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Glucose 6-phosphate stimulation of MgATP-dependent Ca^{2+} uptake by rat kidney microsomes

Rosella Fulceri, Andrea Romani, Alfonso Pompella and Angelo Benedetti

Istituto di Patologia Generale dell'Università di Siena, Siena (Italy)

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(1) The features of MgATP-dependent Ca^{2+} accumulation under stimulation with glucose 6-phosphate were studied in rat kidney microsomes. (2) Ca^{2+} accumulated in the presence of MgATP alone does not exceed approx. 2 nmol/mg protein. (3) Glucose 6-phosphate markedly stimulates Ca^{2+} accumulation, up to steady-state levels approx. 15-fold higher than in its absence. (4) The hydrolysis of glucose 6-phosphate by glucose-6-phosphatase is essential for the stimulation, as shown by inhibiting the glucose 6-phosphate hydrolysis with adequate concentrations of vanadate. Inorganic phosphate is accumulated in microsomal vesicles during glucose 6-phosphate-stimulated Ca^{2+} uptake in equimolar amounts with respects to Ca^{2+}. (5) Increasing concentrations of glucose 6-phosphate result in increasing stimulations of Ca^{2+} uptake, until a maximal Ca^{2+}-loading capacity of approx. 27 nmol/mg microsomal protein is reached. It is suggested that the enlargement of the kidney microsomal Ca^{2+} pool induced by glucose 6-phosphate (an important metabolite in kidney) might play a role in the regulation of Ca^{2+} homeostatis in kidney tubular cells.

It is well known that microsomal fractions from various tissues, including kidney [1], possess an energy-dependent Ca^{2+}-sequestering activity. In the case of liver, it has previously been shown that glucose-6-*P* markedly stimulates the MgATP-dependent Ca^{2+} uptake by isolated microsomal fractions [2,3] and by the endoplasmic reticulum of permeabilized hepatocytes [4]. In addition, other results have shown that such a stimulatory effect depends upon the hydrolysis of glucose-6-*P* [3–5] mediated by glucose-6-phosphatase (EC 3.1.3.9) at the luminal aspect of the microsomal membrane [6], which likely represents a source of phosphate anions within the microsomal vesicle. We suggested that such phosphate anions may act as intravesicular trapping agents for the actively transported Ca^{2+} [7]. Other authors have shown that glucose-6-*P* increases the ATP-dependent Ca^{2+} content of the endoplasmic reticulum in pancreatic islet cells [5], where the microsomal glucose-6-phosphatase system has been characterized [8].

Glucose-6-phosphatase activity is known to occur also in kidney [9]. Kidney microsomal glucose-6-phosphatase, like liver glucose-6-phosphatase, appears to be a multicomponent system, consisting of a transporter for glucose-6-*P* (which allows it to enter the microsomal vesicle) and a glucose-6-*P* phosphohydrolase, located at the luminal aspect of the microsomal membrane [10,11]. Against this background, in the present report the possibility that glucose-6-*P* may favour active Ca^{2+} accumulation in kidney microsomal fractions as well has been investigated.

Microsomes were isolated from kidneys of 12-hours fasted, male Sprague-Dawley rats (180–200 g), after the procedure of Landon and Norris [12], as modified by Moore et al. [1], in sucrose media containing 1 mM dithiotreitol. 'Light' microsomes yielded by such procedure (originally used for studies on kidney microsomal ATPase activity and calcium pumps) have been extensively characterized as to their Ca^{2+}-sequestering capacity [13].

After isolation, microsomes were washed and resuspended in a medium of the following composition (mM): KCl, 100; NaCl, 20.0; $MgCl_2$, 5.0; Mops, 10.0 (pH 7.2), containing 1 mM dithiotreitol. In the microsomal preparations employed, the enrichment factors versus whole homogenate for the marker enzymes glucose-6-phosphatase, cytochrome-*c* oxidase and 5′-nucleotidase [3] were about 1.9 ± 0.3, 0.2 ± 0.02 and 0.8 ± 0.2, respec-

Abbreviations: glucose-6-*P*, glucose 6-phosphate; Mops, 3-(*N*-morpholino)propanesulfonic acid.

Correspondence: A. Benedetti, Istituto di Patologia Generale, Università di Siena, Via del Laterino 8, 53100 Siena, Italy.

tively (means ± S.E. of 3–8 determinations); these values are in agreement with previous data [13]. The integrity of the membrane of microsomal vesicles was ascertained by measuring the latency of mannose-6-phosphatase activity [14], which was anyway lower than 15% with respect to the activity of fully disrupted microsomal vesicles.

For evaluation of MgATP-dependent Ca^{2+} accumulation, kidney microsomes were incubated (0.1–0.3 mg protein/ml) in the medium described above, without dithiotreitol, in the presence of: 1 mM ATP plus an ATP-generating system (5 mM creatine phosphate and 5 μM units/ml creatine phosphokinase), 20 μM $CaCl_2$ plus 1 μCi/ml $^{45}Ca^{2+}$, and 2 μM ruthenium red or 5 mM NaN_3 (as mitochondrial inhibitors). $^{45}Ca^{2+}$ accumulated by microsomes was measured by using a rapid filtration technique [2]. The individual Ca^{2+} uptake assays were corrected for the non-specifically bound Ca^{2+}, by subtracting the amounts of Ca^{2+} becoming associated with microsomes in the absence of ATP; these values were anyway lower than 0.7 nmol Ca^{2+}/mg protein. In some experiments, the concentration of free Ca^{2+} in the incubation medium was measured instead, by means of a selective Ca^{2+}-electrode constructed after Affolter and Sigel [15], and calibrated after Tsien [16]. Background Ca^{2+} concentrations in the media used for incubations, (i.e., calcium detectable as contaminant before any exogenous addition) were in the range of 15–20 nmol/ml, as determined by atomic absorption spectroscopy.

Inorganic phosphates accumulated by microsomes were determined by the same rapid filtration technique used for Ca^{2+} determinations. Microsomes harvested on filters were rapidly washed with 0.25 M sucrose and extracted 15 min with 1 M HCl; inorganic phosphate was determined in aliquots of the acid extract [17]. Glucose-6-phosphatase activity was assayed by measuring the amounts of glucose released from glucose-6-*P* enzymatically [2], or (with low glucose-6-phosphatase activities) by measuring [^{14}C]glucose released from [^{14}C]glucose-6-*P* [18]. Protein was determined according to Lowry et al. [19].

As can be seen in Fig. 1, in the presence of MgATP kidney microsomes accumulate Ca^{2+} up to steady-state levels of approx. 1.7 nmol Ca^{2+}/mg protein. The subsequent addition of glucose-6-*P* (2 mM) causes a further, much higher accumulation of Ca^{2+} into microsomes, up to a new steady-state level of approx. 27 nmol Ca^{2+}/mg protein, and the concomitant hydrolysis of glucose-6-*P* by kidney microsomes increases linearly with time. Ca^{2+} accumulated in the presence of glucose-6-*P* was fully releasable by the Ca^{2+}-ionophore A23187 (2 μM), as already observed in the case of liver microsomes [20]. The effect of various concentrations of glucose-6-*P* on the MgATP-dependent Ca^{2+} accumulation by kidney microsomes and on their glucose-6-phosphatase activity

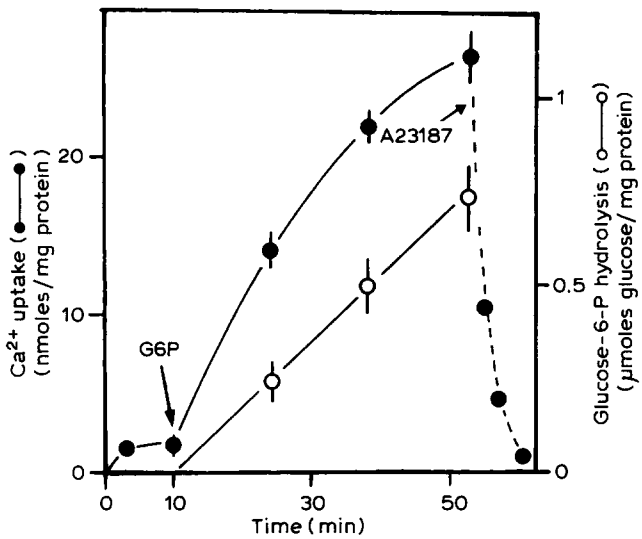

Fig. 1. Glucose 6-*P* (G6P)-stimulation of MgATP-dependent Ca^{2+} uptake and concomitant glucose-6-*P* hydrolysis in rat kidney microsomes. Microsomes were incubated (0.2 mg protein/ml) at 37 C in Mops buffer (pH 7.2) containing 100 mM KCl, 20 mM NaCl, 5 mM $MgCl_2$, 20 μM $CaCl_2$, 1.0 μCi/ml $^{45}CaCl_2$, 2 μM ruthenium red and 1 mM ATP, plus an ATP-regenerating system (5 mM creatine phosphate and 5 μM units/ml creatine phosphokinase). 10 min later glucose 6-phospate (2 mM, final concentration) was added. $^{45}Ca^{2+}$ uptake by microsomes was determined by a rapid filtration technique. Non-specifically bound Ca^{2+} (i.e., Ca^{2+} associated to microsomes in the absence of ATP) was subtracted, so to obtain net active Ca^{2+} accumulation values. Glucose-6-*P* hydrolysis was determined by measuring the glucose released following the addition of glucose-6-*P* (see text). Data reported represent means ± S.E. of four experiments.

is shown in Fig. 2. Increasing concentrations of glucose-6-*P* (0.5 to 2 mM) resulted in higher degrees of stimulation on Ca^{2+} uptake along with higher glucose-6-*P* hydrolysis. Concentrations of glucose-6-*P* higher than 2 mM did not result in further stimulation of Ca^{2+} uptake, although the glucose-6-phosphatase activity increased linearly up to 10 mM glucose-6-*P* (not shown). The observed maximal Ca^{2+} accumulation under glucose-6-*P* stimulation may represent the maximal Ca^{2+}-loading capacity of rat kidney microsomal vesicles. This capacity of rat kidney microsomal vesicles (approx. 30 nmol Ca^{2+}/mg protein) is nevertheless several fold lower than that observed with liver microsomes (100–150 nmol Ca^{2+}/mg protein) [2].

With liver microsomes, it has been shown that the hydrolysis of glucose 6-phosphate is essential for the stimulation of Ca^{2+} accumulation, and that inorganic phosphate ions yielded by the hydrolysis are accumulated intravesicularly, in an equimolar ratio with respect to accumulated Ca^{2+} [2]. Similar results were obtained with kidney microsomes (Table I). Besides, the addition of increasing concentrations (5 to 10 μM) of vanadate, adequate as to inhibit the phosphohydrolase component of glucose-6-phosphatase [21] (but leaving the MgATP-dependent Ca^{2+} accumulation nearly unmodified), resulted in a progressive inhibition of glu-

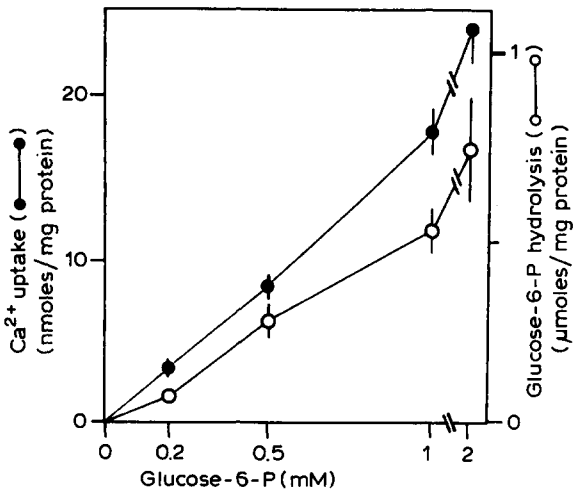

Fig. 2. MgATP-dependent Ca^{2+} accumulation (●) and glucose-6-*P* hydrolysis (○) by kidney microsomes as functions of glucose-6-*P* concentration. Microsomes were incubated as described in the legend to Fig. 1, for 30 min, in the presence of the indicated glucose-6-*P* concentrations. At the end of the incubations Ca^{2+} uptake and the amount of released glucose were determined (see text). Ca^{2+} accumulated in the presence of MgATP alone (1.7 ± 0.1 nmol/mg protein) was subtracted from glucose-6-*P*-stimulated uptakes. Data reported represent means ± S.E. of three or four experiments.

cose-6-phosphatase activity, which was parallelled by a corresponding decrease of the stimulatory effect of glucose-6-*P* on Ca^{2+} accumulation (Fig. 3).

By measuring the free Ca^{2+} levels in the incubation mixture by means of a Ca^{2+}-electrode, additional data were obtained concerning the rate of Ca^{2+} accumulation under stimulation by glucose-6-*P*. As can be seen in Fig. 4, the initial rate of glucose-6-*P*-stimulated Ca^{2+} accumulation increased with increasing glucose-6-*P* concentrations (as previously observed with liver microsomal fractions [20]) and appeared in all instances quite high, so that metabolic fluctuations of glucose-6-*P* levels

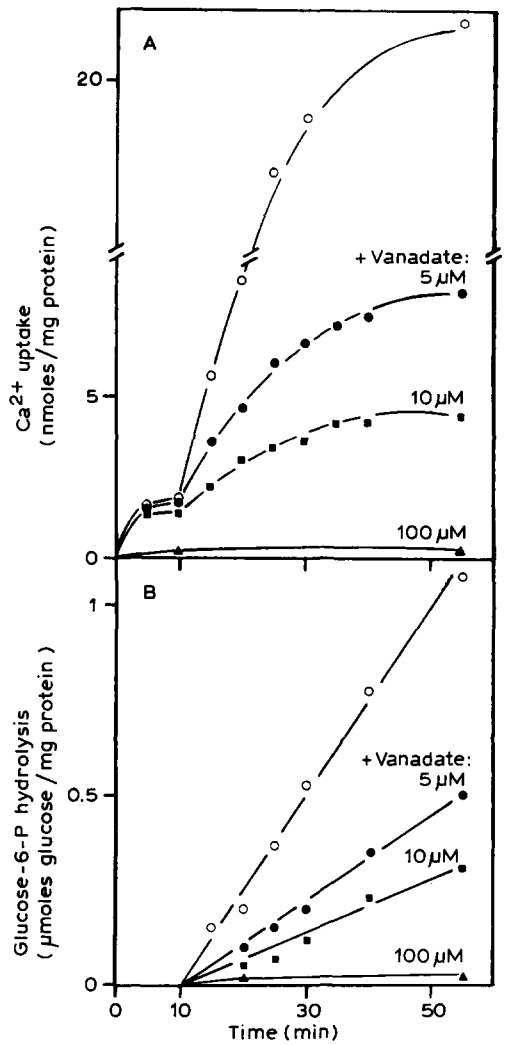

Fig. 3. Inhibition of glucose-6-*P* (G6P)-stimulated Ca^{2+} accumulation (A) and glucose-6-*P* hydrolysis (B) by vanadate in kidney microsomes. Incubation of microsomes and measurement of microsomal Ca^{2+} uptake were performed as described in the legend to Fig. 1. Glucose-6-*P* was added at 2 mM, final concentration. Where indicated, Na_3VO_4 was present in the incubation mixture at the indicated concentrations. A typical experiment out of three is reported.

TABLE I

Ca^{2+} and inorganic phosphate (P_i) contents of rat kidney microsomal vesicles incubated for MgATP-dependent, glucose-6-P-stimulated Ca^{2+} uptake

Data are expressed as nmol/mg protein, and are means ± S.E. of three or four experiments. Microsomes were incubated as reported in the legend to Fig. 1. Where indicated, 2 mM glucose-6-*P* and/or 1 mM ATP plus an ATP-regenerating system (see text) were present in the incubation mixture. The values for P_i measured at zero-time ranged 0.90 to 5.5 nmol/mg protein, in each experiment, and were subtracted in order to have the net P_i accumulation value.

Additions		Incubation time			
MgATP	glucose-6-*P*	20 min		30 min	
		Ca^{2+}	P_i	Ca^{2+}	P_i
+	+	25.3 ± 2.8	19.5 ± 2.1	28.8 ± 3.6	27.0 ± 3.5
+	−	1.8 ± 0.2	0	1.7 ± 0.2	0.4
−	+	0	0.2	0	0.3

in the cytosol could affect the microsomal Ca^{2+} content. The efficient glucose-6-*P* concentrations (100–200 μM, see Fig. 4) are comparable with those reported for rat kidney tissue [22,23].

The observed similarities between the characteristics of glucose-6-*P*-stimulated Ca^{2+} accumulation in rat liver and kidney microsomes suggest that the glucose-6-phosphatase multicomponent systems of the two tissues might be identical, as proposed by others [10,11]. In addition, it appears that also in kidney tissue one of the functions of this enzymatic complex in vivo might be the one of providing phosphate ions inside the endoplasmic reticulum lumen, where they can act as trapping agents for Ca^{2+} ions inward transported. In this respect, it should be mentioned that kidney glucose-6-phosphatase activity has been shown to be restricted to

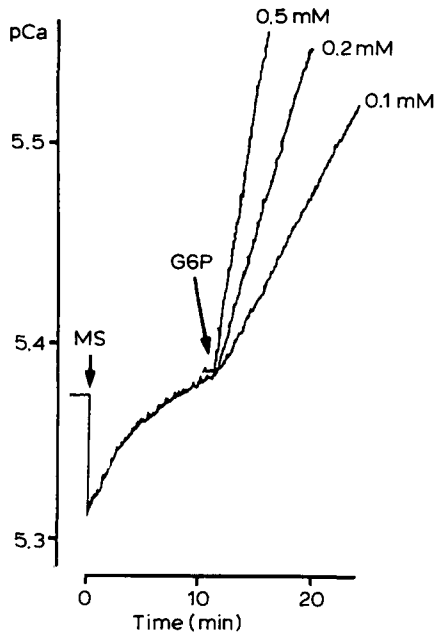

Fig. 4. Initial rate of MgATP-dependent Ca^{2+} uptake by kidney microsomes under stimulation by varying concentrations of glucose-6-*P*. Ca^{2+} uptake was monitored by measuring the free Ca^{2+} concentration in the incubation mixture with a Ca^{2+}-electrode. The incubation mixture was as reported in the legend to Fig. 1, except for ruthenium red, which was replaced by 5 mM NaN_3, and microsomal protein, which was 1 mg/ml. No $CaCl_2$ was added. Total Ca^{2+} present in incubates as contaminant was 15–18 μM, as determined by atomic absorption spectroscopy. Where indicated, glucose-6-*P* was added to the incubation medium. A typical set of experiments out of three is reported.

the proximal tubular epithelium [24], and this indeed is the site of glucose production in kidney [25]. Thus, it is conceivable that the glucose-6-*P*-induced stimulation of active Ca^{2+} accumulation observed in kidney microsomes is actually reflecting events that are allegedly restricted in vivo to the reticular compartment of tubular cells only.

As reported in Fig. 1, Ca^{2+} accumulated by kidney microsomes in the presence of MgATP alone does not exceed 2 nmol/mg protein, which represents a remarkably low value as compared to those of microsomal preparations from several non-muscle cell types. In fact, maximal MgATP-dependent Ca^{2+} accumulations by microsomes as high as 10–15, approx. 35, 15–50 and 25 nmol/mg protein have been reported for liver [2,20,26], brain [27], platelets [28] and parotid gland [29,30], respectively. Comparably low maximal MgATP-dependent Ca^{2+}-accumulations (2.3 ± 0.5 nmol Ca^{2+}/mg protein) were exhibited also by kidney microsomes prepared differently, i.e., with the procedure previously used for liver microsomes [2]. Accordingly, due to such low maximal Ca^{2+}-loading capacity, measurements of free Ca^{2+} levels in the incubation mixture (Fig. 4) showed only minimal variations of p*Ca* when 1 mg microsomal protein/ml was added, in the presence of MgATP alone and excess Ca^{2+} in the medium (17–18 μM). On the contrary, provided that the ratio microsomes/medium Ca^{2+} was increased, kidney microsomes did prove able to lower the external free Ca^{2+} concentration (with MgATP alone) down to 0.1 μM (data not shown). Thus, it appears that the affinity of kidney microsomes for Ca^{2+} ions is indeed comparable with that of microsomes from other tissues, despite being accompanied by a lower Ca^{2+}-loading capacity. This limitation of kidney microsomal active calcium accumulation is efficiently overcome in the case of glucose-6-*P* stimulation, which allows kidney microsomes to attain a Ca^{2+}-loading capacity comparable with that of microsomes from other cell types. From a speculative point of view, the observed stimulation of microsomal Ca^{2+} uptake by glucose 6-phosphate may represent an important regulatory mechanism of cellular calcium homeostasis also in the kidney, besides liver [6] and pancreatic islet cells [5,8]. It should be considered in fact that glucose-6-*P* is currently available in kidney tissue [22,23], and that its concentration is likely to change following the challenge of kidney tubular cells with hormones activating the gluconeogenetic pathways [31,32] through mechanisms involving elevations of cytosolic Ca^{2+} [32].

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