Volume 314, number 1, 17–19 FEBS 11818 December 1992

Initiator–promotor coupling of phospholipases D and A_2 in platelets upon cholesterol incorporation

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Received 13 October 1992

Since phospholipases exist within a membrane lipid environment, it is not unreasonable to assume that cholesterol capable of changing the lipid environment can effect the coupling relationship among the signal transducing components. Our previous study showed that the 'molecular switch' through which membrane cholesterol modulates cyclic nucleotide levels and Na^+/H^+ exchange within human platelets is phospholipase A_2. We demonstrate here that membrane cholesterol initiates the activation of phosphatidyl choline phospholipase D and phosphatidic acid thus generated promotes the activation of its phospholipase A_2 in the presence of extraplatelet calcium. More important, inhibition of phospholipase D by zinc blocks the activation of phosphatidic acid phospholipase A_2 in platelets upon cholesterol incorporation. Our result led us to postulate that membrane cholesterol induced initiation promotion coupling of phospholipases D and A_2 in human platelets may be responsible for the hypersensitized state of platelets in hypercholesterolemic patients.

Cholesterol; Phospholipase D; Phospholipase A_2; Initiation–promotion coupling; Platelet

1. INTRODUCTION

For the last decade or so interest in human platelets was generated not only because of their increased sensitivity to aggregating agents in hypercholesterolemic patients [1,2] but also they exhibit an excellent and unique human cellular model for the understanding of transmembrane signalling pathway. Consequently our recent studies [3,4], directed to understand the complex role of membrane cholesterol in the regulation of the transmembrane signalling pathway, sought to establish that the 'molecular switch' through which membrane cholesterol modulates cyclic nucleotide levels and Na^+/H^+ exchange within human platelets may be phospholipase A_2 which is totally dependent upon extraplatelet calcium and apparently specific for phosphatidyl choline for its activity. These findings prompted us to explore the nature of the interrelationship between phospholipases, especially phospholipase A_2, and phospholipases D in human platelets upon cholesterol incorporation.

2. MATERIALS AND METHODS

2.1. *Materials*

Cholesterol, lecithin, phosphatidic acid and other chemicals were purchased from Sigma. Radioactive nickel was purchased from BARC Bombay (India). All other reagents were of the highest quality available.

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2.2. *Platelet incubation experiments*

As reported previously [3], variation in the platelet-membrane cholesterol content (without any significant change in either the total phospholipid or protein content of platelets) was specifically achieved by incubating platelet-rich plasma with various types of liposomes in Tyrode's solution for up to 5 h, and five types of platelet preparation (having $c/p \approx 0.15$ to 1.2) were subjected to lipid analysis by thin-layer chromatography [5–7] in order to explore the type of phospholipases affected by the variation in the platelet-membrane cholesterol content.

2.3. *Phospholipase assay*

Phosphatidyl choline-specific phospholipase D and phosphatidic acid-specific phospholipase A_2 activities in cholesterol-enriched platelets ($c/p \approx 1.2$) were studied using standard methods [8]. The platelets were enriched with cholesterol in the presence of varying concentrations of zinc (0–4 mM) and subsequently analysed for phospholipase D and A_2 activities. Phospholipase A_2 activity (specific for phosphatidic acid) was also assayed in normal platelets exposed to varying concentrations of phosphatidic acid (0–50 μM) in the presence and absence of 3 mM EGTA.

3. RESULTS

In agreement with our previous results [3], the present study reveals that the acquisition or depletion of cholesterol from platelets was highly selective and there was no change in various types of phospholipids or phosphoinositides present in the platelets (Figs. 1 and 2) However, there was a significant increase in phosphatidic acid generation in platelets upon cholesterol incorporation in the absence of extraplatelet calcium (Fig. 1B). Further, in cholesterol-enriched platelets, (a) there was significant increase in phosphatidyl choline-specific and calcium-independent phospholipase D activity and this activity was blocked by zinc in a dose-dependent fashion (Fig. 3), and (b) there was

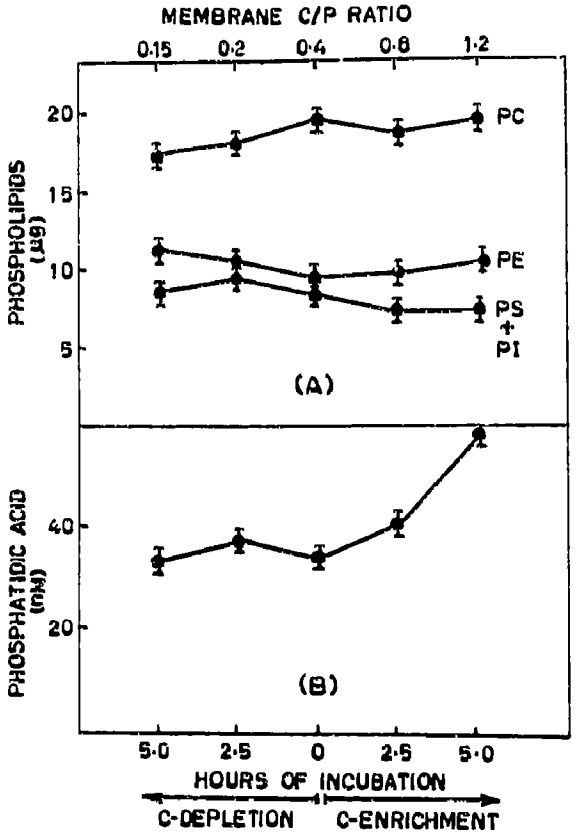

Fig. 1. Effect of depletion and acquisition of cholesterol by human platelets on their phospholipid composition and phosphatidic acid generation studied in the absence of extraplatelet calcium. Each value in (A) and (B) represents the mean ± S.D. of experiments done in triplicate.

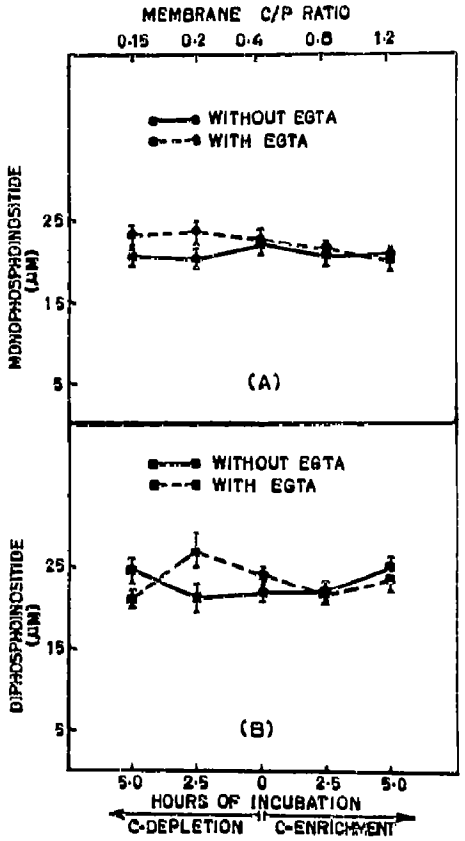

Fig. 2. Effect of depletion and acquisition of cholesterol by human platelets on their phosphoinositide composition studied in the presence and absence of extraplatelet calcium. Each value in (A) and (B) represents the mean ± S.D. of experiments done in triplicate.

significant increase in phosphatidic acid-specific phospholipase A_2 activity and this activity was abolished by zinc in a dose-dependent fashion (Fig. 3). Unmodified normal platelets, when exposed to increasing concentrations of phosphatidic acid (0–50 μM) coupled with or without 3 mM EGTA, exhibited extra-platelet calcium-sensitive increased phosphatidic acid-specific phospholipase A_2 activity as a function of extra-platelet phosphatidic acid concentration availability (Fig. 4).

4. DISCUSSION

Platelet activity in hypercholesterolemic human subjects has been shown to be elevated with regard to platelet adhesion, aggregation, release of increased amounts of malondialdehyde (MDA) and thromboxane A_2 (TxA_2) [9]. These changes in platelet activity are paralleled by the observation of raised levels of cholesterol to phospholipid ratio in platelets of these patients [10]. It is in this context that the results reported here as well as reported earlier [3] may be of importance in defining the membrane-cholesterol modulated transmembrane signalling pathway responsible for platelet hypersensitivity phenomenon observed in hypercholesterolemic human subjects. The results reported here clearly indicate that an increase in the platelet membrane-cholesterol content results in the activation of phospholipase D and the phosphatidic acid thus generated acts as an ionophore for calcium leading to the activation of phospholipase A_2 specific for phosphatidic acid (Fig. 5). This

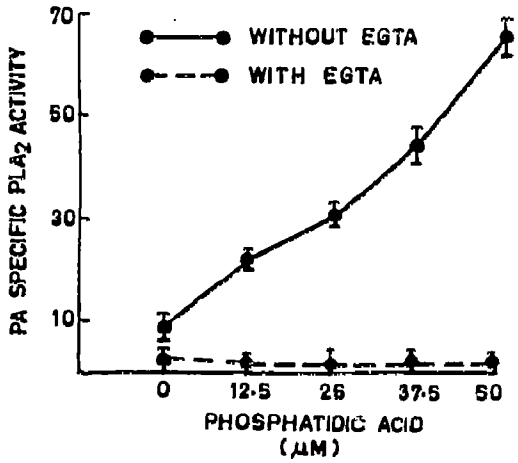

Fig. 3. Effect of different concentrations of extraplatelet phosphatidic acid on PA-specific PLA_2 activity in the presence and absence of EGTA. Each value represents the mean ± S.D. of experiments done in triplicate.

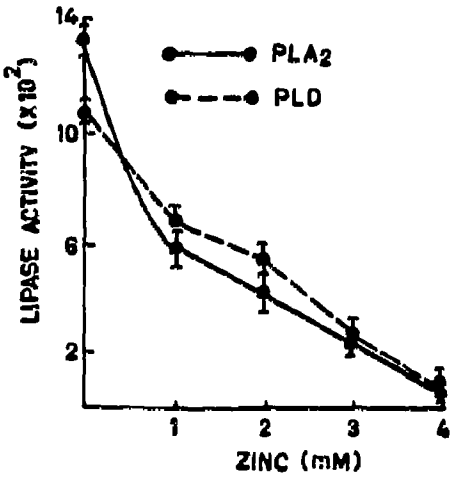

Fig. 4. Effect of different concentrations of zinc on the lipase activity of cholesterol-enriched platelets having $c/p \approx 1.2$. Each value represents the mean ± S.D. of experiments done in triplicate.

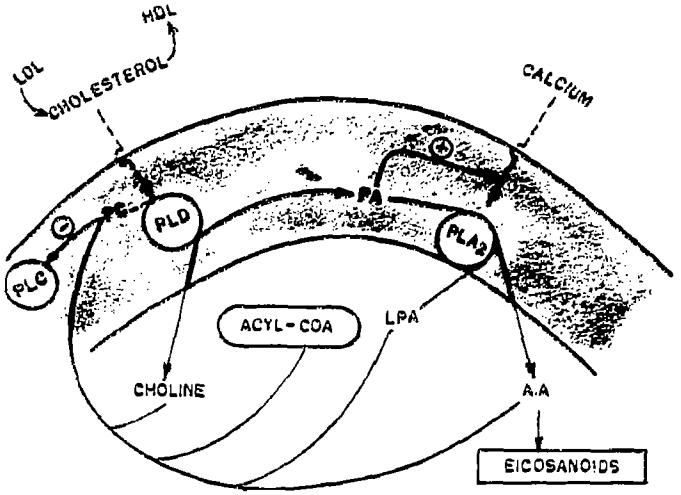

Fig. 5. Initiator–promotor coupling model in platelets upon cholesterol incorporation.

initiation–promotion coupling of phospholipase D and A_2 results in the regulation of cyclic nucleotide levels and Na^+/H^+ exchange within platelets observed by us recently [3,4]. The lysophosphatidic acid generated through this coupling of phospholipase D and A_2 is recycled back to phosphatidyl choline (Fig. 5) because over all phospholipid composition did not change in platelets exposed to cholesterol-rich or cholesterol-poor liposomes.

In conclusion, we propose that membrane cholesterol-induced initiation–promotion coupling of phospholipase D and A_2 in human platelets may be the basic molecular mechanism responsible for their increased sensitivity to various agonists in hypercholesterolemic human subjects.

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