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A dual effect of arachidonic acid on Ca^{2+} transport systems in lymphocytes

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Abstract A sustained increase of cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) in lymphocytes was induced by a potent inhibitor of the intracellular Ca^{2+}-pump 2,5-di-(tert-butyl)-1,4-hydroquinone (BHQ) that effectively depletes Ca^{2+} stores and produces Ca^{2+} influx. The addition of low concentrations of arachidonic acid (AA) (less than 10 μM) in the presence of a BHQ-induced Ca^{2+} response produced a $[Ca^{2+}]_i$ decrease. At the same time at higher concentrations, AA induced an additional increase of $[Ca^{2+}]_i$. The unidirectional uptake of Mn^{2+} into fura-2 loaded thymocytes was used to examine the effects of AA and BHQ on Ca^{2+} permeability of the plasma membrane. Judging by the rate of Mn^{2+}-induced fura-2 fluorescence quenching, AA causes a concentration-dependent increase of the divalent cations' permeability both in the control solution and in the presence of BHQ. These results allowed us to suggest that the decrease of BHQ-induced $[Ca^{2+}]_i$ stationary levels caused by low AA concentrations is due to the enhancement of Ca^{2+} extrusion from the cell, whereas the additional $[Ca^{2+}]_i$ increase at high AA concentrations results from an elevation of cell membrane Ca^{2+} permeability.

Key words: Arachidonic acid; Ca^{2+}, cytosolic free; Divalent cation transport; Ca^{2+}-store; Rat thymocyte; Human lymphocyte

1. Introduction

Earlier [1] in experiments on rat thymocytes it has been shown that exogenous arachidonic acid (AA) (3 μM) produces a complete blockade of $[Ca^{2+}]_i$ and pH_i responses induced by lectin mitogens. The purpose of the present study was to examine the effect of exogenous AA on membrane Ca^{2+}-transport activated by the depletion of the intracellular Ca^{2+}-stores. The latter was induced by 2,5-di-(tert-butyl)-1,4-hydroquinone (BHQ), an inhibitor of intracellular Ca^{2+}-stores Ca^{2+}-ATPase, that effectively depletes Ca^{2+}-stores and promotes Ca^{2+} influx [2,3].

The experiments performed on rat thymic lymphocytes and human peripheral blood lymphocytes revealed a dual effect of AA on $[Ca^{2+}]_i$ response induced by BHQ: a decrease of this response at AA low concentrations (less than 10 μM) and the additional $[Ca^{2+}]_i$ increase at high concentrations of AA. The analysis of the nature of these effects of AA allowed us to suggest that the former ('inhibitory') effect of AA was apparently due to the acceleration of Ca^{2+} extrusion from the cell, whereas the latter (enhancing) effect results from an increase of cell membrane Ca^{2+} permeability.

2. Materials and methods

Thymocytes were obtained from Wistar rats (150–180 g) by teasing out the thymus tissue through a nylon mesh into medium 199 [4]. Then the cells were washed twice by centrifugation (at 800 × *g* for 3 min) and resuspended in standard buffered saline containing (mM): NaCl, 140; KCl, 5.4; $CaCl_2$, 1.2; Na_2PO_4, 1; KH_2PO_4, 1; $MgSO_4$, 1; $NaHCO_3$, 4; glucose, 6; HEPES 10 mM (pH 7.3). Prior to the experiment the cells were incubated at 37°C for 40 min with 1 μM fura-2-acetoxymethyl ester (fura-2/AM). Then the cells were washed twice and placed in medium free of the dyes. The fluorescence was measured on a Hitachi F-4000 spectrofluorimeter at 37°C under continuous stirring. The cell concentration in the 2 ml cuvette was 5×10^6 cells/ml. The excitation and emission wavelengths were 335 and 500 nm, respectively. The fluorescence of fura-2 was calibrated using digitonin (DIG) and Mn^{2+} as described [5–8]. Cells were lysed with DIG (50 μM) to saturate the dye and establish maximal fluorescence. Mn^{2+} (1 mM) was added to reduce dye fluorescence to a minimum value. A dissociation constant of 224 nM for the fura-2 · Ca^{2+} complex was used to evaluate $[Ca^{2+}]_i$ [5].

In the experiments on determination of Mn^{2+} influx (200 μM $MnCl_2$ was added directly to the cuvette with cell suspension), Mn^{2+} uptake was monitored as the rate of quenching of fura-2 fluorescence measured at the Ca^{2+}-insensitive wavelengths (excitation 360 nm and emission 500 nm). The rate of fluorescence decrease, measured at the Ca^{2+} isobestic point, provides a relative measure of the divalent cations permeability [2].

Fura-2/AM and BHQ were obtained from Calbiochem; AA from Serva; DIG from Merck.

3. Results and discussion

The ability of AA to alter the effect of BHQ on $[Ca^{2+}]_i$ in lymphocytes was examined using the fluorescent Ca^{2+} probe fura-2. BHQ was used in a concentration of 10 μM, which induced a maximal Ca^{2+}-response of thymic lymphocytes in normal Ca^{2+}-containing medium and maximal Ca^{2+} release from the intracellular Ca^{2+}-stores in Ca^{2+}-free medium [2]. In our experiments the addition of 10 μM BHQ to rat thymocytes increased $[Ca^{2+}]_i$ by 108 ± 7 nM (n = 27). After maximal elevation of $[Ca^{2+}]_i$ by BHQ, AA in concentrations less than 10 μM produced an abrupt decrease of $[Ca^{2+}]_i$ to a new plateau level (Fig. 1A,B,C). In contrast, at concentrations higher than 11 μM AA induced an additional sustained elevation of $[Ca^{2+}]_i$ as shown in Fig. 1E,F. Qualitatively similar effects of AA were observed in 20 experiments.

The effects of the combined application of BHQ and AA on $[Ca^{2+}]_i$ were also examined on human peripheral blood lymphocytes. The addition of AA on a background of 10 μM BHQ action produced dual changes in $[Ca^{2+}]_i$ very similar to those observed in rat thymocytes (data not shown). These results led us to investigate the concentration dependence of the effects of AA on $[Ca^{2+}]_i$ in resting (non-treated) cells.

Fig. 2 demonstrates the effects of AA on $[Ca^{2+}]_i$. It can be seen that at 'low' AA concentrations, $[Ca^{2+}]_i$ at first increased

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Abbreviations: AA, arachidonic acid; $[Ca^{2+}]_i$, cytosolic free calcium concentration; pH_i, cytosolic H^+; BHQ, 2,5-di-(tert-butyl)-1,4-hydroquinone; BSA, bovine serum albumin; fura-2/AM, fura-2-acetoxymethyl ester; DIG, digitonin.

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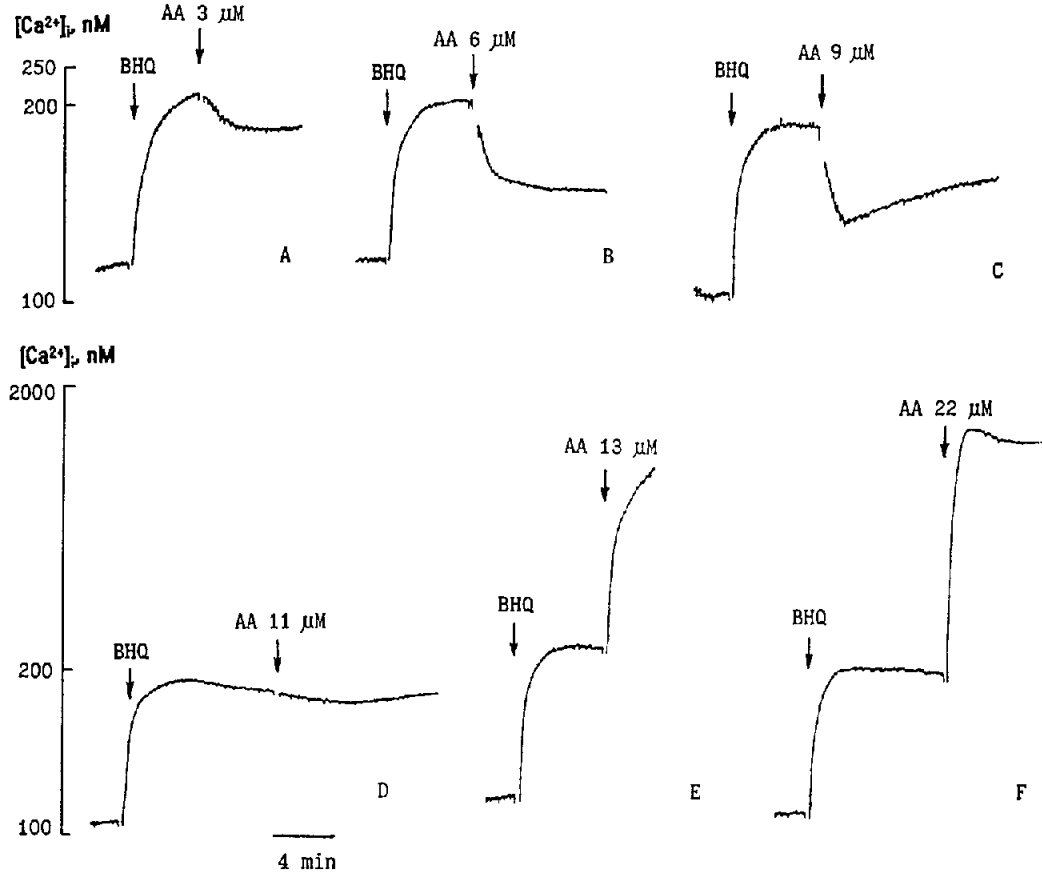

Fig. 1. Effect of AA on the $[Ca^{2+}]_i$ rise induced by BHQ (10 μM) in rat thymocytes. The traces represent at least four experiments.

to its peak values (Fig. 2A,B) and then decreased to the plateau level. This decrease diminished with the increase of AA concentration (Fig. 2C) and finally (at AA higher than 15 μM) often disappeared completely (Fig. 2D).

Since in Ca^{2+}-free medium AA induces only a transient $[Ca^{2+}]_i$ increase [9,10], it is reasonable to suppose that the peak component of the Ca^{2+} response induced by low concentrations of AA is mainly due to Ca^{2+} release from intracellular Ca^{2+}-stores, while the sustained plateau level results from an increase of plasma membrane Ca^{2+} permeability. At higher AA concentrations the latter component becomes dominating and masks the initial one.

In order to study the effects of AA on Ca^{2+} permeability of the plasma membrane the unidirectional uptake of Mn^{2+} into fura-2-loaded thymocytes was measured. Mn^{2+}, which is widely used as a probe for Ca^{2+} influx pathways in different non-excitable cells [11–13], enters the unstimulated lymphocytes. Fig. 3B shows that the rate of fura-2 quenching produced by Mn^{2+} was slightly accelerated by the addition of 3 μM AA. This effect was further increased by the consequent elevation of the AA concentration up to 10 μM and 20 μM, which revealed the activation of a divalent cation permeability pathway in the plasma membrane. The final addition of DIG (50 μM) to the cell suspension had resulted in the effective influx of Mn^{2+} which rapidly abolished the remaining fluorescence. All these effects of AA cannot be explained by the non-specific action of ethanol (Et) used for the dissolving of AA since the application of high ethanol concentrations to thymocytes did not effect the

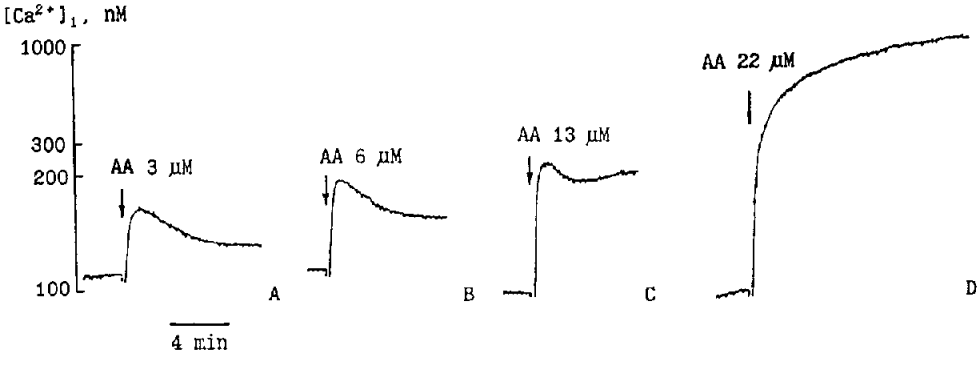

Fig. 2. Effect of AA on $[Ca^{2+}]_i$ in rat thymocytes. The traces represent at least four experiments.

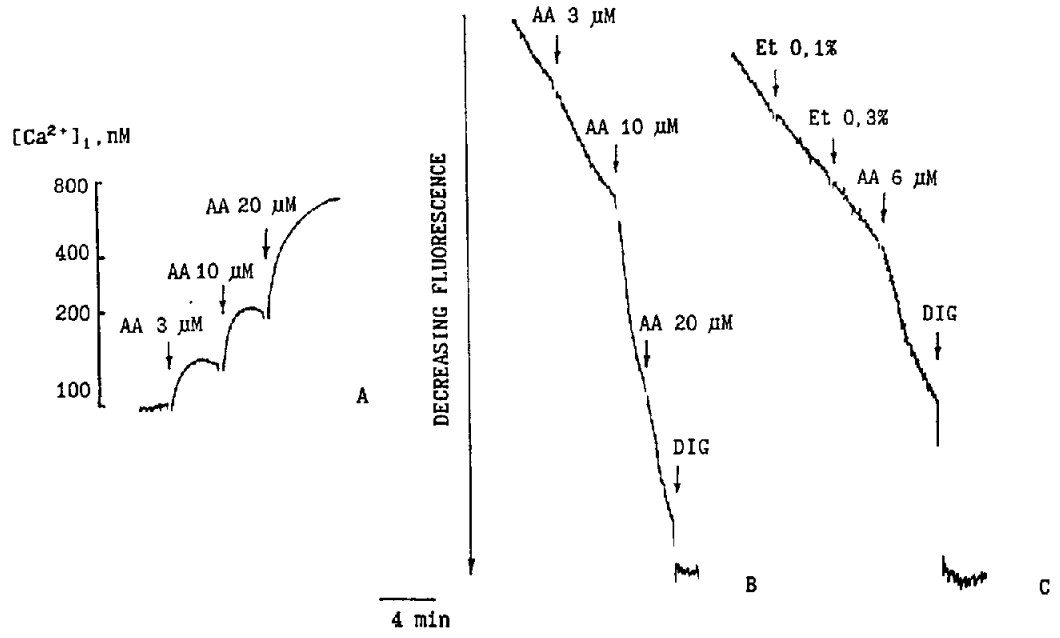

Fig. 3. Effect of AA on $[Ca^{2+}]_i$ (A) and on the rate of Mn^{2+} uptake (B,C). To initiate the experiment $MnCl_2$ (200 μM) was added to the suspension of fura-2-loaded thymocytes and the fluorescence was monitored at the Ca^{2+}-isobestic wavelengths (excitation 360 nm, emission 500 nm). DIG, 50 μM. C: control application of ethanol (Et). The traces represent at least three similar experiments.

Mn^{2+} influx (Fig. 3C). These effects of AA on Mn^{2+} influx in thymocytes were compared with the corresponding AA induced changes of $[Ca^{2+}]_i$ in the sister portions of the same cell suspension (Fig. 3A). It becomes clear that AA at all concentrations used increased $[Ca^{2+}]_i$ that was at least partially due to the increase of cell membrane Ca^{2+} permeability.

If so, what can be the reason for a stable $[Ca^{2+}]_i$ decrease induced by low concentrations of AA applied on a background

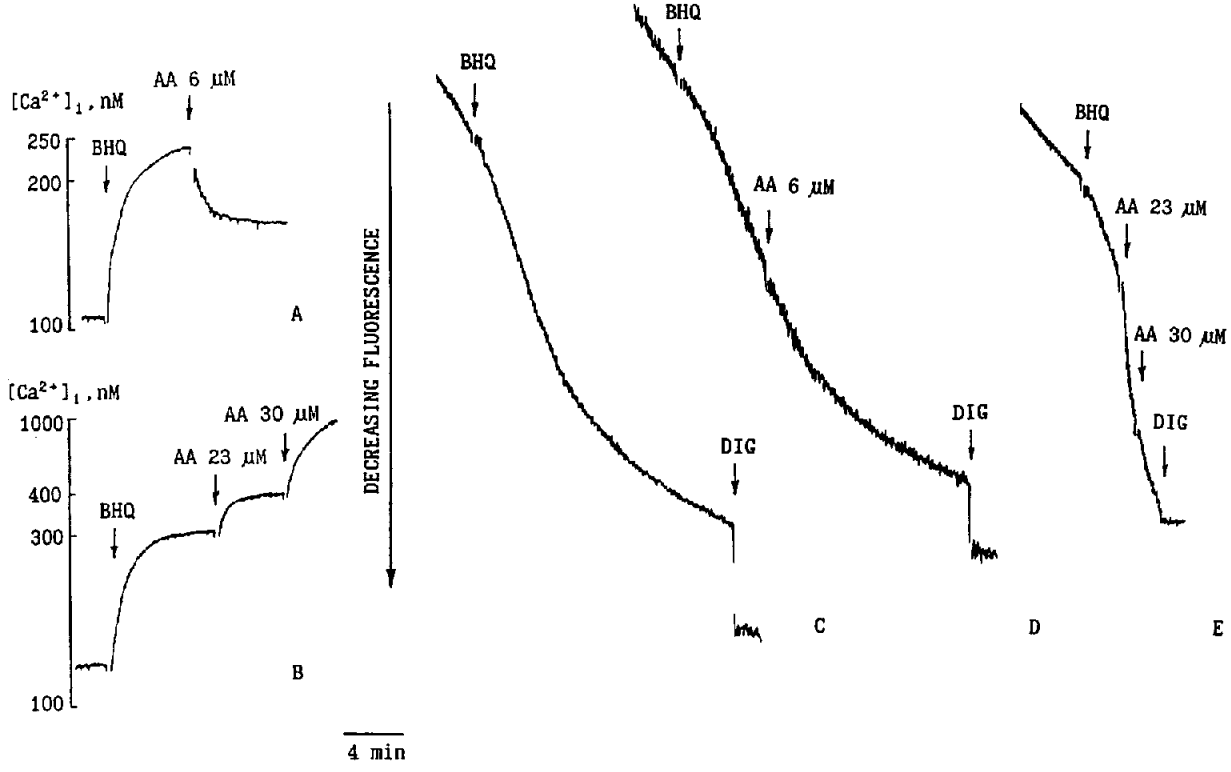

Fig. 4. Effects of BHQ (10 μM) and AA on $[Ca^{2+}]_i$ (A,B), and on the rate of Mn^{2+} uptake (C,D,E). To initiate the experiment $MnCl_2$ (200 μM) was added to the suspension of fura-2-loaded thymocytes and the fluorescence was monitored at the Ca^{2+}-isobestic wavelengths. DIG, 50 μM. The traces represent at least three similar experiments.

of BHQ action (see Fig. 1)? Two possibilities should be considered: (i) AA blocks the Ca^{2+} influx through Ca^{2+} channels, activated by the BHQ-induced depletion of the intracellular Ca^{2+}-stores; or (ii) AA accelerates the efflux of Ca^{2+} through the plasma membrane influencing Ca^{2+}-ATPase. In order to examine the first possibility we have studied the combined effect of BHQ and AA on Mn^{2+} influx. Our experiments have shown that BHQ increased the influx of Mn^{2+} revealing an increase in the permeability of cell membranes for divalent cations. These data correlate with those of Mason et al. [2]. Fig. 4 illustrates this effect of BHQ. The subsequent addition of 6 μM AA which has been shown to decrease the level of the BHQ-induced $[Ca^{2+}]_i$ plateau (Fig. 4A) did not produce any inhibitory effect on the entry of divalent cations (Fig. 4D). Treatment with high concentrations of AA (23 μM) in the presence of BHQ resulted in a more pronounced acceleration of the Mn^{2+} induced fura-2 fluorescence quenching rate (Fig. 4E). Thus neither high nor low AA concentrations inhibit the Ca^{2+} influx through the plasma membrane induced by BHQ.

Hence in order to explain the inhibitory effects of low AA concentrations on BHQ-induced Ca^{2+}-response it is reasonable to hypothesize that AA stimulates Ca^{2+} extrusion from the cell. Some data in the literature seem to support this suggestion. Thus Yurkiv et al. [14] have shown that polyunsaturated fatty acids produce an increase of plasma membrane Ca^{2+}-dependent ATPase activity in enterocytes.

As to the second effect of AA, an increase in the membrane permeability for Ca^{2+}, it is probably due to the physical perturbation of membrane lipids by polyunsaturated fatty acids [15].

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