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A single cell observation of staurosporine effect on the Ca^{2+} signals in rat basophilic leukemia cells

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A digital imaging fluorescence microscope was used to study the effect of a protein kinase inhibitor staurosporine on the antigen-dependent calcium signals in an individual rat basophilic leukemia cell (RBL-2H3). Although dose dependency of staurosporine was different from one cell to another, staurosporine inhibited, at low concentration, the calcium influx from the external medium into RBL-2H3 cells. At high concentration, however, it inhibited both the removal of calcium ion from internal stores and the calcium influx from the external medium. These results indicated that staurosporine is necessary for the inhibition of the calcium influx from the external medium and that a protein kinase (possibly protein kinase C) is involved in the calcium influx from the external medium into the cytoplasm.

RBL-2H3; Calcium signal; Staurosporine; Microscopic image analysis

1. INTRODUCTION

The release of histamine and other inflammatory mediators from tissue mast cells and blood basophils is the primary event in a variety of acute allergic and inflammatory conditions [1]. The release of granules from these cells is an energy- and calcium-dependent process which is initiated by the interaction of antigen with membrane-bound IgE [2]. Rat basophilic leukemia cells (RBL-2H3) contain, like mast cells and blood basophils, receptors for IgE and can be stimulated to secrete histamine by aggregation of the receptors with antigens [3]. Although the intracellular free calcium ions for cell activation are known to be derived from both internal stores and external medium [4,5], it is still unknown how protein kinases contribute to the regulation of the intracellular free calcium concentration $[Ca^{2+}]_i$.

We intended in the present paper to elucidate roles of protein kinases in regulating the $[Ca^{2+}]_i$ using a protein kinase inhibitor staurosporine [6]. By video-imaging fluorescence microscopy [7] we were able to obtain information of the dose dependency of staurosporine on the calcium signals in an individual RBL-2H3 cell. The results explain the role of protein kinases in regulating the calcium influx in RBL-2H3 cells.

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2. MATERIALS AND METHODS

Fura-2-AM [8] was obtained from Dojindo (Kumamoto, Japan). Staurosporine was from Kyowa Hakko kogyo (Tokyo, Japan). Mouse anti-dinitrophenyl monoclonal IgE (IgE-53-569) [9] was provided by Prof. T. Kishimoto (Osaka University). Preparation of dinitrophenylated bovine serum albumin (DNP_7-BSA) was described in the previous paper [10].

A secreting subline of rat basophilic leukemia cells (RBL-2H3) was used [3], which was given by Prof. T. Ishizaka (Johns Hopkins). Pipes buffer contained 140 mM NaCl, 5 mM KCl, 0.6 mM $MgCl_2$, 1 mM $CaCl_2$, 5.5 mM glucose, 0.1% BSA and 5 mM piperazine-*N,N'-bis*-(2-ethanesulfonic acid) at pH 7.4.

Single cell observation was done following procedure. RBL-2H3 cells were harvested from culture dishes and they were transferred to an observation chamber. The chamber was then settled in a CO_2 incubator overnight. After incubation cells were treated with a medium containing fura-2-AM (10 μM) and anti-DNP-IgE (50 μM). They were then washed with Pipes buffer to remove both free fura-2-AM and unbound IgE in solution.

Fluorescence microscopic images of RBL-2H3 cells were taken under an inverted epifluorescence microscope (Nikon TMD-EFQ) with a silicon intensified target TV camera (Hamamatsu photonics 2400-08) and a U-matic video tape recorder (Sony). The fluorescence images (excitation at 340 and 360 nm, emission at 500 nm) was analyzed with a digital image processor (Image Sigma II and TVIP-2000, Avionics), which was controlled by a microcomputer (NEC PC-9801VX). The digital frame memory has 512 × 480 pixels. Each pixel is 8 bit in depth (256 steps for full scale gray level).

3. RESULTS

When fura-2 loaded RBL-2H3 cells were stimulated with DNP-BSA (50 nM), we observed the fluorescence ratio images. Ratios of fluorescence images at 340 and

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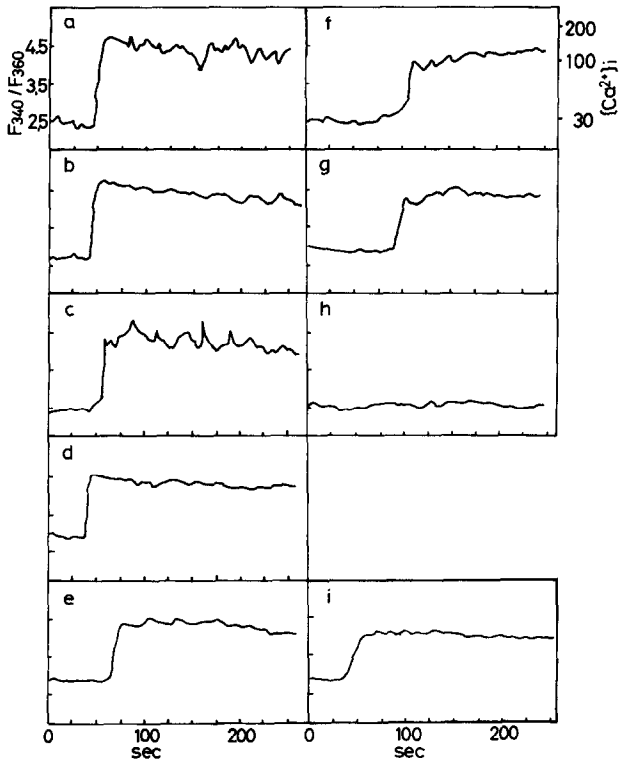

Fig. 1. Time courses of changes of the $[Ca^{2+}]_i$ in fura-2 loaded RBL-2H3 cells in response to DNP-BSA (50 nM) at 37°C. Ratios of the fluorescence images at 340 and 360 nm were taken at intervals of 3.6 s. Eight typical traces of an individual time course (a)–(h) and an averaged time course (i) of 30 cells are shown. The abscissa shows the time after antigen stimulation.

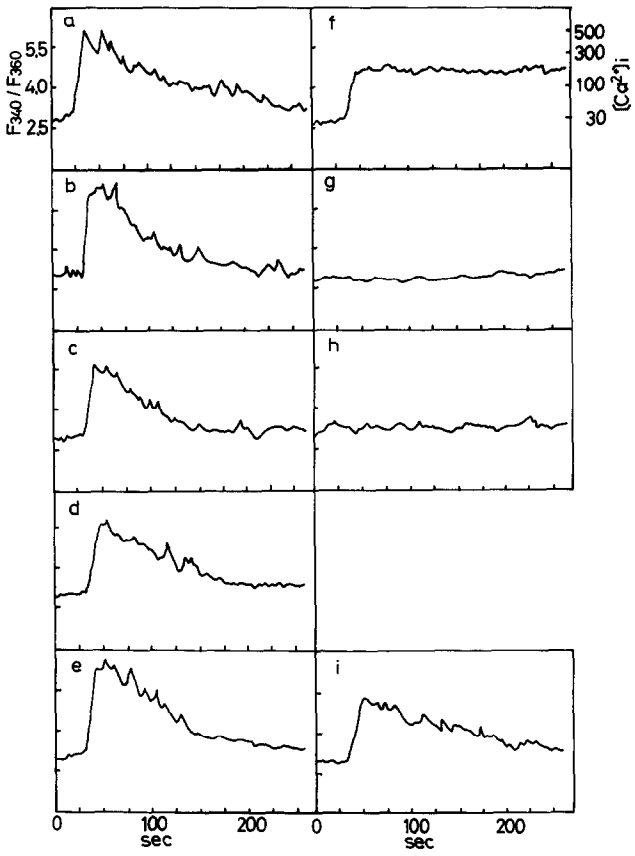

Fig. 2. Effects of staurosporine (30 nM) on the $[Ca^{2+}]_i$ of RBL-2H3 cells after DNP-BSA (50 nM) stimulation at 37°C. Fura-2 loaded RBL-2H3 cells were preincubated with staurosporine (30 nM) for 2 min at 37°C. Then they were stimulated with DNP-BSA. Eight typical traces of an individual time course (a)–(h) and an averaged time course (i) of 30 cells are shown.

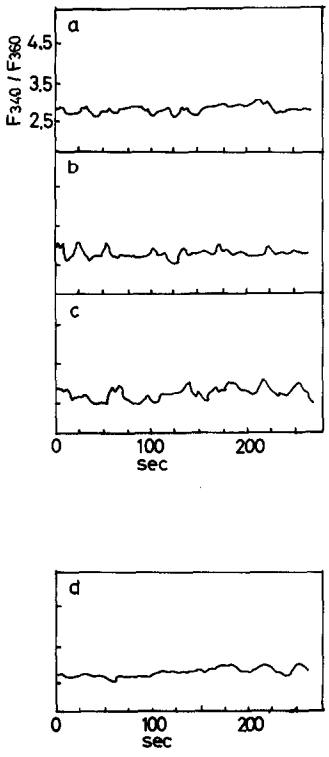

Fig. 3. Effects of staurosporine (300 nM) on the $[Ca^{2+}]_i$ of RBL-2H3 cells after DNP-BSA (50 nM) stimulation at 37°C. Fura-2 loaded RBL-2H3 cells were preincubated with staurosporine (300 nM) for 2 min at 37°C. Then they were stimulated with DNP-BSA. Three typical traces of an individual time course (a)–(c) and an averaged time course (d) of 30 cells are shown.

360 nm gave $[Ca^{2+}]_i$ images. After antigen stimulation the $[Ca^{2+}]_i$ in RBL-2H3 cells increased with short lag-times [7,11,12]. The individual time courses of the $[Ca^{2+}]_i$ are plotted in Fig. 1a–h. The averaged time course of individual cells (Fig. 1i) provides a trace which can be compared with fluorometric measurements of the mean time courses of a suspension of RBL-2H3 cells (data not shown).

When we pretreated RBL-2H3 cells with staurosporine, time courses of the $[Ca^{2+}]_i$ in RBL-2H3 cells changed drastically depending on the concentration of staurosporine. We showed two examples of this in Figs. 2 and 3. At a low concentration of staurosporine (30 nM), as shown in Fig. 2a–e, the $[Ca^{2+}]_i$ of more than half the cells showed rapid rises and falls of much

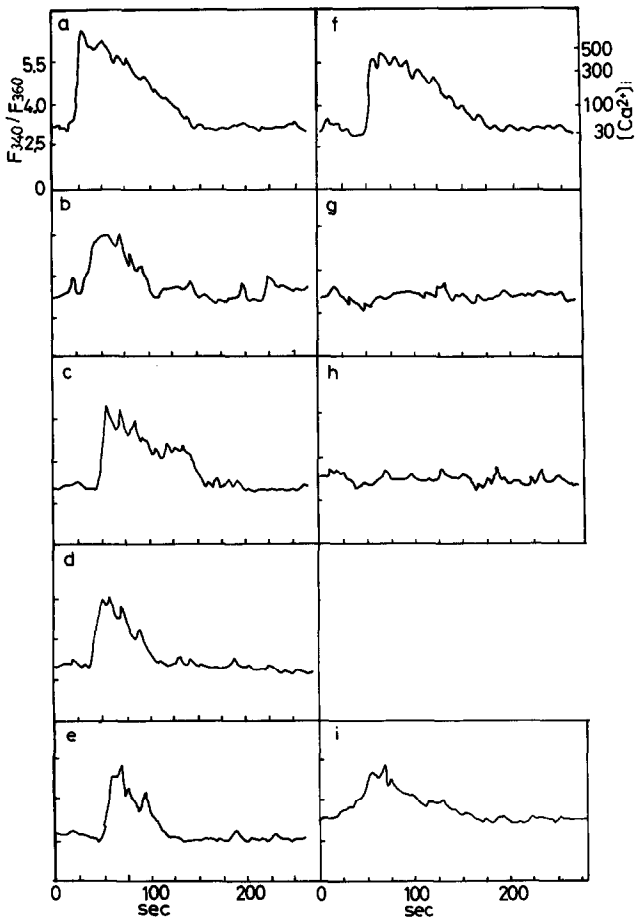

Fig. 4. Effects of staurosporine (30 nM) on the $[Ca^{2+}]_i$ of RBL-2H3 cells after DNP-BSA stimulation in the presence of 1 mM EGTA at 37°C. Fura-2 loaded RBL-2H3 cells were washed with Pipes buffer including 1 mM EGTA and were preincubated with staurosporine (30 nM) for 2 min. Then they were stimulated with DNP-BSA. Eight typical traces of an individual time course (a)-(h) and an averaged time course (i) of 30 cells are shown.

shorter periods during the time course of the response without staurosporine. Other cells (10–20%) showed the usual $[Ca^{2+}]_i$ which showed rapid rises and falls of much longer periods (Fig. 2f). The remaining cells (about 30%) did not show the calcium response within 4 min after antigen stimulation (Fig. 2g, 2h). At a high concentration of staurosporine (300 nM), none of the cells showed detectable $[Ca^{2+}]_i$ rises as shown in Fig. 3.

In the presence of 30 nM staurosporine and 1 mM EGTA, where no extracellular calcium ions were available, the $[Ca^{2+}]_i$ of half the cells showed the rapid rises and falls of much shorter periods during the time course of the usual response without staurosporine (Fig. 4). In this condition, however, none of the cells showed the pattern which was seen in Fig. 1a–g and in Fig. 2f. This suggests that 30 nM staurosporine did not inhibit the calcium removal from internal stores. All data of $[Ca^{2+}]_i$ response in RBL-2H3 cells are shown in Table I.

Table I

Summary of the effect of staurosporine on the $[Ca^{2+}]_i$ response in RBL-2H3 cells stimulated with antigen

Treatment		Pa-1	Pa-2	Pa-3
Antigen stimulation	Staurosporine concentration (nM)	↓	↓	↓
With ambient Ca^{2+}	0	73	0	27
	30	13	54	33
	300	0	0	100
Without ambient Ca^{2+}	0	0	47	53
	30	0	53	47

Values are the percentage of the number of cells in each pattern per 30 cells

4. DISCUSSION

As shown in Table I, we classified RBL-2H3 cells into 3 populations according to the $[Ca^{2+}]_i$ response after antigen stimulation. In pattern 1, the $[Ca^{2+}]_i$ shows the rapid rise and fall of much longer periods, which is maintained by the removal of calcium ions from the internal stores and the calcium influx from the external medium. In pattern 2, the $[Ca^{2+}]_i$ shows a rapid rise and fall of much shorter period during the time course of the pattern 1, which is maintained by the removal of calcium ions from internal stores. In pattern 3, the $[Ca^{2+}]_i$ does not increase at all because neither the removal of calcium ion from internal stores nor the calcium influx from the external medium does occur.

Pattern 1 was decreased from 73% to 13% in the presence of 30 nM staurosporine and it was also decreased to 0% in the presence of both 1 mM EGTA and 30 nM staurosporine. However, the percentage of pattern 2 was not changed with or without the external calcium ions at 30 nM staurosporine and it was decreased to 0% at 300 nM staurosporine. These results indicate that at low concentration staurosporine works as an inhibitor of the calcium influx from the external medium. At a high concentration the drug works as an inhibitor of both the calcium influx from the external medium and the calcium removal from internal stores. Thus, staurosporine is necessary for the inhibition of the calcium influx from the external medium and a protein kinase (possibly protein kinase C) is involved in the calcium influx from the external medium into the cytoplasm.

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