HISTAMINE INDUCES A FREE CYTOPLASMIC CALCIUM RISE IN INDO 1-LOADED HeLa CELLS

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Human cell line HeLa, obtained from an epidermoid carcinoma of the cervix uteri, is a favorite object in molecular and cellular biology. Research into cytogenetics [15], radiobiology [3], biochemistry of protein synthesis [13], etc., is conducted on cells of this line. Recently these cells have attracted the attention of electrophysiologists.

It has been found by the patch-clamp method that the plasma membrane of HeLa cells contains potassium channels [5]. Experiments using the outside-out configuration have shown that the activity of these channels depends on the Ca^{++} concentration in the pipet. It has been concluded that Ca^{++}-activated K-channels exist in these cells. Measurement of the membrane potential of HeLa cells has shown that the addition of histamine to the incubation medium evokes stroke and reversible membrane hyperpolarization [6]. This effect is absolished by quinine, a blocker of Ca-activated K channels. It can be postulated on the basis of these data that an increase in the Ca^{++} concentration takes place in the cytoplasm of HeLa cells under the influence of histamine, and in turn, this leads to activation of Ca-activated K-channels and hyperpolarization of the plasma membrane. A shortcoming of this scheme was the fact that there were no direct data on an increase in the free cytoplasmic calcium concentration (Ca^{++}_{cyt}) in HeLa cells under the influence of histamine. To study this topic we used the fluorescent calcium indicator Indo 1, which is able to penetrate into intact cells.

EXPERIMENTAL METHOD

HeLa cells were cultured in 50-ml plastic flasks ("Nunclon") in Eagle's medium containing 10% calf serum. The night before the experiment the cells were removed from the substrate by treatment with 0.05% trypsin and 0.02% ethylenediaminetetraacetate and seeded at the rate of 50,000 cells in 1 ml into 3-ml plastic cuvettes ("Kone," Finland), which were incubated sloping so that the cells could adhere to the side wall. Before the experiment a 1 mM solution of acetoxymethyl ester of Indo 1 in dimethyl sulfoxide was added to the cuvette up to a final concentration of 10 μM. After incubation for 1 h the cells were washed three times with growth medium. All manipulations with the cells were conducted at 37°C.

To measure the concentration of free cytoplasmic Ca^{++} the method described earlier [11] was used. A block diagram of the exeprimental equipment is shown in Fig. 1. To excite fluorescence, an M2100 pulsed laser ("Parc," USA) with wavelength of 337 nm, pulse energy 550 μJ, and pulse duration 10 msec, was used [1]. The beam was focused on the front of the thermostatically controlled cuvette, on which the adherent cells were located. Light of fluorescence, passing through the polychromator, was converted into a spectrum, which fell on a 1254EW vidicon ("Parc"). The spectrum from the vidicon was counted, converted in to digital form, and stored in a microcomputer. In these experiments we analyzed fluorescence within the range from 350 to 600 nm. Intervals between measurements were 4 sec. During measurement the contents of the cuvette were stirred by a mechanical mixer. The background fluorescence, consisting of autofluorescence of the cells, medium, and cuvette, and fluorescence of residual amounts of unhydrolyzed Indo 1, was determined in each preparation. For this purpose, at the end of each measurement ionomycin was added up to 5 μM and $MnCl_2$ up to 1 mM to quench the fluorescence of the free dye Indo 1. The background values were subtracted automatically. The final fluorescent spectrum of indo 1 was the sum of two spectra, corresponding to Ca^{+}-bound (C_b) and Ca^{++}-free (C_f) forms of Indo 1 [11]. $[Ca^{++}]_{cyt}$ was determined by the formula

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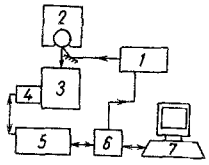

Fig. 1. Diagram of experimental set up for measuring Ca^{++}_{cyt}. 1) Laser; 2) cuvette unit; 3) polychromator; 4) multichannel optical detector (vidicon); 5) vidicon controller; 6) parallel interface; 7) computer.

$$[Ca^{++}]_{cyt} = K_d \cdot (C_b/C_f)$$

where K_d = 250 M [11].

Data were obtained in at least two different experiments and in two parallel determinations.

The cultural reagent used in the work were obtained from the Research Institute of Vaccines and Sera; histamine, phorbol esters, prostaglandin E1, and $CaCl_2$ were from "Sigma" (USA), and the Indo-1/AM was from "Calbiochem" (USA).

EXPERIMENTAL RESULTS

Measurements made by the method described above show that the concentration of free cytoplasmic Ca^{++} in HeLa cells at rest is 100-150 nM. This value is typical of mammalian cells [8]. Addition of histamine (to 10^{-4} M) to the incubation medium led to a rapid rise of $[Ca^{++}]_{cyt}$ for 30 min followed by a return to its initial level in 60-90 sec (Fig. 2a). The amplitude of the calcium response to histamine in medium containing 1 mM $CaCl_2$ was 825 ± 33 nM. There are two types of histamine receptors. Receptors of type H2 are linked with the adenylate cyclase system, those of type H1 with phospholipase C and calcium channels [4]. The data of Hazama et al. [6], showing that activation of Ca-activated K-channels by histamine in HeLa cells is blocked by pyrilamine, a selective blocker of H1-receptors, are evidence in support of the functioning of H1-receptors in HeLa cells.

We know that $[Ca^{++}]_{cyt}$ can be raised through the action of hormones either by release of Ca^{++} from the endoplasmic reticulum or by the entry of these ions from outside. The amplitude of the response of Ca to histamine in HeLa cells in calcium-free medium was 185 ± 21 nM (Fig. 2b), which is significantly lower ($p < 0.002$) than in medium containing Ca^{++} in the hormonal response, the main contribution to the rise of the Ca^{++} level being made by external calcium.

There are two main types of Ca-channels in the plasma membrane: voltage- and receptor-dependent. Both these channels can be regulated by hormones and mediators [14]. An essential condition for opening of voltage-dependent calcium channels is depolarized of the plasma membrane. As was shown in [6], the action of histamine on HeLa cells does not lead to the development of depolarization. The more likely mechanism of the inflow of Ca^{++} into the cells we studied is therefore activation of the so-called "receptor-dependent" or "receptor-control" channels (Ca-RCC) [2].

We found that 4β-phorbol-12β-myristate-13α-acetate (PMA) in a concentration of 10^{-7} M inhibits the histamine-stimulated rise of the $[Ca^{++}]_{cyt}$ level in HeLa cells (Fig. 3b). The amplitude of the calcium response in this case fell from 825 ± 23 to 121 ± 23 nM ($p < 0.001$). It is generally accepted that PMA exerts its action through activation of Ca, phospholipid-dependent protein kinase (protein kinase C) [9]. Another phorbol ester, namely 4α-phorbol-12,13-didecanoate, has no such action on protein kinase C and does not affect the calcium response (results not shown). This indicates the specificity of the observed effect of PMA. A similar action of protein kinase C activator on Ca-RCC was found previously in many different cells: platelets [2, 8], astrocytoma cells [10], endothelial cells of the pulmonary vessels [12], etc. The mechanism of the inhibitory action of protein kinase C is unknown, but according to one hypothesis, it may consist of phosphorylation of GTP-binding proteins, linking Ca-RCC with receptors [7]. Since activation of Ca-RCC by hormones in most, if not all, cases is accompanied by activation of protein kinase C, it was suggested that the blocking action of protein kinase C on Ca-RCC acts as negative feedback, terminating the calcium response [1, 8].

Ca-RCC in platelets are blocked, not only by PMA, but also by agents raising the intracellular cAMP level [8]. It will be clear from Fig. 3 that simultaneous treatment of HeLa cells with 10^{-5} M forskolin, activating the catalytic component of adenylate cyclase, and 10^{-4} M isobutylmethylxanthine, inhibiting cyclic nucleotide phosphodiesterase, did not lead

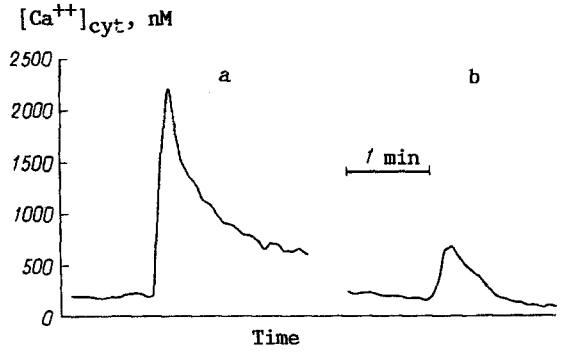

Fig. 2

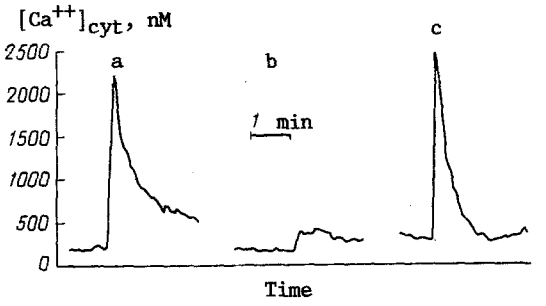

Fig. 3

Fig. 2. Effect of histamine on $[Ca^{++}]_{cyt}$ in HeLa cells in medium containing calcium (a) and in calcium-free medium (b).

Fig. 3. Effect of protein kinase C activator and of substances raising intracellular cAMP level on histamine-stimulated rise of $[Ca^{++}]_{cyt}$ level. a) Control; b) preincubation with PMA for 3 min; 3) preincubation with forskolin. and isobutylmethylxanthine for 1 h.

to any significant change in amplitude of the calcium response. Increasing the duration of incubation with these preparations (up to 1 h) likewise had no effect. Similar treatment of platelets (up to 1 h) likewise had no effect. Similar treatment of platelets led to the almost complete disappearance of the calcium response (results not shown). Data obtained on HeLa cells are in agreement with data obtained on endothelial cells of the pulmonary blood vessels, in which substances raising the cAMP level did not affect the calcium response [12]. Thus factors raising the intracellular cAMP level, unlike protein kinase C activators, are not universal inhibitors of receptor-controlled Ca channels.

This investigation showed that HeLa cells are very convenient objects with which to study hormonal regulation of free cytoplasmic calcium. These cells are very undemanding in culture. They are easily loaded with the calcium indicator and react to histamine by a strong rise of the Ca^{++}_{cyt} level. This rise is the sum of two components: Ca^{++} relased from the endoplasmic reticulum and Ca^{++} entering from outside, so that both these processes can be studied. Activation of HeLa cells by histamine is blocked by phorbol esters, so that it is possible to study interaction between phosphoinositide metabolism and Ca-RCC. Because these cells are large enough and because their fusion can easily be induced [6], they are particularly promising for the electrophysiological study of the properties of receptor-controlled calcium channels.

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