Lysis of chromaffin granules by phospholipase A_2-treated plasma membranes

A cell-free model for exocytosis in adrenal medulla

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Received 12 December 1985

The possible involvement of phospholipase A_2 (PLA_2) in the release of catecholamines was examined in a cell-free model system, using isolated chromaffin granules and plasma membranes of adrenal medulla cells. Plasma membranes treated with PLA_2 in the presence of Ca^{2+} caused lysis of chromaffin granules which was also dependent on Ca^{2+}. This finding suggests that Ca^{2+} acts in two steps of exocytosis, namely in the transformation of plasma membranes into a lytic form by PLA_2 and in the interaction of chromaffin granules with plasma membranes. These findings show good agreement with recently reported findings in leaky adrenal medulla cells, and suggest the involvement of PLA_2 in the release of catecholamines.

Ca^{2+} *Catecholamine* *Chromaffin granule* *Exocytosis* *Phospholipase A_2* *Plasma membrane*

1. INTRODUCTION

Ca^{2+} plays a pivotal role in the exocytotic secretion of catecholamines from the adrenal medulla. Stimulation of the acetylcholine receptor evokes a rapid influx of Ca^{2+} [1,2] and a transient rise in cellular Ca^{2+}, concentration [3] which triggers the secretory process. In leaky adrenal medulla cells [4–6] which were rendered permeable to Ca^{2+}, micromolar Ca^{2+} was shown to cause exocytotic secretion of catecholamines. Phospholipase A_2 (PLA_2, EC 3.1.1.4) is a Ca^{2+}-dependent enzyme which releases unsaturated fatty acid from the *sn*-2-position of membrane phospholipids. Because lysophospholipids [7] and unsaturated fatty acids such as arachidonic acid [8] are known to be fusogens, it is possible that this enzyme plays a role in exocytosis. Recently, in leaky adrenal medulla cells, Frye and Holz [9] reported that Ca^{2+} caused the release of arachidonic acid in parallel with catecholamine release and suggested the possible involvement of PLA_2 in secretion of catecholamines. We reported previously that cytoplasmic protein which was partially associated to microsomes caused an all-or-none type lysis of chromaffin granules [10] and that lysis of chromaffin granules was augmented by PLA_2 in a Ca^{2+}-dependent manner [11]. Here, in an attempt to develop a cell-free experimental system for the study of the action of Ca^{2+} in exocytosis, we treated plasma membranes from bovine adrenal medulla with exogenous PLA_2 and investigated their interaction with chromaffin granules under controlled free Ca^{2+} concentrations in the medium.

2. EXPERIMENTAL

Fresh bovine adrenal medulla was used as the starting material. Chromaffin granules were isolated as in [12]. Plasma membranes were isolated according to Meyer and Burger [13]. Plasma membranes (5 mg protein/ml medium) were treated

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00145793/86/$3.50

with PLA_2 (porcine pancreas, Boehringer, 3 U/ml) for 5 min at 37°C in a medium consisting of 150 mM KCl, 50 mM Tris (pH 7.4) and 5 mM EGTA or Ca-EGTA (p*Ca* 7.37–5.77). After incubation, plasma membranes were sedimented by centrifugation (105 000 × *g*, 1 h) and washed once with distilled water. Release of catecholamines was examined as follows. Chromaffin granules were incubated with PLA_2-treated plasma membranes (50 μg/ml) in 150 mM KCl, 50 mM Tris (pH 7.4), 5 mM Ca-EGTA (p*Ca* 7.37–5.77) for 5 min at 37°C. After incubation, non-lysed granules and lysed granule membrane components were sedimented by centrifugation (20 000 × *g*, 10 min). The supernatant was subjected to assay of catecholamines [14] and dopamine β-hydroxylase [15].

3. RESULTS AND DISCUSSION

Konings and De Potter [16–18] reported that plasma membranes from adrenal medulla cells caused, by themselves, a Ca^{2+}-dependent release of catecholamines from the granules. However, Burgoyne [19] has indicated that they were unable to confirm this finding. In our experiment, plasma membranes which were not treated with PLA_2 and those treated with PLA_2 in the presence of EGTA did not cause the release of catecholamines from chromaffin granules. In contrast, plasma membranes treated with PLA_2 in the presence of free Ca^{2+} (p*Ca* > 6.79) caused the release of catecholamines (fig.1A). The half-maximal concentration of Ca^{2+} which was required in PLA_2 treatment was p*Ca* 6.7. The interaction of chromaffin granules with PLA_2-treated plasma membranes was also dependent on free Ca^{2+}, the half-maximal concentration being p*Ca* 6.4 (fig.1B). These findings suggest the possibility that Ca^{2+} has at least two actions in the release of catecholamines, the activation of PLA_2 and the interaction of chromaffin granules with plasma membranes. Recently, in leaky adrenal medulla cells, micromolar Ca^{2+} has been shown to cause the release of arachidonic acid and catecholamines [9]. In these cells, the concentration of Ca^{2+} which was required for release of arachidonic acid was lower than that for catecholamine release, showing a good agreement with our findings in the cell-free system.

Release of catecholamines caused by PLA_2-treated plasma membranes was inhibited by high

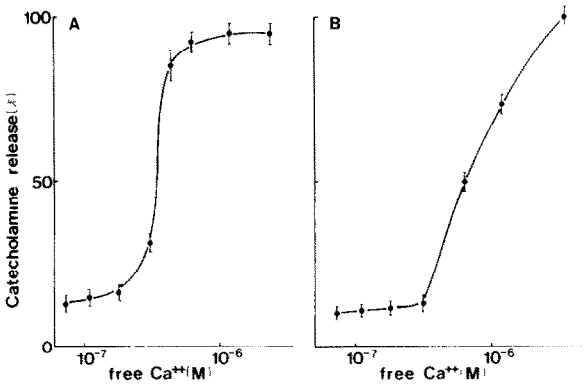

Fig.1. Release of catecholamines from chromaffin granules induced by PLA_2-treated plasma membranes. (A) Plasma membranes were treated with PLA_2 at various free Ca^{2+} concentrations. Incubation with chromaffin granules was carried out for 5 min at 37°C. The free Ca^{2+} concentration during catecholamine release was adjusted to p*Ca* 5.77. (B) Plasma membranes were treated with PLA_2 in the presence of fixed Ca^{2+} (p*Ca* 5.77). Release of catecholamines was examined at various concentrations of free Ca^{2+} in the medium. Catecholamine release was expressed by percent release. Values are the means from 5 experiments each; SD shown by the vertical bars.

concentration of Mg^{2+} (fig.2A). However, plasma membranes treated with PLA_2 in the absence or presence of Mg^{2+} showed the same lytic potency suggesting that Mg^{2+} did not inhibit the action of PLA_2 (fig.2B). This result again agrees with the finding in leaky cells that Mg^{2+} inhibited the release of catecholamines but not of arachidonic acid [9]. Therefore, Mg^{2+} seems to antagonize preferentially the interaction of chromaffin granules with plasma membranes.

Dopamine β-hydroxylase, a large molecular marker of the soluble component of the granules, was released into the medium along with catecholamines upon incubation with PLA_2-treated plasma membranes (fig.3). This shows that release of catecholamines caused by PLA_2-treated plasma membranes was due to the lysis of chromaffin granules. In exocytotic secretion of catecholamines, the soluble content of the granules is entirely extruded directly into the extracellular space. The all-or-none type lysis of granules observed in this experiment may represent the change in granule structure characteristic of exocytosis. In intact

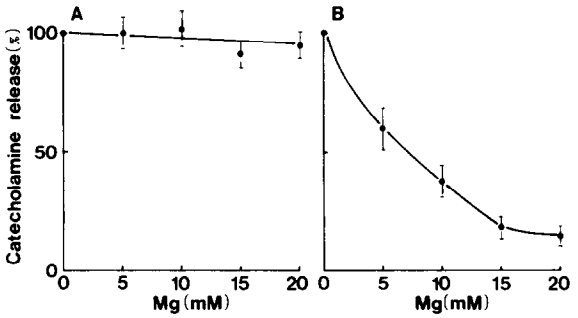

Fig.2. Effect of Mg^{2+} on the release of catecholamines induced by PLA_2-treated plasma membranes. (A) Plasma membranes were treated with PLA_2 at various concentrations of $MgCl_2$. Plasma membranes were collected by centrifugation and incubated with chromaffin granules in the absence of Mg^{2+}. The free Ca^{2+} concentration of the medium was p*Ca* 5.77 throughout the experiment. (B) Chromaffin granules were incubated with PLA_2-treated plasma membranes at various concentrations of $MgCl_2$ in the medium. The free Ca^{2+} concentration in the medium was p*Ca* 5.77. Values are the means from 4 experiments each; SD shown by the vertical bars.

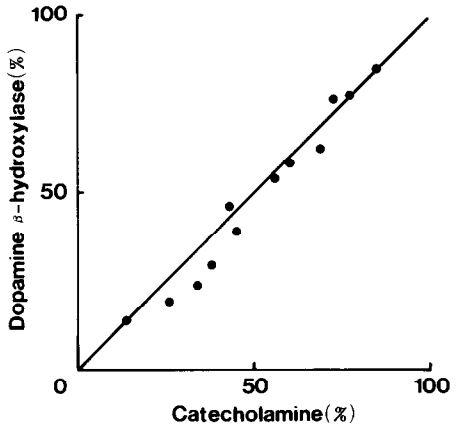

Fig.3. Release of catecholamines and dopamine β-hydroxylase from chromaffin granules induced by PLA_2-treated plasma membranes. Chromaffin granules were incubated with various amounts of PLA_2-treated plasma membranes (5–50μg protein/ml incubation medium). Catecholamines and dopamine β-hydroxylase were assayed and expressed by percent release. Release of dopamine β-hydroxylase was plotted vs release of catecholamines.

adrenal medulla cells, influx of Ca^{2+} evoked by stimulation of acetylcholine receptor may activate endogenous PLA_2 of the cells and convert the plasma membranes into the lytic form, and Ca^{2+} also plays an indispensable role in the interaction of granules with plasma membranes.

Recently accumulated evidence shows that PLA_2 activation is involved in several types of secretory processes [20]. Moskowitz et al. [21] suggested that synaptic vesicle PLA_2 activation may be an important mechanism underlying Ca^{2+}-mediated neurotransmitter release. In rat brain synaptosomes, Bradford et al. [22] suggested that Ca^{2+} influx by synaptosomal depolarization activated endogenous PLA_2 and modulated the secretion of catecholamines. Activation of PLA_2 by Ca^{2+} might be considered as a preliminary step in triggering of the release process. In addition, our results suggest that Ca^{2+} also plays an indispensable role in the fusion of chromaffin granules with plasma membranes.

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