INTERACTION BETWEEN NERVE GROWTH FACTOR AND LYSOPHOSPHATIDYLSERINE ON RAT PERITONEAL MAST CELLS

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1. Introduction

Lysophosphatidylserine induces hyperglycaemia and brain glucose accumulation in mice and rats. These effects are thought to originate from an induced secretion of histamine and adrenaline [1–3]. Experiments on isolated mast cells support this possibility since lysophosphatidylserine enhances the histamine release induced by antigens, concanavalin A or dextran [4,5]. Moreover, a cationic protein eliciting histamine release from peritoneal mast cells in the presence of lysophosphatidylserine has been detected in mouse plasma [3,6]. Attempts to further purify this protein led to the unexpected finding that the effect on mast cells can be seen when the mouse blood is collected by a cut in the neck region and not by heart or tail puncture. Since contamination by nerve growth factor is common in blood of male mice collected from the neck region [7], a likely explanation for the difference was the presence of nerve growth factor in the active plasma preparations. These experiments confirm this possibility and demonstrate a powerful synergism between lysophosphatidylserine and nerve growth factor on rat peritoneal mast cells.

2. Material and methods

2.1. *Preparation of nerve growth factors*

Mouse β-nerve growth factor (β-NGF) was obtained from submaxillary glands of adult male albino mice as in [8]. Cobra (*Naja naja*) nerve growth factor was prepared as in [9]. Polyacrylamide gel electrophoresis of the 2 preparations was performed in 6% acrylamide, 0.1% SDS. The samples were heated 3 min at 100°C in 5 mM phosphate buffer (pH 7) containing 0.5% SDS, 8 M urea. The single band of mouse β-NGF had M_r 12 500–13 500 when assessed by a titration curve with standard proteins. Cobra nerve growth yielded a major band of 25 000–26 000 M_r. A minor band of 12 500–13 500 M_r indicated the partial splitting of the dimer under these conditions. Minor contaminations of high M_r were also detected. On dissociated sensory ganglia from 8-day-old chick embryos [10], mouse β-NGF elicited survival and fiber outgrowth at 0.5 ng/ml, cobra nerve growth factor at 2 ng/ml.

2.2. *Rat peritoneal mast cells*

Mast cells were collected from the peritoneum of male albino rats and purified on a concentrated serum albumin solution as in [11]. About 3×10^5 mast cells (3–5 μg histamine) were added to 1 ml balanced saline solution [11] containing 1 mM $CaCl_2$, 10 mM glucose and 1 mg/ml serum albumin. When indicated, albumin was omitted. After the addition of nerve growth factors the incubation was 15 min at 37°C. Lysophosphatidylserine (0.5 μM) was added to the incubation medium before the nerve growth factor and equilibrated 5 min with the mast cells. After centrifugation, the histamine was determined [12] in the supernatant and the release expressed as percent of total histamine obtained by heating the mast cells 5 min at 100°C in 0.1 N HCl.

2.3. *Lysophospholipids*

Lysophosphatidylserine [1] and acetimidyl-lysophosphatidylserine [4] were prepared as described. Lysophosphatidylethanolamine was purchased from General Biochemicals. All phospholipids yielded a single spot when tested (0.1 μmol) on thin-layer chromatography with chloroform, methanol, acetic acid, water (25:15:4:2, by vol.) as developing solvent.

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3. Results and discussion

When the homogenate of submaxillary glands from adult male mice was tested on rat peritoneal mast cells, high histamine releasing activity was detected both in the presence and in the absence of lysophosphatidylserine. Reducing protein to 5–10 μg/ml, the activity diminished but could be fully restored by the addition of 0.5 μM lysophosphatidylserine. The phospholipid-dependent activity copurified with the nerve growth factor when the procedure in [13] was followed. To further investigate the interaction between lysophosphatidylserine and nerve growth factor a purified preparation of the β-subunit was obtained. This subunit is the active moiety of this complex protein [8,14,15]. As a comparison, cobra nerve growth factor was prepared. Nerve growth factor from cobra venom is known to have the same biological activity as mouse protein but is poorly recognized by antibodies directed against it [9]. When the mouse β-NGF was tested on mast cells 2 effects could be observed (fig.1 A). The first led to histamine release in the absence of lysophosphatidylserine and reached its maximum at the highest concentrations employed (0.5–1.0 μg/ml). The second was manifest only in the presence of lysophosphatidylserine and led to a histamine secretion at physiological β-NGF concentrations (i.e., those active on dorsal root ganglia of chick embryos, 1.0–10 ng/ml). As indicated by the calcium dependence, by the lack of lactate dehydrogenase activity in the supernatant and by the decrease of histamine release at 0°C, the 2 effects did not result from

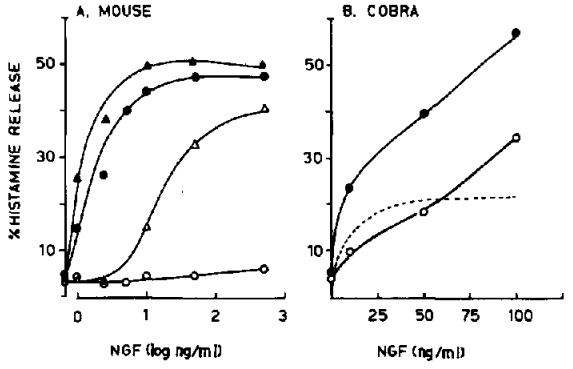

Fig.1. Interaction on mast cells. (A) Mouse β-NGF without (△) and with (○) 1 mg/ml serum albumin; mouse β-NGF + 0.5 μM lysophosphatidylserine without (▲) and with (●) serum albumin. (B) Cobra nerve growth factor without (○) and with (●) lysophosphatidylserine (the dashed line is the difference between the 2 curves); serum albumin was always added.

mast cell damage. The addition of serum albumin to the incubation mixture allowed to control the first effect suggesting that aggregation of mast cells was involved in this case. Indeed, β-NGF is known to increase cellular adhesiveness at a rather high concentration [16]. Albumin did not prevent the histamine release occurring in the presence of lysophosphatidylserine. Under these conditions, half-maximal stimulation by the β-NGF was reached at 1–2 ng/ml corresponding to $\sim 5 \times 10^{-11}$ M on the basis of a dimer M_r 26 000. Lysophosphatidylserine which was used at 1/37th of its critical micellar concentration (18.8 μM, [4]), did not elicit histamine release in the absence of β-NGF. In a comparison with compound 48/80, the histamine secretion induced by lysophosphatidylserine plus β-NGF was 70% of the maximum release promoted by this agent (70–75%). When cobra nerve growth factor substituted for the mouse proteins, the activity in the absence of lysophosphatidylserine was higher and could not be completely controlled by the addition of serum albumin. However, the stimulation by lysophosphatidylserine was always manifest (fig.1B). In this case the lysophosphatidylserine-dependent activity (dashed line) reached its maximum at 50 ng/ml. As shown in table 1, the effect of lysophosphatidylserine was dependent on the structure of the headgroup. When the amino group of the serine was blocked (acetimidyl-lysophosphatidylserine) or when the carboxyl group was absent (lysophosphatidylethanolamine) little or no activity was manifest. This structure–activity relationship was similar to that observed in the enhancement of concanavalin A-induced histamine release [4]. When mast cells were preincubated with β-NGF and washed, lysophosphatidylserine elicited histamine release without the addition of the protein. This showed that the anionic phospholipid headgroup was not required for the binding of β-NGF to mast cells. The interaction between β-NGF and lysophosphatidylserine could be observed also in vivo. After local administration in the paw of rats the histamine content was measured 18 h later. In 17 determinations 20 μg lysophosphatidylserine and 10 μg β-NGF injected separately reduced the histamine content from a control value of 82.31 ± 2.65 μg/g wet wt to 59.00 ± 2.63 and 75.63 ± 2.51, a decrease of 28.3% and 8.1%, respectively. When the 2 compounds were associated, the histamine content dropped to 44.01 ± 2.38, a significant ($P < 0.01$) reduction of 46.4%, even higher than the value expected from the simple additive effect. The interaction between lyso-

Table 1
Role of lysophosphatidylserine headgroup (% histamine release)

System	without β-NGF	+10 ng β-NGF/ml
Untreated mast cells (11)	2.9 (0.3–9.5)	4.1 (2.9–11.0)
+0.5 μM lysoPS (11)	4.3 (0.2–10.6)	42.0 (35.9–59.0)
+0.5 μM lysoPEA (6)	3.2 (1.5–8.3)	8.5 (4.0–12.8)
+0.5 μM acetimidyl-lysoPS (4)	5.4 (2.3–10.0)	9.3 (7.3–14.2)
β-NGF-treated mast cells (6)	3.6 (0.8–5.5)	3.7 (0.0–9.6)
+0.5 μM lysoPS (6)	38.0 (26.9–69.0)	50.6 (33.1–68.0)

1.5×10^6 purified mast cells were treated with 0.1 μg β-NGF 5 min at 20°C. The cells were centrifuged and washed once with a saline solution containing 1 mM $CaCl_2$, 10 mM glucose and 1 mg/ml bovine serum albumin. The data are mean values (range) of the experiments indicated in parenthesis

phosphatidylserine and β-NGF in vivo could be relevant to explain the systemic effects elicited by the phospholipid administration. However, preliminary tests do not support this possibility since lysophosphatidylserine retains full effectiveness in mice after bilateral sialoadenectomy.

The present results suggest that rat peritoneal mast cells possess receptors for nerve growth factor. These receptors are able to initiate the sequence of histamine secretion in the presence of lysophosphatidylserine and a minimal concentration (5×10^{-11} M) of the ligand. The possibility that the receptors originate from bound IgE anti-mouse nerve growth factor seems unlikely since the rats had no contact with mice. Furthermore, rat serum did not prevent the effect of mouse nerve growth factor and both the mouse and the cobra preparations were active. The interaction between lysophosphatidylserine and nerve growth factor on rat peritoneal mast cells provides a simple, sensitive method to assay the biological activity of these compounds. In view of the involvement of phospholipase A_2 in the inflammatory response [17] and considering the possibility of nerve growth factor production in peripheral tissues [15], this test is suitable to investigate the participation of lysophosphatidylserine and nerve growth factor-like proteins in a system of mast cell activation.

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