhibited by the H1 anti-histamine tripelenamine, plasma prolactin level drops below the basal line. Repeated lysophosphatidylserine administrations induce mast cell desensitisation, thus reducing also the pituitary response. Under these conditions a decrease in prolactin basal level is still observed, although the pituitary stores of this hormone are preserved. Control tests *in vitro* with lysophosphatidylserine, show that the diacyl lysophosphatidylserine derivative amplifies the inhibitory effect of dopamine on prolactin secretion from isolated pituitaries. The data suggests that lysophosphatidylserine induces prolactin secretion through mast cell activation. After this event, the reacylation of this phospholipid into lysophosphatidylserine in the pituitary membrane may enhance the inhibitory control by dopamine.

1. Introduction

The intravenous injection of lysophosphatidylserine (lysoPS) into mice and rats is followed by hyperglycemia and increased brain glucose content (Bigon et al. 1979). As shown by subsequent studies (Bruni et al. 1984), these effects are the consequence of the increased blood histamine level due to mast cell activation. In accord the increase in blood histamine level and the concomitant changes in carbohydrate distribution are not observed in a strain of mice free of mast cells (Chang et al. 1988). Confirming these findings, several *in vitro* experiments illustrate the specific and stereoselective activation induced by lysoPS in rodent mast cells (Boarato et al. 1984; Chang et al. 1988). Parallel lines of investigation show that the injection of histamine into rats affects the secretory pattern of pituitary hormones, (Tuomisto et al. 1985; Fleckenstein et al. 1994; Kjaer et al. 1994) including prolactin release which is increased (Libertun and Mc Cann 1976; Donoso et al. 1976). To extend these observations, we have investigated whether the lysoPS-induced mast cell activation elicits prolactin secretion. Such a finding would indicate that the influence of this phospholipid on the immunological cells is not confined to mast cells. Indeed, previous



Phosphatidylserine
(PS)



Lysophosphatidylserine
(LysoPS)

reports show that prolactin activates the lymphocyte responsiveness to immunological stimuli (Hiestand et al. 1986; Bernton et al. 1988). Receptors for prolactin have been found in human lymphocytes from which it is displaced by the immunosuppressive peptide cyclosporin A, but not by the inactive parent compound, cyclosporin H (Russel et al. 1985). Consistently, in the hypophysectomized rats the immune response is decreased but it can be restored by a treatment with prolactin or growth hormon (Nagy et al. 1983).

Further interest in this study originates from the finding that the diacyl analogue of lysoPS, phosphatidylserine (PS) shows hypoprolactinemic effects on rats (Canonico et al. 1981; Bonetti et al. 1985; Galbiati et al. 1986). If the opposite effect is demonstrated with lysoPS, a complex modulatory influence by the exogenous serine phospholipids on pituitary cells would be indicated.

2. Investigations and results

As shown in Table 1 the intravenous injection of lysoPS induced a large increase in the histamine blood level. As expected, this effect was concomitant with a significant increase in plasma prolactin. The lysoPS-induced prolactin surge was inhibited by increasing doses of the H_1 antihistamine tripelenamine, thus indicating a causal relationship between the mast cell and the pituitary response (Fig. 1). To further investigate this relationship, compound 48/80, a mast cell secretagogue alternative to LysoPS was also tested. At the dose of 0.1 mg/kg i.v., compound 48/80 elicited histamine secretion reaching a blood level of 521 ± 84 ng/ml after 15–30 min. At the same time the plasma prolactin level rose to 45.5 ± 3 ng/ml from an initial value of 1.4 ± 0.1 ng/ml ($n = 4$). Previous studies demonstrated that a first injection of LysoPS causes prolonged mast cell desensitisation (Bruni et al. 1984). Accordingly, in Fig. 2 it is seen that a second administration of LysoPS after 5 h did not induce prolactin secretion. The system recovered responsiveness 24 h later. As shown in Fig. 2, a noticeable feature of LysoPS effect during the desensitisation period was a significant decrease in plasma prolactin level. The LysoPS-induced oversuppression of prolactin secretion during the desensitisation period was not due to the exhaustion of prolactin pituitary reserves, since these were found undepleted in spite of a prior secretory event (Table 2).

Since parenteral phosphatidylserine administration *in vivo* manifests hypoprolactinemic activity it was thought that the delayed decrease in plasma prolactin level induced by LysoPS was the consequence of a conversion of this phospholipid into phosphatidylserine. To test this possibility phosphatidylserine was added to pituitaries incubated *in*

Table 1: Effect of lysophosphatidylserine on blood histamine and plasma prolactin

Treatment[a]	Blood histamine (ng/ml)	Plasma prolactin (ng/ml)
None	130 ± 40	21.9 ± 4.0
LysoPS (5 mg/kg)	$340 \pm 80^*$	$69.7 \pm 12.1^*$
LysoPS (10 mg/kg)	$640 \pm 30^*$	$70.4 \pm 9.7^*$

[a] The indicated doses of lysoPS were injected i.v. into rats. Blood was taken 10 min later
Mean ± S.E. for 8 rats. * $p < 0.01$ vs. none

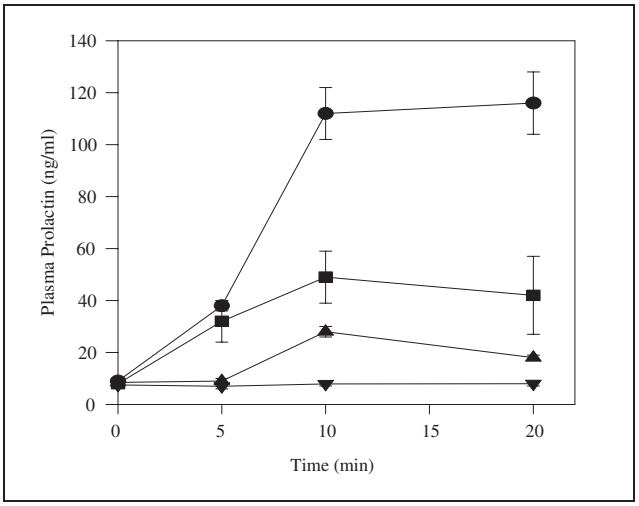


Fig. 1: Antagonism by tripelenamine in rats. The anti H_1 anti-histamine tripelenamine was administered i.v. 15 min before the injection of lysoPS (10 mg/kg i.v.); (●) without the treatment of tripelenamine; (■) 0.4 mg/kg of tripelenamine; (▲) 2mg/kg of tripelenamine; (▼) 4 mg/kg of tripelenamine. Vertical bars denote S : E: for at least 4 rats

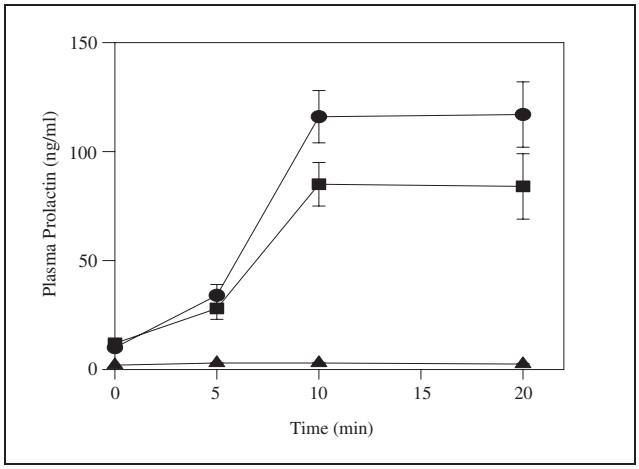


Fig. 2: Desensitisation to lysophosphatydilserine (lysoPS). Two consecutive lysoPS administrations (10 mg/Kg, i.v.) were given to chronically cannulated rats. Plasma prolactin was tested 5, 10 and 20 min later. (●) effect of the first lysoPS injection; (▲) effect of a second lysoPS injection, 5 h after the first; (■) effect of a second lysoPS injection, 24 h after the first. Mean ± S.E. for 4–6 rats

Table 2: Delayed hypoprolactinemic effect of lysophosphatidylserine

Treatment[a]	Blood histamine (ng/ml)	Plasma prolactin (ng/ml)	Pituitary prolactin (ng/mg wet wt)
None	154 ± 9	30 ± 5.0	1412 ± 121
LysoPS	$1102 \pm 123^{**}$	$61 \pm 10^{**}$	1344 ± 101
LysoPS, LysoPS	$310 \pm 60^*$	$15 \pm 2.5^{**}$	1316 ± 113

[a] A single or two consecutive Lyso PS administrations at two hours interval (10 mg/Kg i.v.) were given to rats. 10 min after the lysoPS treatment blood histamine, plasma prolactin and the pituitary stores of the hormone were measured.
Mean ± S.E. for 10–13 rats. * $p < 0.05$; ** $p < 0.01$ vs. none

Table 3: Synergism between dopamine and phosphatidylserine

Prolactin release (ng/μg protein)			
	Without dopamine	Plus dopamine[a]	Dopamine-induced inhibition (%)
None	598 ± 50	474 ± 31	20
0.6 μM PS	559 ± 45	429 ± 30	30
6.0 μM PS	611 ± 45	$366 \pm 22^*$	40^*

[a] dopamine 10 nM
Mean ± S.E. for 5 glands. * $p < 0.05$; vs. none

Table 4: Distribution (%) of [^{3}H]arachidonate in the phospholipids of anterior pituitary of rat.

Treatment[a]	Phospholipids			
	PC[b]	PE	PS	PI
None	61.2 ± 2.6	20.3 ± 0.5	3.5 ± 0.1	7.7 ± 1.0
LysoPS 10μM	48.4 ± 0.7**	28.4 ± 1.3**	10.9 ± 1.1**	6.0 ± 0.6

[a] [^{3}H]arachidonate (1 μCi) were added in the absence or presence of lysoPS to one pituitary gland incubated in vitro, as described in the experimental section.
[b] PC = phosphatidylcholine; PI = phosphatidylinositol; PS = phosphatidylserine; PE = phosphatidylethanolamine
Mean ± S.E. for 5 pituitaries. ** $p < 0.01$ vs. none

vitro. Although phosphatidylserine did not influence the secretion of prolactin, the effect of a concentration of dopamine producing submaximal inhibition of prolactin release was significantly enhanced (Table 3).
To investigate whether lysoPS is converted into the corresponding diacyl analogoue by reacylation, it was added to pituitaries incubated *in vitro* in the presence of [^{3}H]arachidonate, an unsaturated fatty acid largely present in the phospholipids of pituitary cellular membranes. As shown in Table 4 a significative incorporation of [^{3}H]arachidonate was found in the phosphatidylserine and phosphatidylethanolamine fractions, indicating that in this tissue the lysophospholipid is efficiently incorporated and reacylated.

3. Discussion

Early data shows that the intravenous administration of histamine elicits prolactin secretion (Libertun et al. 1976). Based on the observation of a concomitant decrease in portal blood dopamine, it has been proposed that the histamine induced activation of H_1 receptors located in the tubero-infundibular area has a negative modulatory influence on dopamine release. Prolactin secretion follows as a consequence (Fleckenstein et al. 1994; Gibbs et al. 1984). Extending these observation our results show that prolactin secretion occurs in response to potent mast cell secretagogues such as lysoPS. The antagonism by an H_1-anti-histamine supports the conclusion that histamine is responsible for the pituitary response. Furthermore, we observe that mast cell desensitisation to the phospholipid is associated with the disappearance of the induced prolactin secretion. Confirming the involvement of mast cells, it was found that compound 48/80, a drug inducing histamine release, reproduces the effect of LysoPS. The histamine mediated functional interaction between mast cells and the prolactin secreting cells may become operative when pathological stimuli cause the release of histamine into the systemic circulation. Since histamine has a depressive influence on T-lymphocytes activity whereas prolactin has a stimulatory effect, this interaction may serve the purpose of avoiding unnecessary negative modulation of these cells during pathological states associated with extensive mast cell activation. A possible role of LysoPS in promoting the mast cell-pituitary interaction under pathophysiological circumstances is suggested by the observation that this phospholipid is produced at sites of damaged tissue (Mietto et al. 1987; Hara et al. 1994; Lloret and Moreno 1995). An additional observation of our study is the oversuppression of prolactin secretion following the initial lysoPS induced release. This effect becomes especially manifest when mast cell refractoriness is induced by repeated lysoPS administrations. Since the decrease in prolactin plasma leveles is produced in the absence of a discernible decrement of prolactin pituitary stores, we conclude that LysoPS has a delayed inhibitory effect on the secretion of this hormone. We attempted to characterize this effect by the use of phosphatidylserine, the main product of lysoPS metabolism in mammalian cells. Indeed, the incorporation of exogenous lysophospholipids into several tissues and their conversion into the corresponding diacylphospholipids is a well known event in the pharmacokinetics of these compounds (Palatini et al. 1991). Our results clearly demonstrate also in the pituitary that exogenously added lysoPS is efficiently incorporated and reacylated to the corresponding diacyl analogue. Furthermore, phosphatidylserine has been previously shown to have an inhibitory effect on prolactin secretion *in vivo* (Canonico et al. 1981; Bonetti et al. 1985; Galbiati et al. 1986). The data shows that phosphatidylserine enhances the inhibitory effect of dopamine on prolactin secretion.
This finding suggests that lysoPS has a dual influence on prolactin-producing cells: a histamine-mediated stimulatory effect and a negative modulatory influence mediated by its conversion into phosphatidylserine at the pituitary level. Other possibilities such as the exhaustion of a promptly releasable prolactin pool or the enhanced production of dopamine as a consequence of the plasma prolactin peak may also explain the lysoPS-induced oversuppression. Further experiments are needed to evaluate the relative contribution of these events.

4. Experimental

4.1. Drugs

Compound 48/80, tripelenamine and dopamine, were from Sigma Chemical Co. (St. Louis, Mo). Phosphatidylserine was obtained from bovine brain. The preparation was dispersed by sonication in 50 mM Tris-HCl buffer pH = 7.4. Chromatographically pure lysoPS was prepared from phosphatidylserine as described (Viola et al. 1993). This phospholipid was dissolved in the same buffer with the help of a brief (2 min) sonication. [^{3}H]arachidonic acid (76 Ci mmol^{-1}) was from New England Nuclear.

4.2. Animals

Young adult (3 month), male Sprague Dawley rats were used in this study. The animals received food and water *ad libitum* and were hield in a temperature controlled environment (12 : 12-ligth : dark cycles). At the time of sacrifice all rats showed a stable body weight and were free of any observable disease. Blood samples were obtained from trunk after decapitation or from chronically cannulated rats.
Freely moving rats bearing a polyethylene cannula inserted into the right atria through the jugular vein were used for some experiments. The animals underwent surgery 4 days before the experiment and were housed in specially designed single cages. This procedure allowed us to avoid animal handling during the repeated blood withdrawn. For each sample 0.1 ml of blood was collected into a syringe and immediately replaced with an equal volume of sterile isotonic saline. At the end of the experiments the animals were inspected to exclude the presence of disease or weight loss.

4.3. Blood histamine assay

Histamine was determined in the whole blood, since this procedure allowed the detection of the fraction of histamine bound to the erythrocytes (Bruni et al. 1984). EDTA was added to blood samples to avoid clotting. Perchloric acid extracts was purified by the butanol extraction procedure and assayed by a fluorimetric method (Shore et al. 1959). For a time-course experiment histamine was assayed using a commercial RIA-Kit purchased from Sorin (Saluggia, Italy). For this purpose, heparinized whole blood samples were diluted 1 : 100 with distilled water to obtain the destruction of cells before the amine determination. Basal blood histamine levels detected by the two different methods were superimposable.

4.4. Plasma and pituitary prolactin assay

Heparinized blood was centrifuged at 3500 rpm for 15 min. Plasma was separated and kept frozen at −20 °C until prolactin assay. The anterior pituitary glands of lysoPS treated rats, were separated from the intermediates lobes, weighed and homogeneized in 1 ml of 0.01 M phosphate buffer pH = 7.4, containing 1% Triton, using a Potter-Elvehjem homogenizer.

The homogenate was centrifuged at 10000 rpm at 4 °C for 20 min, the supernatant was collected and stored frozen for prolactin assay. The results were normalized for the protein content (Peterson 1977).

For the *in vitro* experiments the anterior pituitary gland was separated from neurointermediate lobe then hemisected and weighed. The tissue was incubated, using a Dubnoff shaker, as described previously (Galbiati et al. 1986). After an incubation period of 1 h the medium was discarded and replaced with fresh medium containing either test substances or vehicle. Flasks were maintained in an atmosphere of 95% of O_2 and 5% of CO_2 throughout the incubation period.

This assay was performed by a double antibody RIA using material and protocols kindly supplied by the NIDDK rat pituitary hormone distribution program (Bethesda, MD USA). The results are expressed in terms of rat prolactin (RP-3) standard. The intra-assay variations were less than 6%, the inter-assay variations are around 10%.

4.5 Incorporation of [^{3}H]arachidonate

Anterior pituitaries were collected and incubated in the presence of lysoPS at the concentration of 10 μM, and [^{3}H]arachidonate as described above. After 1 h of incubation the pituitaries were homogeneized in 1 ml of 0.01 M phosphate buffer pH = 7.4. The lipid content of the gland was extracted and analysed as described elsewhere in detail (Viola et al. 1993). Briefly, after homogeneization, the phospholipids were extracted with 20 volumes of chloroform 2 : 1 (v/v) as described (Folch et al. 1957). The organic extract was washed with 0.2 volumes of 0.05 M HCl. Phospholipids were resolved by two dimensional thin layer chromatography (Silica gel G plates, Merck) as described previously (Punzi et al. 1986).

4.6 Statistics

Results are expressed as mean ± S.E. The statistical analysis was performed by Duncan's "t" test.

References

Bernton EW, Meltzer MS, Holaday JW (1988) Suppression of macrophage activation and T-lymphocyte function in hypoprolactinemic mice. Science 239: 401–404.

Bigon E, Boarato E, Bruni A, Leon A, Toffano G (1979) Pharmacological effects of phosphatidylserine liposomes: the role of lysophosphatidylserine. Br J Pharmacol 67: 611–616.

Boarato E, Mietto L, Toffano G, Bigon E, Bruni A (1984) Different response of rodent mast cells to lysophosphatidylserine. Agent Actions 14: 613–618.

Bonetti AC, Canonico PL, Mac Leod RM (1985) Brain cortex phosphatidylserine inhibits phosphatidylinositol turnover in rat anterior pituitary glands. Proc Soc Exp Biol Med 180: 79–120.

Bruni A, Bigon E, Battistella A, Boarato E, Mietto L, Toffano G (1984). Lysophosphatidylserine as histamine releaser in mice and rats. Agent Actions 14: 619–625.

Canonico PL, Annunziato L, Toffano G, Bernardini R, Stanzioni S, Foti M, Clementi G, Drago F, Scapagnini U (1981) *In vivo* and *in vitro* interference of phosphatidylserine liposome on prolactin secretion in the rat. Neuroendocrinology 33: 358–362.

Chang HW, Inoue K, Bruni A, Boarato E, Toffano G (1988) Stereoselective effects of lysophosphatidylserine in rodents. Br J Pharmacol 93: 647–653.

Donoso AO, Banzan AM, Borzino MI (1976) Prolactin and luteinizing hormone release after intraventricular injection of histamine in rats. J Endocrinol 68: 171–172.

Fleckenstein A E, Lookingland KJ, Moore KE (1994) Differential effects of histamine on the activity of hypothalamic dopaminergic neurons in the rat. J Pharmacol Exp Ther 268: 270–276.

Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and the purification of total lipids from animal tissues. J Biol Chem 226: 497–509.

Galbiati E, Bonetti AC, Bellini F, Calderini G Effects of phosphatidylserine on prolactin secretion in rat. in: Biggio G, Spano PF, Toffano G, Gessa GL (Eds.) Modulation of central and peripheral transmitter functions,. Liviana Press, Padova, pp. 383–391.

Galbiati G, Calderini G, Viola G, Toffano G (1986) Phorbol 12-myristate 13-acetate induces prolactin secretion from rat anterior pituitary gland by the activation of protein kinase-C Pharm. Res Comm 18: 687–698.

Gibbs DM, Plotsky PM, de Greef WJ, Neil JD (1979) Effect of histamine and acetylcholine on hypophyseal stalk plasma dopamine and peripheral plasma prolactin level Life Sci., 24: 2063–2070.

Hara K, Komatsu H, Tsutsumi N, Ujiie A, Ikeda S, Kobayashi T, Kudo I, Inoue K (1994) Suppressive effects of the anti-allergic drugs, tranilast and azelastine, on the lysophosphatidylserine-dependent activation of rat mast cells. Biol Pharm Bull 17: 1121–1123.

Hiestand CP, Meckler P, Nordmann R, Grieder A, Permmongkol C. (1986) Prolactin as modulator of lymphocyte responsiveness provides a possible mechanism of action for cyclosporine. Proc Natl Acad Sci (USA) 83: 2599–2603.

Kjaer A, Larsen PJ, Knigge U, Warberg J (1994) Histaminergic activation of the hypothalamic-pituitary-adrenal axis. Endocrinology. 135: 1171–1177.

Libertun C. McCann SM (1976) The possible role of histamine in the control of prolactin release and gonadotropin release. Neuroendocrinology 20: 110–120.

Lloret S, Moreno JJ (1995) Ca^{2+} influx, phosphoinositide hydrolysis, and histamine release induced by lysophosphatidylserine in mast cells. J Cell Physiol 165: 89–95.

Mietto L, Boarato E, Toffano G, Bruni A (1987) Lysophosphatidylserine-dependent interaction between rat leukocytes and mast cells. Biochim Biophys Acta 930: 145–153.

Nagy E, Berczi I, Friesen HG, (1983) Regulation of immunity in rats by lactogenic and growth hormones. Acta Endocrinol 102: 351–357.

Palatini P, Viola G, Bigon E, Menegus AM, Bruni A (1991) Pharmacokinetic characterization of phosphatidylserine liposomes in the rat. Br J Pharmacol 102: 345–350.

Peterson GL (1977) A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal Biochem 83: 346–356.

Punzi L, Todesco S, Toffano G, Catena R, Bigon E, Bruni A (1986) Phospholipids in inflammatory synovial effusions. Rheumatol Int 6: 7–11.

Russel D H, Kibler R, Mattrisian L, Larson DF, Poulos B, Magun BE (1985) Prolactin receptors on human T and B lymphocytes: antagonism of prolactin binding by cyclosporine,. J Immunol 134: 3027–3031.

Shore PA, Burkhalter A, Cohn VH (1959) A method for the fluorimetric assay of histamine in tissue. J Pharmacol Exp Ther 127: 182–185.

Tuomisto J, Mannistö P (1985) Neurotransmitter regulation of anterior pituitary hormones. Pharmacol Rev 37: 249–332.

Viola G, Mietto L, Secchi EF, Ping L, Bruni A (1993) Absorption and distribution of arachidonate in rats receiving lysophospholipids by oral route. J Lipid Res 34: 1843–1852.