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Inhibitors of arachidonic acid metabolism eliminate the increase in cytosolic free calcium induced by the mitogen concanavalin A in rat thymocytes

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Using inhibitors of arachidonic acid (AA) metabolism, the possible involvement of AA products in the generation of $[Ca^{2+}]_i$ and the pH_i rise induced by the mitogen concanavalin A (Con A) in rat thymocytes has been studied. The lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA, 10 μM) and the phospholipase A_2 inhibitor bromophenacyl bromide (10 μM) eliminated the $[Ca^{2+}]_i$ signal induced by Con A; the cyclooxygenase blocker indomethacin also inhibited it. However, neither NDGA nor indomethacin suppressed the pH_i rise stimulated by Con A. Exogenous AA induced an increase in $[Ca^{2+}]_i$ but not in the pH_i. These results indicate that AA metabolites, probably of the lipoxygenase pathway, take part in the generation of the $[Ca^{2+}]_i$ response to the mitogen. In contrast, they appear not to be involved in the pH_i rise evoked by Con A.

Arachidonic acid; Ca^{2+}, cytosolic free; Concanavalin A; (Rat thymocyte)

1. INTRODUCTION

Changes in ion transport and intracellular concentration are one of the key events in lymphocyte activation [1,2]. An increase in $[Ca^{2+}]_i$ and pH_i is observed during the first minutes of stimulation of rat thymocytes with the mitogenic lectin Con A [3,4]. The mechanism of generation of these ionic signals and their relation to another crucial event in lymphocyte activation, the stimulation of phospholipid metabolism, remains unclear [3–6].

AA and its metabolites formed by the lipoxygenase or cyclooxygenase pathways play an important role in regulation of various physiological processes [7,8]. Stimulation of AA liberation via PLA_2 or phospholipase C followed by diacylglycerol lipase has been observed in a number of receptor-operated processes accompanied by an increase in $[Ca^{2+}]_i$ [9,10]. Mitogens have been shown to stimulate AA liberation in thymocytes and blood lymphocytes [11,12]. However, the role of AA and its metabolites in the generation of ionic signals under the action of mitogens has not been investigated.

In the present work we have studied the influence of inhibitors of AA metabolism on $[Ca^{2+}]_i$ and the pH_i rise induced by Con A in rat thymocytes. Evidence is obtained which indicates the involvement of AA metabolites, probably of the lipoxygenase pathway, in the formation of the Ca response to the mitogen. In contrast, the pH_i rise appears not to be mediated by AA or its metabolites.

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Abbreviations: AA, arachidonic acid; NDGA, nordihydroguaiaretic acid; Con A, concanavalin A; BCECF, 2',7'-biscarboxyethyl-5(6)-carboxyfluoresceine; $[Ca^{2+}]_i$, cytosolic free Ca^{2+} concentration; pH_i, cytosolic pH; PLA_2, phospholipase A_2

2. MATERIALS AND METHODS

Thymocytes were obtained from Wistar rats as described in [4]. $[Ca^{2+}]_i$ and pH_i were measured using the fluorescent probes

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quin-2 and BCECF, respectively. Prior to the experiment the cells were resuspended in standard buffered saline containing 140 mM NaCl, 5.4 mM KCl, 1.3 mM $CaCl_2$, 1 mM $MgSO_4$, 1 mM KH_2PO_4, 1 mM Na_2HPO_4, 4 mM $NaHCO_3$, 6 mM glucose, and 10 mM Hepes, pH 7.2, and incubated at 37°C for 40 min with 10 μM quin-2 acetoxymethyl ester or 1 μM BCECF acetoxymethyl ester. Then the thymocytes were washed twice and placed in the medium free of the dyes. The fluorescence was measured in a special spectrofluorimeter [3,4] at 37°C and continuous stirring; the cell concentration in the 2 ml cuvette was $(1-2) \times 10^7$ ml^{-1}. The excitation and emission wavelengths were 337 and 495 nm for quin-2 and 500 and 530 nm for BCECF, respectively. The $[Ca^{2+}]_i$ and pH_i values were calculated as in [3,4].

Con A was obtained from Sigma; quin-2 and BCECF acetoxymethyl esters from Calbiochem; NDGA, AA, bromophenacyl bromide, indomethacin from Serva; U46619 from Upjohn Co., Kalamazoo, MI.

3. RESULTS AND DISCUSSION

NDGA, an inhibitor of the lipoxygenase pathway of AA metabolism [13], blocks the Ca response to Con A although it does not change the $[Ca^{2+}]_i$ resting level (fig.1). In the presence of 5 μM NDGA the $[Ca^{2+}]_i$ rise is both weakened and retarded, 10 μM NDGA completely eliminates the Ca response. Indomethacin also inhibits the Ca response to Con A; however, its effect is pronounced only at concentrations of about 10 μM which cannot only block the cyclooxygenase but also the lipoxygenase pathway. At a concentration of 1–2 μM, at which indomethacin selectively blocks cyclooxygenase [8,12,14], its inhibitory effect on the Ca response is rather weak (fig.1).

The data presented indicate that in rat thymocytes AA metabolites, and in particular lipoxygenase metabolites, are involved in the formation of the Ca response to the mitogen.

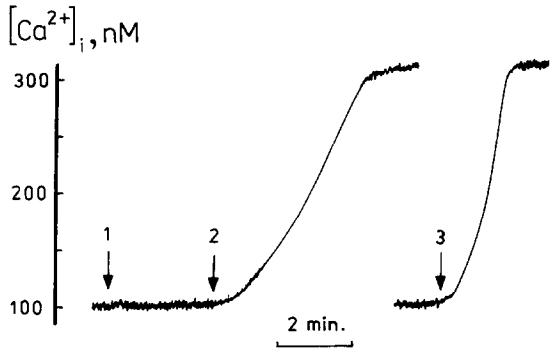

Fig.2. Changes in $[Ca^{2+}]_i$ induced by 2 μM U46619 (1); 15 μg/ml Con A (2); 4 μM AA (3).

The Con A-induced $[Ca^{2+}]_i$ rise is also eliminated by bromophenacyl bromide, an inhibitor of PLA_2 (fig.1). This suggests that PLA_2 may mediate the Ca response to the mitogen. (Note, however, that in some cells bromophenacyl bromide inhibits not only PLA_2 but also phospholipase C [8,15]).

It has been found that platelets generate a two-phase Ca response to a number of stimuli [16–18]. Collagen, for example, first stimulates the production of a cyclooxygenase AA metabolite, thromboxane A_2 (TXA_2), and this stage proceeds without an increase of $[Ca^{2+}]_i$. During the second phase TXA_2 is released from the cell and stimulates, via interaction with a receptor on the

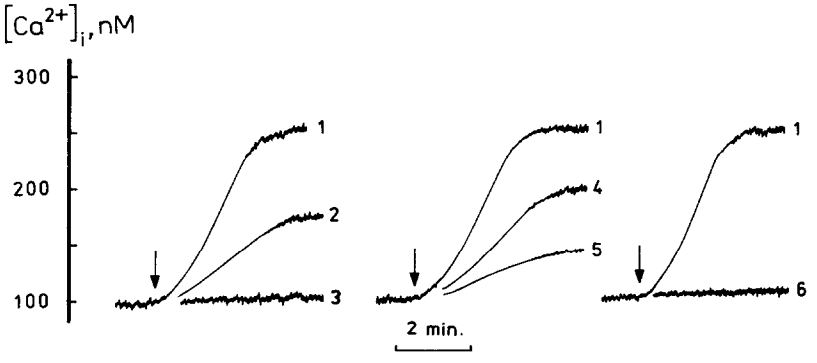

Fig.1. Changes in $[Ca^{2+}]_i$ evoked by 15 μg/ml Con A (arrow) in the absence (1) or presence of inhibitors of AA metabolism (2–5) and PLA_2 inhibitor (6). 2 min prior to application of Con A the inhibitors were added: 5 μM (2) or 10 μM (3) NDGA; 2 μM (4) or 10 μM (5) indomethacin; 10 μM bromophenacyl bromide (6). This and other figures are examples of four to five similar experiments on minimally three thymocyte preparations.

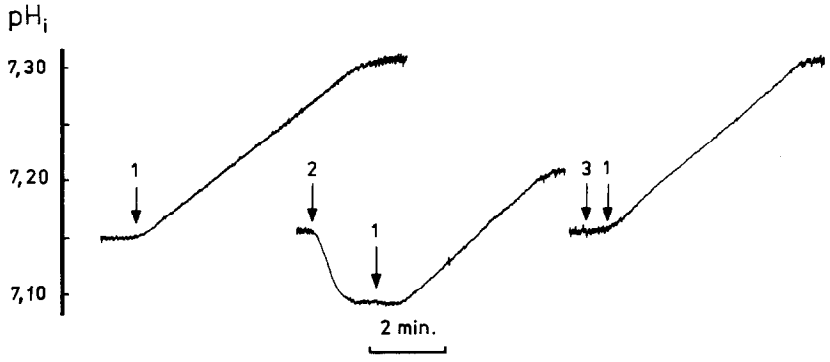

Fig.3. Changes in pH_i induced by 15 μg/ml Con A (1); 10 μM NDGA (2); 2 μM indomethacin (3).

plasma membrane, the phosphoinositide turnover which results in a $[Ca^{2+}]_i$ increase necessary for platelet secretory response to collagen. The same Ca response can be generated by TXA_2 or its analogues, instead of collagen [16]. It might be thought that a similar mechanism is realized in rat thymocytes. However, as distinct from platelets, in these cells TXA_2 analogue U46619 produces no $[Ca^{2+}]_i$ increase (fig.2). Thus, in thymocytes the Ca response to Con A seems to be mediated by other AA metabolites.

Exogenous AA evokes a concentration-dependent increase in $[Ca^{2+}]_i$ (fig.2). At AA concentrations above 10 μM $[Ca^{2+}]_i$ rises to a level above 1 μM, i.e. to a complete saturation of quin-2 with Ca^{2+}. At 4 μM exogenous AA the magnitude of the Ca signal is close to that induced by Con A.

In contrast to the Ca signal, neither NDGA nor indomethacin prevents the Con A-induced pH_i rise (fig.3). Hence, AA metabolites appear not to be involved in the stimulation of Na^+/H^+ exchange effected by the mitogen. It is worth noting that NDGA causes a slight decrease in the resting pH_i ($\approx$0.05 units), and indomethacin (1–2 μM) does not change it (fig.3). Exogenous AA does not increase the pH_i resting level, it even produces a weak acidification of the cytoplasm which is potentiated by NDGA (fig.4).

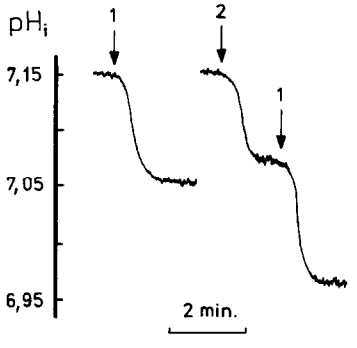

Fig.4. Changes in pH_i induced by 4 μM AA (1) and 5 μM NDGA (2).

The results obtained suggest that AA metabolites, probably of the lipoxygenase pathway, are involved in realization of the Ca response to Con A: in the presence of AA metabolism inhibitors the mitogen induces no $[Ca^{2+}]_i$ increase. The $[Ca^{2+}]_i$ and pH_i signals seem to be generated by different mechanisms since the latter is not eliminated by the inhibitors of AA metabolism.

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