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INHIBITION BY GLUCOSE AND DEOXYGLUCOSE OF PHOSPHATIDYLSERINE SYNTHESIS IN EHRLICH ASCITES TUMOR CELLS: A possible relation to the Crabtree effect and depletion of endoplasmic reticulum Ca^{2+} stores

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Incorporation of [^{14}C]serine into phosphatidylserine by the base exchange reaction in Ehrlich ascites tumor cells is inhibited by 30% by glucose and by 60% by 2-deoxyglucose (10 mM each). The inhibition by thapsigargin (0.2 μM) amounts to 80%. This inhibition is interpreted as being due to depletion of calcium stores in the endoplasmic reticulum and is compatible with the previous observation [Teplova, V.V., Bogucka, K., Czyż, A., Evtodienko, Yu.V., Duszyński, J., and Wojtczak, L. (1993) Biochem. Biophys. Res. Commun. 196, 1148-1154] that glucose and deoxyglucose elicit an increase of cytoplasmic [Ca^{2+}] at the expense of intracellular Ca^{2+} stores and with the finding [Barańska, J. (1989) FEBS Lett. 256, 33-37] that the base exchange reaction requires high concentration of Ca^{2+} within the endoplasmic reticulum lumen.

Glucose and its non-metabolizable analogue, 2-deoxyglucose, produce in numerous tumors and some non-malignant highly glycolytic cells a partial inhibition of respiration, known as the Crabtree effect (1,2). In our previous studies on its mechanism we have found that these sugars elicit an increase of cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$) in Ehrlich ascites tumor cells. This was observed using both cell suspensions (3) and single cells applying a video imaging system (4). Elevated $[Ca^{2+}]_i$ results in an increased accumulation of Ca^{2+} by mitochondria and, consequently, inhibition of their coupled respiration (5). As shown recently (6), the latter inhibition is most likely due to Ca^{2+}-promoted association of the mitochondrial F_1F_o complex (H^+-ATPase) with its natural

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protein inhibitory subunit. Ca^{2+} appearing in the cytoplasm derives primarily from the endoplasmic reticulum stores, since its increase in response to glucose or deoxyglucose was considerably reduced in cells pretreated with thapsigargin (3,4), the inhibitor of endoplasmic Ca^{2+}-ATPase (7).

Endoplasmic reticulum is the locus of phosphatidylserine synthesis by the base exchange reaction (8). This reaction is strongly dependent on the presence of relatively high (in the millimolar range) concentrations of Ca^{2+} and is independent on metabolic energy. However, under specific conditions and in the presence of low Ca^{2+} concentrations, incorporation of labelled serine into phosphatidylserine is stimulated by ATP (for reviews see Refs. 9,10). Barańska and Czarny (11,12) have clearly demonstrated that this effect of ATP is due to the accumulation, inside microsomal vesicles, of Ca^{2+} high enough to enable the base exchange reaction. Subsequently, the same authors (13) as well as Pelassy et al. (14) have shown that depletion of intracellular Ca^{2+} stores in intact cells also results in diminution of phosphatidylserine synthesis.

If glucose and deoxyglucose increase cytoplasmic [Ca^{2+}] in Ehrlich ascites tumor cells at the expense of Ca^{2+} stored in the endoplasmic reticulum, one should expect a decrease of phosphatidylserine synthesis under such conditions. The present experiments show that this is indeed the case, thus confirming our previous conclusions (3,4). They also point to a possibility that in tumor cells glucose not only elicits the inhibition of respiration (known as the Crabtree effect) but may also produce changes in the lipid metabolism.

Materials and Methods

Ehrlich ascites tumor cells were cultivated in the peritoneal cavity of Swiss albino mice and harvested as described previously (5,6). The incubation medium contained 150 mM NaCl, 3 mM KCl, 1.5 mM Na-phosphate, 0.6 mM $MgCl_2$, 25 mM 4-(2-hydroxy-ethyl)-1-piperazineethanesulfonate (Hepes, Na salt, pH 7.4) and, unless indicated otherwise, 0.1 mM $CaCl_2$. The typical experimental protocol was as follows. To 1.0 ml of this medium 0.1 ml of the cell suspension, containing 30-50 million cells, was added and this mixture was equilibrated at 37°C for 5 min. Thereafter, D-glucose, 2-deoxyglucose or thapsigargin was added, followed by [3-^{14}C]L-serine (0.5 μCi, 10 nmol) 1 min later and the incubation was continued for 15 min at 37°C. The base exchange reaction was terminated by addition of 0.15 ml 6 M perchloric acid. The precipitate was sedimented by centrifugation, washed three times with a few ml of unlabelled 2 mM serine, and the lipids were extracted with a mixture of chloroform and methanol (2:1, v/v). After evaporation of the solvents, the extracted lipids were dissolved in a known volume of chloroform and methanol (4:1, v/v) and aliquots counted for total radioactivity incorporated into lipids. Parallel aliquots were subjected to thin layer chromatography on

aluminum sheets covered with Silica gel 60 (Merck, Darmstadt, Germany). The chromatograms were developed in chloroform/methanol/glacial acetic acid (65:25:8, v/v). The spots were visualized by autoradiography on X-ray plates, scrapped out and counted for radioactivity. Identification of the spots were made using commercial or self-prepared phospholipid standards.

[3-^{14}C]L-Serine, specific radioactivity 55 mCi/mmol, was from Amersham (U.K.) and thapsigargin was a gift of Dr. Michele Chiesi (Ciba-Geigy, Basel, Switzerland).

Results

Preliminary analysis showed that 75-90% of the label from [^{14}C]serine incorporated into the lipidic fraction from Ehrlich ascites tumor cells was found in phospholipids, the remaining 10-25% being tentatively identified as free fatty acids, originating, most likely, from the carbon skeleton of [^{14}C]serine after its deamination. Therefore, to assess the magnitude of the base exchange reaction, thin layer chromatography of extracted lipids had to be applied as a routine procedure. It was also found that phosphatidylserine constituted 85% of the labelled phospholipids, with only minor contribution from phosphatidylethanolamine, dimethylphosphatidylethanolamine, lysophosphatidylethanolamine and phosphatidylcholine. Further examination of the labelled phospholipids, using mild alkaline hydrolysis, revealed that practically all their radioactivity was present in the nitrogen base moiety.

Because the maximal increase of $[Ca^{2+}]_i$ produced by glucose or deoxyglucose in Ehrlich ascites tumor cells occurred with some delay (3,4), the base exchange reaction was initiated by addition of [^{14}C]serine 1 min following addition of the sugar. It was found (Table I) that glucose and even more so deoxyglucose decreased the incorporation of [^{14}C]serine into the phospholipid fraction and thapsigargin produced a very strong inhibition. No significant changes in the distribution of the label between phosphatidylserine and its decarboxylation and methylation products after addition of glucose or deoxyglucose were observed (not shown). This is compatible with our assumption that these sugars affected the base exchange reaction rather than the metabolism of phosphatidylserine already formed.

Because labelling of phosphatidylserine in control samples approached saturation after 15 min incubation, whereas that in the presence of glucose and deoxyglucose was approximately linear (Fig. 1), percentage of the inhibition measured within shorter incubation times was even higher than calculated after 15 min. The inhibition by glucose and deoxyglucose was independent of whether the medium contained Ca^{2+} or was nominally Ca^{2+}-free (Fig. 2).

Table I

Effect of glucose, deoxyglucose and thapsigargin on the incorporation of [^{14}C]serine into phospholipids

Addition	[^{14}C]Serine incorporation (% of the control)
Glucose (10 mM)	69 ± 14 (6)
Deoxyglucose (10 mM)	42 ± 7 (6)
Thapsigargin (0.2 μM)	18 ± 4 (3)

[^{14}C]Serine incorporation in the control (without additions) amounted to 95 ± 27 (6) pmol/15 min per 50 million cells. All values are means ± SD for the number of experiments indicated in parentheses.

Although the effects shown in the present paper were obtained with 10 mM glucose or deoxyglucose, the concentration used in previous studies on the mechanism of the Crabtree effect (3,4), both sugars were effective already at 0.1 mM concentration and an almost maximal inhibitory effect on phosphatidylserine synthesis was obtained with 1 mM glucose and deoxyglucose (not shown).

Discussion

In the light of our previous finding (3,4) that glucose and deoxyglucose increase the concentration of free Ca^{2+} in the cytoplasm of Ehrlich ascites tumor cells at the expense of the endoplasmic reticulum stores, the present results fully confirm the thesis of Barańska et al. (10-12) that the synthesis of phosphatidylserine by the base exchange reaction requires high concentration of this cation within the endoplasmic reticulum. They are also compatible with the concept that the base exchange reaction in mammalian cells can be regulated by extracellular factors such as agonists and ionophores (13,14). The degree of inhibition of phosphatidylserine synthesis by thapsigargin and the two hexoses used was compatible with their effectiveness in depleting intracellular Ca^{2+} stores. As shown previously (3,4), deoxyglucose produced a higher increase of $[Ca^{2+}]_i$ than glucose, thus presumably being more effective in decreasing Ca^{2+} accumulated inside the endoplasmic reticulum, whereas thapsigargin, a potent blocker of Ca^{2+}-pumping ATPase of the endoplasmic reticulum (7), inhibited phosphatidylserine synthesis almost completely.

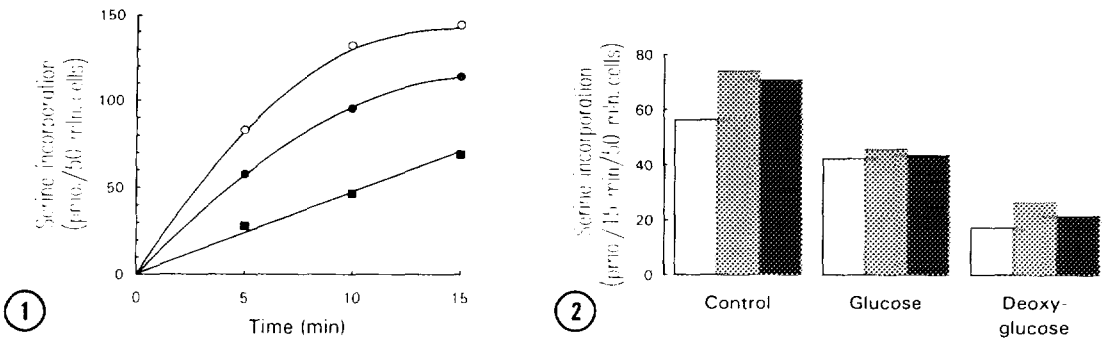

Fig. 1. Time course of [^{14}C]serine incorporation into phospholipids and the effect of glucose and deoxyglucose. The cells were incubated as described under Materials and Methods in the presence of 0.1 mM $CaCl_2$. ○, Control (without additions); ●, with 10 mM glucose; ■, with 10 mM deoxyglucose.

Fig. 2. Incorporation of [^{14}C]serine into phospholipids in the presence and absence of Ca^{2+} in the medium. White column, 1 mM EGTA; dashed columns, 0.1 mM $CaCl_2$; black columns, 2 mM $CaCl_2$. The sugars were added to 10 mM final concentration.

We want to stress two aspects of the present observations. Firstly, they confirm the previous assumption (3,4) that the increase of $[Ca^{2+}]_i$ accompanying the Crabtree effect and presumably involved in its mechanism (5,6) occurs at the expense of Ca^{2+} stores in the endoplasmic reticulum. Secondly, they point to a possibility that glucose and some of its analogues not only elicit inhibition of respiration of malignant cells but may also modulate their lipid metabolism. The importance of the base exchange reaction, in particular that involving serine, is not well understood (see Ref. 9 and references cited therein). Phosphatidylserine synthesized in this reaction in the endoplasmic reticulum is mostly transported to mitochondria where it becomes decarboxylated to phosphatidyl-ethanolamine and then distributed among various cell membranes. On the other hand, phosphatidylserine is a known activator of protein kinase C (15,16). It is therefore tempting to speculate that production of phosphatidylserine in the endoplasmic reticulum may have a crucial role in regulating the activity of this important enzyme. If this is the case, then the Crabtree effect in malignant and some normal tissues and cells may be accompanied by more profound changes of the cellular metabolism.

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