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Effect of glucose and deoxyglucose on cytoplasmic concentration of free Ca^{2+} in Ehrlich ascites tumour: studies on single cells*

Aneta Czyż[a], Vera V. Teplova[a, b], Paweł Sabała[a], Małgorzata Czarny[a], Yuri V. Evtodienko[b] and Lech Wojtczak[a]**

[a]*M. Nencki Institute of Experimental Biology, L. Pasteura 3, 02–093 Warsaw, Poland, and* [b]*Institute of Biological Physics, Russian Academy of Sciences, Pushchino, Moscow Region, Russian Federation*

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Concentration of free cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$) in Ehrlich ascites tumour cells loaded with fura-2 was measured in single cells applying a video imaging system. In resting cells $[Ca^{2+}]_i$ amounted to 60 - 340 nM and was increased after addition of 10 mM D-glucose or D-2-deoxyglucose by 80 - 200 nM. This increase occurred within 30 - 60 s following addition of the sugars and lasted for several minutes. Pretreatment of the cells with thapsigargin resulted in a much smaller $[Ca^{2+}]_i$ increase after addition of glucose or deoxyglucose and, *vice versa*, thapsigargin added after the sugars mobilized less Ca^{2+} than when added before. A possible relation of the $[Ca^{2+}]_i$ rise evoked by glucose and deoxyglucose to the Crabtree effect is discussed.

Cytoplasmic free Ca^{2+} ($[Ca^{2+}]_i$), as the most important second messenger, governs numerous vital functions of the cell and its level is controlled in a subtle and complex way by a number of extracellular factors, like hormones, growth factors, neurotransmitters etc. (for review see [1, 2]). Calcium homeostasis in neoplastic tissues has attracted special attention as it has been believed that some of its features may be characteristic for malignancy [3 - 7]. However, according to Arslan *et al.* [8] malignant cells can differ from normal ones not by the cytoplasmic Ca^{2+} concentration but, rather, by the sensitivity of their cellular processes to this cation.

In a previous report [9] we have shown, using suspensions of isolated Ehrlich ascites cells, that their $[Ca^{2+}]_i$ rises when glucose or deoxyglucose is added to the incubation medium. In the present paper we confirm this finding by observations on single cells using the video imaging techniques. Such approach enabled not only to follow individual difference between cells in response to glucose, deoxyglucose and thapsigargin but, in addition, to determine more precisely the actual level of $[Ca^{2+}]_i$ and its scatter in the total cell population.

MATERIALS AND METHODS

Ehrlich ascites cells, cultivated in the peritoneal cavity of white Swiss female mice, were harvested as described previously [9, 10] and stored on ice in a medium containing 150 mM NaCl, 5 mM KCl and 10 mM Tris/HCl (pH 7.4). The cells were loaded with fura-2 essentially as described by Dubyak [11] for cell suspensions. In brief, the cells were attached to polylysine-coated glass coverslips and left for 20 min in the

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**To whom correspondence should be addressed.

medium containing 150 mM NaCl, 3 mM KCl, 1.5 mM Na-phosphate, 0.6 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM Na-pyruvate, 25 mM Na-4-(2-hydroxyethyl)-1-piperazineethanesulphonate (Hepes), pH 7.4, and 0.5% bovine serum albumin (fatty acid-free). Thereafter, fura-2/acetoxymethyl ester was added to the final concentration of 5 μM and the cells were incubated at 37°C. After 30 min they were washed with the medium without serum albumin and containing 0.1 mM $CaCl_2$. The coverslips were then mounted in a chamber over a Nikon Diaphot inverted microscope equipped with a 40× fluorescence objective lens. The experiments were performed in the medium similar to that used for fura-2 loading but with $[Ca^{2+}]$ reduced to 0.1 mM and containing no serum albumin. Intracellular $[Ca^{2+}]$ was measured using the double excitation wavelength mode (340 and 380 nm excitation wavelengths and 510 nm emission wavelength) [12]. The Joyce-Loeb computerizing image-processing system Magiscan was operated with Tardis version 7.2 programme. It was calibrated using Ehrlich ascites cells made permeable to Ca^{2+} with ionomycin (10 μM final concentration) and scanned in the presence of 2 mM EGTA or 2 mM $CaCl_2$ [12]. These measurements were carried out at 37°C.

Fura-2/acetoxymethyl ester was provided by Molecular Probes (Eugene, Oregon, U.S.A.), ionomycin was from Sigma (St. Louis, MO, U.S.A.) and thapsigargin was a generous gift from Dr. Michele Chiesi (Ciba-Geigy, Basel, Switzerland).

RESULTS

It is generally assumed that the fluorescence of fura-2 entrapped within intact cells reflects the concentration of free Ca^{2+} predominantly in the cytosolic compartment. Examination of about one hundred individual Ehrlich ascites cells showed that in about 85% of the cells this concentration amounted to 100 - 260 nM, with extreme values ranging from 60 to 340 nM (Fig. 1). Such a broad scatter may have partly resulted from a damage of some cells during isolation or loading with the dye but, possibly, can also reflect natural variations in $[Ca^{2+}]_i$, depending, for example, on the actual stadium of the cell cycle.

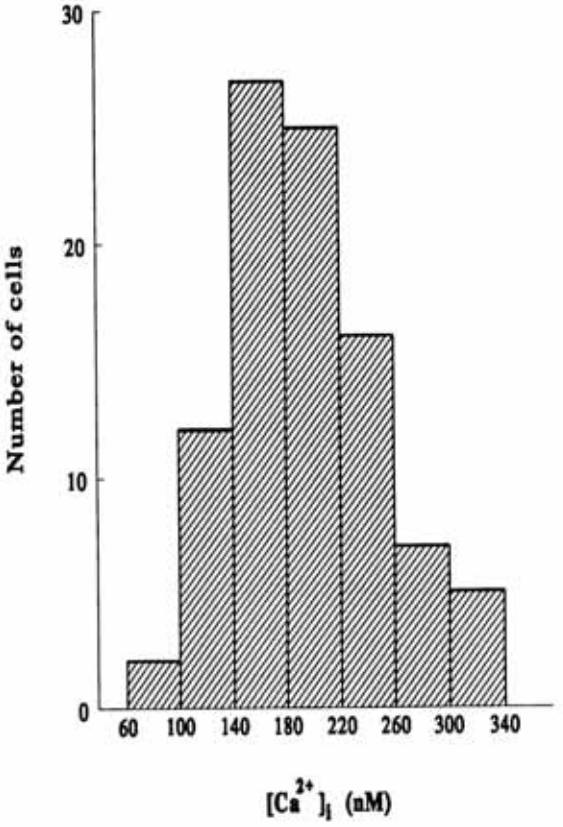

Fig. 1. Histogram showing concentration of cytoplasmic free Ca^{2+} in single "resting" (before treatment with glucose or deoxyglucose) Ehrlich ascites cells, as calculated from fura-2 fluorescence. Ninety two single cells were examined.

Addition of glucose or deoxyglucose to the medium produced a substantial increase of $[Ca^{2+}]_i$. Figure 2 exemplifies two typical experiments. The upper panels show changes of $[Ca^{2+}]_i$ in three selected cells, whereas the lower panels represent the whole microscopic field (average value for about 20 cells). In full agreement with previous results obtained with cell suspensions [9], deoxyglucose elicited a somewhat greater effect than glucose. $[Ca^{2+}]_i$ increase in the three cells shown in Fig. 2 A (after addition of glucose) was between 80 and 160 nM, whereas that for the three cells in Fig. 2 B (after addition of deoxyglucose) was 160 to 200 nM. This difference is also evident in the average values for the whole microscopic fields (lower panels of Fig. 2), 80 and 120 nM, respectively. Another similarity between the present study and previous observations on cell suspension [9] is a faster response to glucose than to deoxyglucose, although the lag after addition of deoxyglucose, very distinct in cell sus-

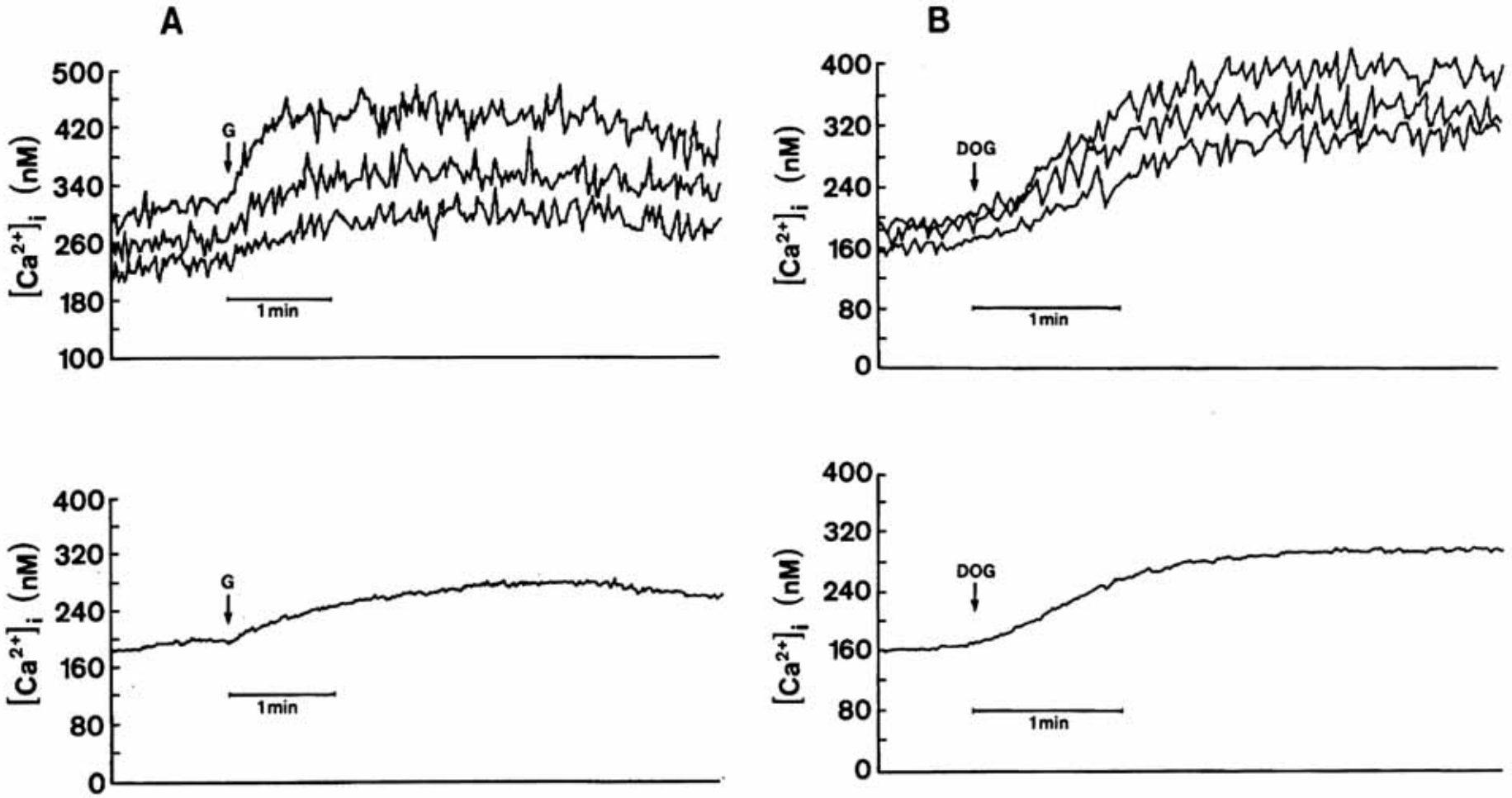

Fig. 2. Effect of glucose and deoxyglucose on cytoplasmic Ca^{2+} concentration in single Ehrlich ascites tumour cells.
Glucose (Panels A; G) or deoxyglucose (Panels B; DOG) was added to 10 mM final concentration. $[Ca^{2+}]_i$ was recorded for three individual cells (upper panels) and for the whole microscopic field containing about 20 cells (lower panels).

pensions [9], is not so much apparent in single cells.

Experiments shown in Fig. 2 were performed in the presence of 0.1 mM Ca^{2+} in the external medium. Nevertheless, the increase of $[Ca^{2+}]_i$ after addition of glucose or deoxyglucose also occurred in the presence of 1 mM EGTA, i.e. in the nominally Ca-free medium (not shown), thus indicating that Ca^{2+} appearing in the cytoplasm originated from intracellular stores.

To further substantiate this point, the cells were poisoned with thapsigargin, the known inhibitor of Ca^{2+}-pumping ATPase in the endoplasmic/sarcoplasmic reticulum [13]. As shown in Fig. 3 A, deoxyglucose added after thapsigargin either had no effect or produced a much smaller $[Ca^{2+}]_i$ increase than when added without the inhibitor (compare Fig. 2 B). Addition of thapsigargin after deoxyglucose (not shown) or glucose (Fig. 3 B) resulted in a small but still visible increase of $[Ca^{2+}]_i$. However, some individual cells (not shown in Fig. 3), which responded very strongly to deoxyglucose, were unable further to increase their $[Ca^{2+}]_i$ upon subsequent addition of thapsigargin.

DISCUSSION

The present results clearly show that glucose and its nonmetabolizable analogue, deoxyglucose, induce a substantial increase of cytoplasmic Ca^{2+} concentration in Ehrlich ascites tumour cells, thus fully confirming the results of experiments on Ehrlich ascites cells in suspension [9]. Analysis of $[Ca^{2+}]_i$ in single cells provides us with additional information not quite apparent when studying cell suspensions. Firstly, it enables a more precise evaluation of $[Ca^{2+}]_i$, not obscured by the presence of fura-2 in the medium (e.g. leaking out from dead cells). Secondly, it shows large variations of the initial $[Ca^{2+}]_i$ between individual cells (Fig. 1). As already commented under Results, this may result from various degree of cell damage but may also reflect individual variations related, for example, to various cell age. Thirdly, it reveals individual differences between the cells in the response of $[Ca^{2+}]_i$ to glucose and deoxyglucose.

The present results fully confirm the previous conclusion [9] that Ca^{2+} appearing in the cyto-

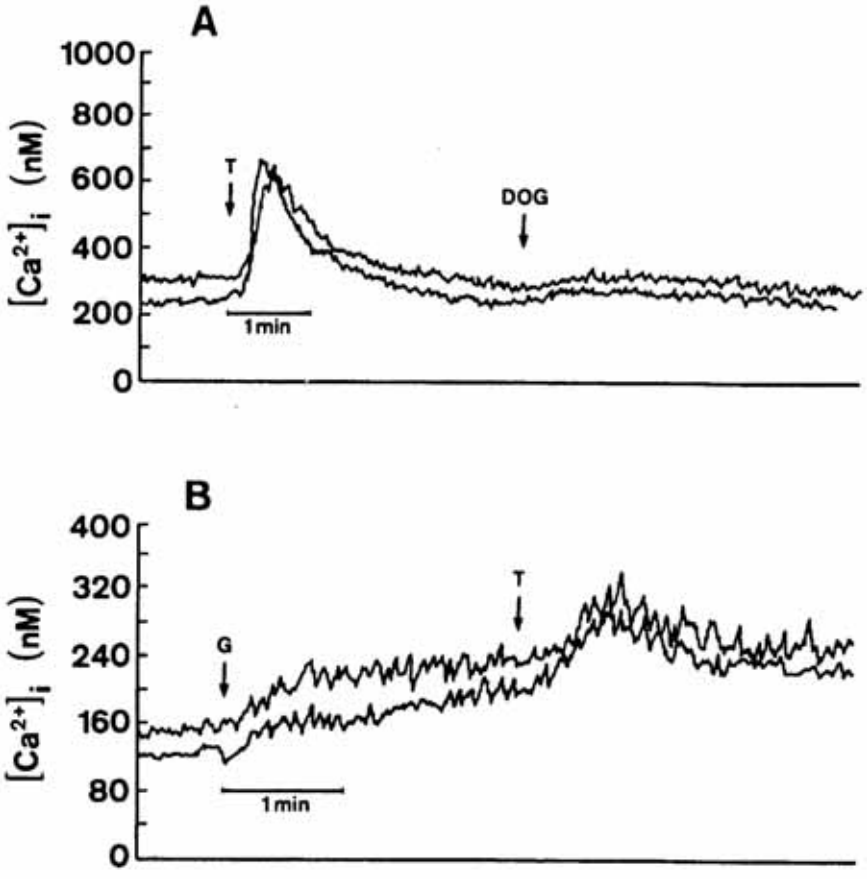

Fig. 3. Effect of thapsigargin, deoxyglucose and glucose on $[Ca^{2+}]_i$ measured in single cells.
Panel A, deoxyglucose was added after thapsigargin; panel B, glucose was added before thapsigargin. Designations: T, thapsigargin, 0.25 μM; DOG, deoxyglucose, 10 mM; G, glucose, 10 mM. Traces for two single cells are shown in each panel. Note difference in concentration scale in panels A and B.

plasm after addition of glucose or deoxyglucose to the incubation medium primarily originates from intracellular stores and not from the external medium. Experiments with thapsigargin show that these stores are, most likely, identical with the endoplasmic reticulum.

In search for the mechanism(s) of $[Ca^{2+}]_i$ increase the following possibilities can be considered: (1) depletion of cytoplasmic ATP; (2) acidification of the cytoplasm; (3) agonist-like cell signalling. As discussed previously [9], the present state of our investigations does not permit to differentiate between these possibilities.

Elevation of cytoplasmic concentration of free Ca^{2+} may have important consequences for the cell metabolism. For example, elevation of $[Ca^{2+}]_i$ within the nanomolar range has been shown to increase aerobic glycolysis in rat adipocytes [14]. On the other hand, accumulation of Ca^{2+} by isolated Ehrlich ascites mitochondria results in inhibition of phosphorylating respiration [15 - 18]. Thus, elevation of $[Ca^{2+}]_i$ elicited by the addition of glucose may switch the energy production from respiratory to glycolytic. It seems, therefore, likely that the well documented inhibition by glucose of the respiration of several tumours [19 - 24] and some non-neoplastic cells [25 - 29], known as the Crabtree effect, may be, at least partly, mediated by $[Ca^{2+}]_i$. In fact, we have recently shown that Ehrlich ascites mitochondria within permeabilized cells, exposed to Ca^{2+} concentrations within the range observed in the present investigation after addition of glucose or deoxyglucose, display inhibited oxidative phosphorylation and the coupled respiration (Evtodienko, Teplova, Duszyński, Bogucka & Wojtczak, manuscript in preparation).

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