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Arachidonic acid abolishes the mitogen-induced increase in cytosolic free Ca^{2+} and intracellular pH_i in rat thymocytes

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The effects of arachidonic acid (AA) and the lectin mitogens, concanavalin A (Con A) and phytohemagglutinin (PHA), on $[Ca^{2+}]_i$ and pH_i in rat thymocytes have been studied by using the intracellular fluorescent probes, Fura-2 and BCECF. It was revealed that exogenous AA (3 μM), in addition to the well-known changes in basal $[Ca^{2+}]_i$ and pH_i, also caused a complete blockade of $[Ca^{2+}]_i$ and pH_i signals induced by Con A (10 μg/ml) and PHA (10 μg/ml). In contrast, exposure of thymocytes to mitogens did not prevent the AA-induced increase in $[Ca^{2+}]_i$ and decrease in pH_i. In experiments with sodium propionate, the similarity between AA action and EIPA (ethylisopropylamiloride), an inhibitor of Na^+/H^+ exchangers, was revealed. It is proposed that the inhibitory effect of AA on mitogen-induced lymphocyte proliferation is due primarily to the blockade of transmembrane $[Ca^{2+}]_i$ and pH_i signals, associated with a sustained cytosolic acidification.

Arachidonic acid; Lectin mitogen; Ca^{2+}; H^+; Cytosolic free; Rat thymocyte

1. INTRODUCTION

Recently it has been established that pretreatment of human and animal lymphocytes with arachidonic acid (AA) inhibits cell proliferation induced by concanavalin A (Con A) and some other T-cell mitogens [1–3]. However, the mechanism of this inhibition has not yet been determined. In order to clarify this problem we have studied the effects of AA on the mitogen-induced early changes in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and pH_i that, according to present views [4], play a key role in triggering the proliferative response in various cells. The results obtained suggest that AA is able to block signal transduction from mitogen receptors to the intracellular systems regulating $[Ca^{2+}]_i$ and pH_i. In addition AA suppresses the Na^+/H^+ exchange system.

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 [5]. 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_2HPO_4 1; KH_2PO_4 1; $MgSO_4$ 1; $NaHCO_3$ 4; glucose 6; pH 7.3; HEPES 10 mM. Prior to the experiment the cells were incubated at 37°C for 40 min with 1 μM Fura-2-acetoxymethyl ester or 1 μM BCECF-acetoxymethyl ester. Then the cells were washed twice and placed in medium free of the dyes. Fluorescence was measured in Hitachi F-4000 spectrofluorimeter at 37°C and continuous stirring. The cell concentration in the 2 ml cuvette was 5×10^6 cells/ml. The excitation and emission wavelengths, respectively, were 335 and 500 nm for Fura-2, and 500 and 530 nm for BCECF. The $[Ca^{2+}]_i$ and pH_i values were calculated as in [6–8].

Con A was obtained from Pharmacia; PHA from Difco; Fura-2 and BCECF acetoxymethyl esters from Calbiochem; AA from Serva; EIPA from Merk, and NaPr from Sigma.

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Abbreviations: AA, arachidonic acid; Con A, concanavalin A; PHA, phytohemagglutinin; $[Ca^{2+}]_i$, cytosolic free calcium concentration; pH_i, cytosolic pH; BCECF, 2′,7′-biscarboxyethyl-5(6)-carboxyfluoresceine; NaPr, sodium propionate; EIPA, ethylisopropylamiloride; PUFAs, polyunsaturated fatty acids; IL2, interleukin-2.

3. RESULTS

Earlier it has been shown that application of Con A and PHA to thymocytes induces an increase in both $[Ca^{2+}]_i$ and pH_i [7,8]. Similar results were obtained in our present study (Figs. 1a,c and 2a,c). Con A (10 μg/ml) increased $[Ca^{2+}]_i$ from its basal level 105 ± 15 nM (mean ± S.D., $n = 20$) to the peak value 196 ± 14 ($n = 20$) and then gradually declined to its plateau level (not illustrated). Unlike Con A, PHA produced a gradual increase in $[Ca^{2+}]_i$ from the basal level to 185 ± 17 nM ($n = 15$) within 10 min. Changes in pH_i induced by Con A (10 μg/ml) and PHA (10 μg/ml) can be characterised as follows. During the first minutes after the addition of Con A (10 μg/ml) to a thymocyte suspension, pH_i increased by 0.11 ± 0.004 pH units ($n = 4$) from the basal level of 7.17 ± 0.01 pH units ($n = 4$) (Fig. 2a). The pH_i response to PHA (10 μg/ml) was weaker: 0.065 ± 0.015 ($n = 4$) (Fig. 2c).

When applied to resting thymocytes, AA (3 μM), like the mitogens, produced an increase in $[Ca^{2+}]_i$ by 116 ± 18 nM, $n = 17$ (Fig. 1b). However, in contrast to mitogens, AA caused a pronounced decrease in pH_i, which agrees with [9]. pH_i decayed from the basal level

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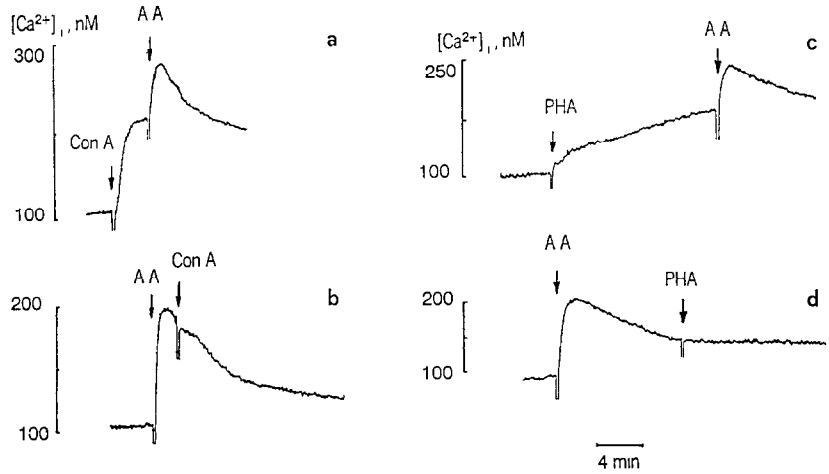

Fig. 1. Changes in $[Ca^{2+}]_i$ evoked by 10 μg/ml Con A (arrow), 10 μg/ml PHA and 3 μM AA.

by 0.16 ± 0.02 pH units ($n = 6$) and remained at this stationary level for 10–11 min of the cell treatment (Figs. 2 and 3).

AA exerted similar effects when applied against the background of Con A and PHA action. Figs. 1a,c and 2a,c demonstrate these $[Ca^{2+}]_i$ and pH_i responses: it is seen that AA induced an additional increase in $[Ca^{2+}]_i$ and a fast decrease in pH_i far below its basal level (by 85 ± 15 nM, $n = 9$ and 0.20 ± 0.03 pH units, $n = 4$ for $[Ca^{2+}]_i$ and pH_i, respectively). Thus mitogen treatment of cells did not appreciably change the effects of AA. Quite the opposite result was obtained in experiments when mitogens were applied against the background of AA action. Figs. 1b,d and 2b,d show that, in this case, both $[Ca^{2+}]_i$ and pH_i effects become completely abolished: both Con A and PHA lose the ability to induce an additional increase in $[Ca^{2+}]_i$ or attenuation of AA-induced cytosolic acidification.

In order to reveal a possible reason for the steady decrease in pH_i produced by AA we have examined its effect on the function of the Na^+/H^+ antiporter. Fig. 3 demonstrates a transitory decrease of pH_i induced by the addition of NaPr (10 mM) to the cell suspension. Relatively fast recovery of pH_i to its basal level is known to result from activation of Na^+/H^+ exchange [4]. Indeed blockade of this exchange with EIPA (20 μM) greatly slows down the recovery of pH_i after its initial decrease caused by NaPr (Fig. 3c). Fig. 3b shows that AA (3 μM) induces a very similar inhibition of pH_i recovery during NaPr application.

4. DISCUSSION

The principal finding of this work is that AA, when applied externally, prevents a mitogen-induced increase in both $[Ca^{2+}]_i$ and pH_i, which are though to play a

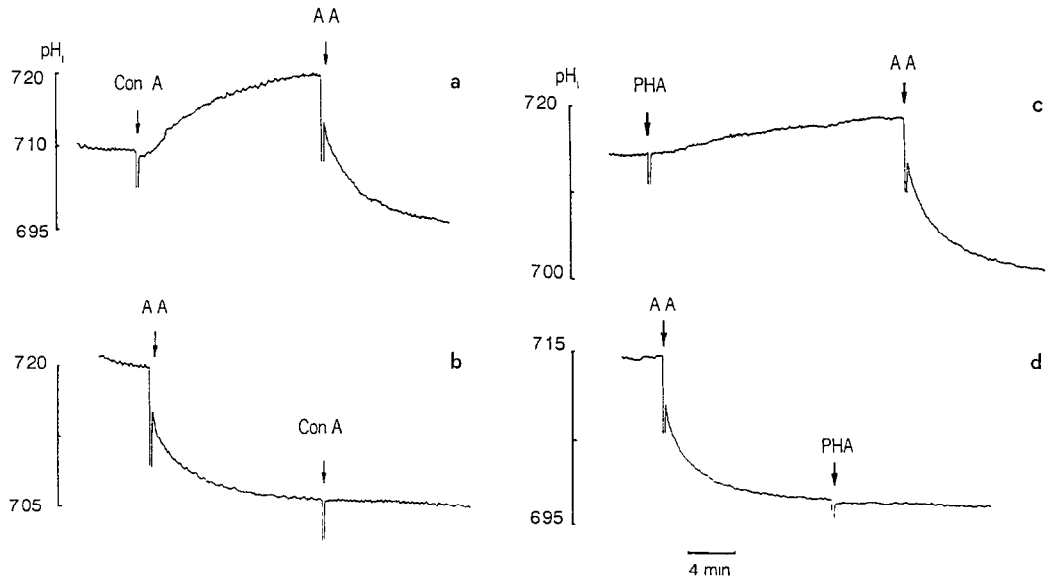

Fig. 2. Changes in pH_i induced by 10 μg/ml Con A, 10 μg/ml PHA and 3 μM AA.

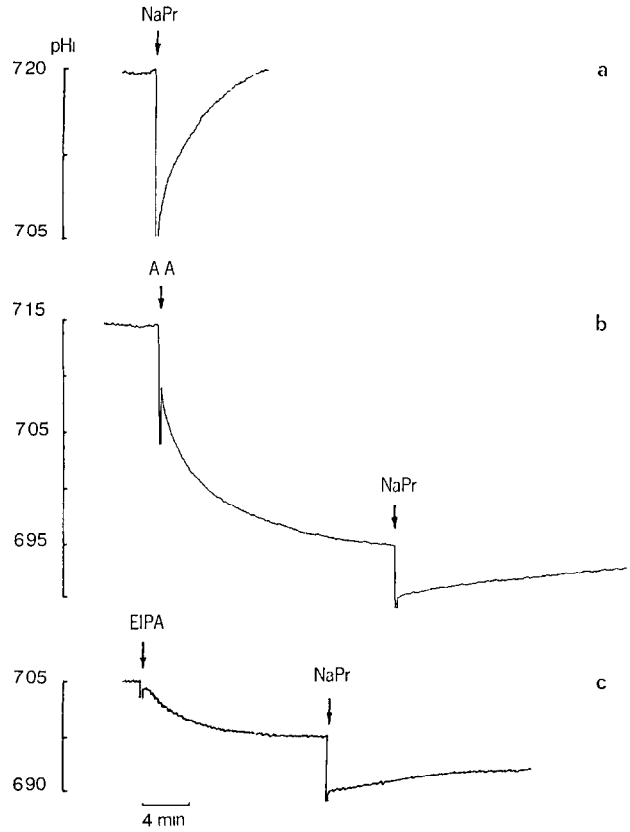

Fig. 3. Changes in pH_i induced by 10 mM NaPr (a, control), 3 μM AA and 20 μM EIPA.

trigger role in cell proliferation [4]. In particular, a rise in $[Ca^{2+}]_i$ was shown to be a prerequisite for interleukin-2 (IL2) secretion in T-lymphocytes [10]. The second principal finding is that the AA-induced sustained decrease in pH_i is associated with inhibition of the Na^+/H^+ exchange system, which apparently makes a considerable contribution to the inhibitory effect of AA on lymphocyte proliferation. Finally we have shown that mitogen-induced changes in $[Ca^{2+}]_i$ and pH_i do not prevent the $[Ca^{2+}]_i$ and pH_i effects of AA. Earlier it has been shown that changes in $[Ca^{2+}]_i$ and pH_i induced by exogenous AA in rat thymocytes are not inhibited by cyclo-oxygenase or lipoxygenase inhibitors, indicating that it is AA itself and not its metabolites that are responsible for the above mentioned changes in $[Ca^{2+}]_i$ and pH_i [13]. Probably AA exerts this effect via changes in membrane fluidity [3], which may disturb the transmembrane signalling. Here it is appropriate to mention that exogenous AA also exerts its action on other membrane processes, including functioning of the sodium pump [14], regulation of phosphate and glucose uptake in the kidney [15], glutamate uptake into glial cells [16]. In all these cases, changes in membrane fluidity have been proposed to underlie the effects of AA. The suggestion that the inhibitory effect of AA on mitogen-induced $[Ca^{2+}]_i$ and pH_i rise results from a direct effect of exogenous AA on membrane physico-chemical properties, is favoured by the fact that, along with AA, some other PUFAs also inhibit the mitogen-induced proliferative response of human and animal lymphocytes to T-cell mitogens [10,11].

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