ISSN 0006-2979, Biochemistry (Moscow), 2008, Vol. 73, No. 10, pp. 1146-1153. © Pleiades Publishing, Ltd., 2008.
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Calcium Uptake in Rat Liver Mitochondria Accompanied by Activation of ATP-Dependent Potassium Channel

O. V. Akopova*, V. I. Nosar, I. N. Mankovskaya, and V. F. Sagach

Bogomolets Institute of Physiology, National Academy of Sciences of Ukraine, Kiev, Ukraine;
E-mail: circul@biph.kiev.ua; mankovsk@biph.kiev.ua

Received June 9, 2007
Revision received March 24, 2008

Abstract—The influence of potassium ions on calcium uptake in rat liver mitochondria is studied. It is shown that an increase in K^+ and Ca^{2+} concentrations in the incubation medium leads to a decrease in calcium uptake in mitochondria together with a simultaneous increase in potassium uptake due to the potential-dependent transport of K^+ in the mitochondrial matrix. Both effects are more pronounced in the presence of an ATP-dependent K^+-channel (K^+_{ATP}-channel) opener, diazoxide (Dz). Activation of the K^+_{ATP}-channel by Dz alters the functional state of mitochondria and leads to an increase in the respiration rate in state 2 and a decrease in the oxygen uptake and the rate of ATP synthesis in state 3. The effect of Dz on oxygen consumption in state 3 is mimicked by valinomycin, but it is opposite to that of the classical protonophore uncoupler CCCP. It is concluded that the potential-dependent uptake of potassium is closely coupled to calcium transport and is an important parameter of energy coupling responsible for complex changes in oxygen consumption and Ca^{2+}-transport properties of mitochondria.

DOI: 10.1134/S000629790810012X

Key words: ATP-dependent K^+-channels, mitochondria, potassium, calcium, transport, oxygen consumption, diazoxide

The accumulation of facts testifying to a key role of mitochondria in cellular physiology has recently caused a sharp increase in interest in these subcellular structures. The results of studies of the last decade have led to substantial reassessment of the role of mitochondria in the processes of cellular functioning. The modern representation is that these organelles are not only the source of ATP, the main power resource of the cell, but also a source of a number of "risk factors" (reactive forms of oxygen and nitrogen, cytochrome *c*, and a wide variety of pro-apoptotic proteins [1, 2]), which are capable of executing programs of cell death under certain conditions.

An important physiological function of mitochondria also fully revealed in rather recent studies is their participation in regulation of cellular calcium homeostasis and in the maintenance of a certain physiological level of cytosolic Ca^{2+}, which involves the transformation of calcium signals from the plasma membrane and endoplasmic reticulum [3]. Therefore, works of recent years have revealed the role of mitochondria as a central part of biological processes responsible for the maintenance of cell viability and also for the realization of cell death programs.

Complex regulation of mitochondrial functions is provided by the coordinated work of mitochondrial uniporters and antiporters, and also by anion carriers supporting electrolyte balance between the mitochondrion and the medium, which is necessary for the normal functioning of the organelles.

It is known that besides the transport system of bivalent cations including Ca^{2+}-uniporter and also Na^+/Ca^{2+} and H^+/Ca^{2+} exchange, mitochondria also possess a rather complex system of transport of monovalent cations [4, 5]. The transport system includes sodium and potassium channels [5, 6] as well as K^+/H^+ and Na^+/H^+ exchangers, which, depending upon conditions, are capable of accumulating or liberating monovalent cations from the organelles [4]. Influx of monovalent cations into the mitochondrial matrix occurs through Na^+- and K^+-channels, and their release into the cytosol under physiological conditions occurs through Na^+/H^+- and K^+/H^+-antiporters.

The ATP-dependent K^+-channel, which was discovered by Inoue in 1991 [7], is probably the most studied

Abbreviations: CCCP) carbonyl cyanide *m*-chlorophenylhydrazone; Dz) diazoxide; ROS) reactive oxygen species.

* To whom correspondence should be addressed.

channel type in mitochondria. It is located in the internal membrane of mitochondria; the matrix-directed transport of K^+ through the K^+_{ATP}-channel is blocked by ATP from the external side of the mitochondrial membrane [5, 6]. It is assumed that under physiological conditions the opening of the K^+_{ATP}-channel occurs on a sharp decrease in the intracellular concentration of ATP, i.e. during ischemia [8]. Mitochondrial K^+_{ATP}-channel is sensitive to pharmacological agents: openers and blockers of plasma membrane K^+_{ATP}-channel. Some of the most often applied openers (pinacidil, cromakalim, nicorandyl, diazoxide, etc.) and blockers (glibenclamide, 5-hydroxydecanoic acid) of K^+_{ATP}-channels possess a relatively high specificity to just mitochondrial K^+_{ATP}-channel (diazoxide, 5-hydroxydecanoic acid).

The mitochondrial K^+_{ATP}-channel has drawn the attention of a broad number of researchers as a target in studies of mechanisms of protection of myocardium from damage under various pathological conditions [8-11]. Thus it is known that the protective effect of K^+_{ATP}-channel openers, defending mitochondria from risks connected with Ca^{2+} overload and the opening of mitochondrial pores under conditions of myocardium ischemia–reperfusion, is similar to effects of preconditioning (a short-term preliminary ischemia) or introduction of a specific inhibitor of mitochondrial pores—cyclosporin A [8-11].

The efficacy of K^+_{ATP}-channel openers is connected with their ability to increase the influx of potassium ions into the mitochondrial matrix [8, 10-12]. Thus according to Garlid [12] a cardioprotective effect of K^+_{ATP}-channel activators is based on the increase in volume and matrix alkalinization due to K^+ influx, which leads to an increased formation of reactive oxygen species (ROS) and activation of protein kinase C (a prospective central part of the protective mechanism). However, despite the accumulated data the conceptions of biochemical mechanisms underlying cytoprotective and in particular cardioprotective actions of these substances in general remain contradictory.

The published data indicate that the K^+ transport activated by the opening of K^+_{ATP}-channel is a strong modulator of basic mitochondrial functions: oxygen consumption [13, 14], generation of proton gradient and transmembrane potential [13, 15], synthesis [13] and hydrolysis [14] of ATP, and thus all main characteristics of the mitochondrial energy state.

It is known that one of the reasons for mitochondrial dysfunction underlying a wide spectrum of pathological conditions is an impairment of Ca^{2+}-homeostasis caused by calcium overload in these organelles. As already mentioned above, the activation of the K^+_{ATP}-channel is a protective mechanism from calcium overload of mitochondria in particular under conditions of ischemia–reperfusion [8, 10, 13]. However, molecular mechanisms underlying the protective effect of K^+_{ATP}-channel openers from calcium overload remain poorly understood [13, 16].

It is assumed that the protection of mitochondria from Ca^{2+} overload consists in the uncoupling of the respiratory chain [14, 17] by these substances and in partial mitochondrial depolarization [11, 13, 17] limiting the access of calcium into the matrix, which in turn should reduce the probability of the opening of mitochondrial pores [11, 13, 14]. However, opposing facts are known indicating an increased accumulation of Ca^{2+} and induction of the mitochondrial pore upon action of the K^+_{ATP}-channel activator diazoxide [18]. Thus, the protective mechanism assumed by many authors [10, 11, 13, 14] does not give a satisfactory explanation of all data on the action of K^+_{ATP}-channel openers on Ca^{2+} accumulation and basic mitochondrial functions—oxygen consumption and ATP synthesis.

Since the efficiency of K^+_{ATP}-channel openers is based on their ability to raise K^+ influx into mitochondria, the purpose of the present work was to study the influence of K^+ on Ca^{2+} accumulation and oxygen consumption in mitochondria from rat liver under conditions of the activation of their transport through the K^+_{ATP}-channel.

MATERIALS AND METHODS

Wistar white rats, body weight of 200-250 g, were used in the experiments. Liver was washed with chilled (4°C) 0.9% KCl solution, crushed, and homogenized in 5-fold volume of medium: 250 mM sucrose, 20 mM Tris-HCl, 1 mM EDTA, pH 7.4. For isolation of mitochondria, the homogenate was centrifuged for 7 min at 700*g* (4°C), and then the supernatant was centrifuged for 15 min at 11,000*g* (4°C). The pellet was resuspended in a small volume of the medium without EDTA added and stored on ice at 4°C.

Calcium accumulation was registered spectrophotometrically at 654 nm in presence of the metallochromic indicator, arsenazo-III (final concentration 70 μM). The quantity of Ca^{2+} added (nmol/mg protein) that was completely absorbed by mitochondria was used as the value of Ca^{2+} capacity. The absorption of calcium ions by the mitochondria was registered upon the disappearance of a characteristic maximum of absorption of Ca^{2+}–arsenazo complexes. Calcium fraction (percent of that added to the medium) accumulated by mitochondria was defined in conditions of incomplete absorption of added Ca^{2+}.

The initial velocity of Ca^{2+} transport was determined from kinetic curves of the absorption change at 654 nm, which is characteristic of Ca^{2+} accumulation. Under conditions of strong swelling caused by K^+ efflux, data corresponding to the change in light absorbance due to mitochondrial swelling were subtracted from the registered curves of absorption.

Potassium transport in the matrix was estimated using the value of swelling for energized mitochondria determined from the absorption at 520 nm taking into

account that swelling reflects the accumulation of the cation in mitochondria [19, 20]. The initial velocity V_0 of K^+ transport was estimated in relative units in relation to the maximal velocity of absorbance change at 520 nm.

The accumulation of potassium ions was studied in the following medium: 1 mM K_2HPO_4, 5 mM sodium glutamate, and 10 mM Tris-HCl buffer, pH 7.4; the concentration of KCl was varied in the range of 120-180 mM. For the experiments on Ca^{2+} transport the medium was supplemented with Ca^{2+} (final concentration 30-70 μM). Mitochondrial concentration corresponded to 1 mg/ml protein. Medium tonicity was achieved by the addition of sucrose up to 350 mosmol/liter. To block Ca^{2+}-induced mitochondrial pores [5] when studying the accumulation of calcium ions, the incubation medium was supplemented with cyclosporin A (10^{-6} M). The K^+_{ATP}-channel opener diazoxide and K^+-ionophore valinomycin were added to concentrations of $5\cdot10^{-4}$ and 10^{-7} M, respectively.

Oxygen consumption was studied under standard conditions by the polarographic method using a Clark oxygen electrode at 26°C in the incubation medium: 120 mM KCl, 10 mM Tris-HCl buffer, pH 7.4, 4 mM sodium glutamate, and 1 mM KH_2PO_4. The final concentration of ADP was 200 μM, and the final concentration of protein was 1 mg/ml.

The following reagents were used in the present work: arsenazo-III, sodium glutamate, Tris (base), carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), valinomycin, and diazoxide (Sigma, Germany); ADP and cyclosporin A (Fluka, Switzerland). Other chemicals were analytical or chemical grade. Solutions were prepared with bidistilled water. The reliability of results was estimated by the Student *t*-criterion; $p < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

It is known that the action of K^+_{ATP}-channel openers on isolated mitochondria is not observed in K^+-free medium [14] and appears in media containing sufficiently high K^+ concentrations characteristic for physiological conditions (potassium concentration in the cell is about 150 mM [21]). According to published data [19], energy-dependent transport of K^+ into mitochondria is performed both by passive diffusion (so-called "potassium leakage", K^+ leak) and by K^+ influx through an ATP-dependent K^+-channel (it should be noted that the molecular structures responsible for the mechanism of K^+ passive diffusion are still not well known). In energized mitochondria, the accumulation of calcium ions occurs simultaneously with the uptake of K^+ from the incubation medium. Therefore, we were going to study not only the influence of the proper K^+_{ATP}-channel activation but also K^+ transport as a whole on Ca^{2+} accumulation over a wide interval of potassium concentrations, 120-180 mM.

To characterize the transport of K^+ over the concentration range of KCl under investigation, we studied the kinetics of mitochondrial swelling using light absorbance [19, 20]. It is known that the transport of electrolytes in the matrix is accompanied by mitochondrial swelling. It was shown [19, 20, 22] that kinetic characteristics of swelling correspond to the kinetics of the transport. It is also well known that an increase in the volume of matrix is accompanied by a decrease in the absorbance of mitochondrial suspensions [20]. The transport was followed from the time of substrate (glutamate) introduction into the mitochondrial suspension.

The data show that the difference in suspension absorbance at the initial point and after the end of the transport process (swelling amplitude) depends upon K^+ content in the incubation medium (Fig. 1a). The quantity of K^+ absorbed by mitochondria increases with an increase in the concentration of K^+ in the medium (Fig. 1a, column *1*). The K^+_{ATP}-channel activator diazoxide increases the swelling in the concentration range ⩽160 mM K^+ (Fig. 1a, column *2*). The maximal value of swelling is observed in the presence of the K^+-ionophore valinomycin and corresponds to the maximum quantity of K^+ absorbed (Fig. 1a, column *3*).

We also studied the dependence of the initial velocity of K^+ transport (by registering absorbance) on K^+ concentration in the control medium as well as in presence of valinomycin and diazoxide. Thus, the highest value of V_0 was taken as 1 in each experimental series, and other velocity values were expressed in relative units (Fig. 1b, curves *1-3*). In all cases the initial velocity of K^+ accumulation increased linearly with increase in K^+ concentration in the medium in the concentration range ⩽160 mM.

It is known that an addition of valinomycin to a suspension of energized mitochondria causes rapid influx of K^+ into the mitochondrial matrix, depending on the value of transmembrane potential $\Delta\Psi_m$ [23]. The relative values of V_0 shown in Fig. 1 give almost identical dependence of the increase in the velocity of K^+ accumulation upon increase in the K^+ concentration in the control medium and in the presence of valinomycin in the concentration range ⩽160 mM K^+ indicating the potential-dependent character of K^+ transport into the matrix under these experimental conditions. To confirm the validity of the given assumption we calculated, also in relative units, the velocity of K^+ transport according to the known equation that connects the velocity of transport of monovalent cation with the value of membrane potential and transmembrane gradient of cation concentration [24]:

$$V = K\Delta\Psi\{[Me^+]_i - [Me^+]_0\cdot\exp(-F\Delta\Psi/RT)\}/$$

$$/\{\exp(-F\Delta\Psi/RT) - 1\},$$

where K is a constant which does not depend on $\Delta\Psi$ value and includes the coefficient of membrane permeability

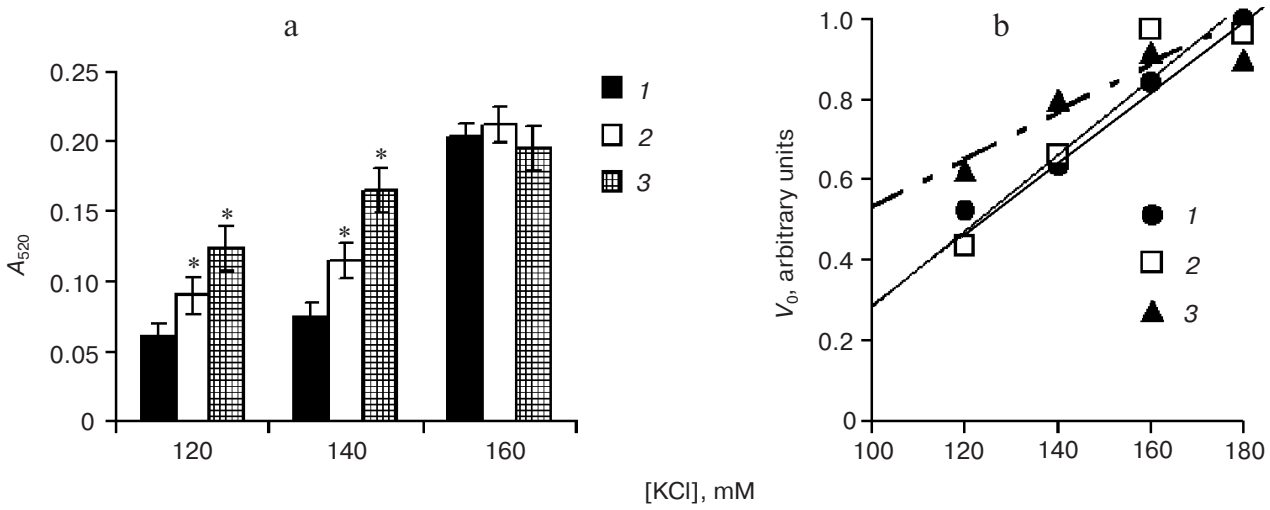

Fig. 1. Influence of K^+ concentration in the medium on the absorbance of suspension of energized mitochondria (a) and on the relative initial velocity of K^+ accumulation (b). Incubation medium: 10 mM Tris-HCl buffer, pH 7.4, 4 mM sodium glutamate, 1 mM KH_2PO_4. Ordinate axis: a) difference in absorbance at 520 nm at the initial point and after the completion of transport (A_{520}); b) initial velocity of K^+ accumulation, V_0, in arbitrary units. The maximal velocity of transport in the control (*1*) and in presence of diazoxide (*2*) and valinomycin (*3*) was determined from absorbance data and taken as 1. Calculated dependence according to the equation is represented by the dashed line. Registration of absorbance was begun upon the addition of glutamate. Diazoxide ($5 \cdot 10^{-4}$ M) and valinomycin (10^{-7} M) were added together with the substrate. $p < 0.05$ (3); * significant compared to control.

for the cation given; $[Me^+]_i$ and $[Me^+]_0$ are cation concentrations in the matrix and in the medium, respectively; T is temperature; F and R are known constants [24]. Under conditions of constant membrane potential, the velocity of cation transport, according to the given equation, is defined by the value of concentration gradient of cation on the two sides of the membrane. The value of V_0 for K^+ transport in the range $[K^+]_0$ = 100-180 mM was calculated taking the value of $\Delta\Psi$ to be equal to 150 mV; according to the literature, $[K^+]_i$ is about 120 mM [22]. Values of V_0 calculated in relative units as above are presented in Fig. 1b (dashed curve). The data shows that the velocity of the accumulation of K^+ in the control (Fig. 1b, curve *1*) as well as in presence of valinomycin and diazoxide (Fig. 1b, curves *2* and *3*, respectively) corresponds well enough to the calculated dependence in the K^+ concentration range ⩽160 mM. Therefore, we conclude that with an increase in K^+ concentration in the medium the potential-dependent transport of K^+ in the matrix leads to increased accumulation of K^+ in mitochondria at K^+ concentrations close to physiological (~150 mM) [21]. The K^+_{ATP}-channel activator diazoxide increases the accumulation of K^+ in the physiological range of cation concentrations.

Calcium accumulation was studied by introducing mitochondria into medium containing given concentrations of $CaCl_2$ (30, 60, and 70 μM) and KCl in presence of 10^{-6} M cyclosporin A, which excludes the induction of Ca^{2+}-dependent mitochondrial pores. The data (Figs. 2a and 2b) show that an increase in the concentration of K^+ in the medium caused a decrease in the quantity of mitochondria-accumulated calcium. An increase in Ca^{2+} concentration from 30 to 70 μM does not increase the accumulation of the cation in mitochondria (Fig. 2a). Even lower level of the accumulation of Ca^{2+} is observed in the presence of $5 \cdot 10^{-4}$ M diazoxide (Fig. 2b). An increase in the concentration of K^+ causes a decrease in both the quantity of Ca^{2+} accumulated by mitochondria and in the initial velocity of accumulation, V_0 (Fig. 2d). Thus, diazoxide also considerably increases the inhibitory action of K^+ on the transport of Ca^{2+} (Fig. 2d, curve *2*).

The results presented in Fig. 2 show that the suppression of Ca^{2+} transport (decrease in V_0 (Fig. 2d) and in the accumulation of cation (Figs. 2a and 2b)) is caused by the total decrease in Ca^{2+} capacity of the mitochondria, which was determined as a maximal quantity of Ca^{2+} completely absorbed by the mitochondria with an increase in K^+ concentration in the medium (Fig. 2c). Thus the maximal value of Ca^{2+} capacity observed at the lowest K^+ concentration of 120 mM (which corresponds to the standard incubation medium) in the range investigated was 108.3 ± 7.4 nmol/mg protein, while in the presence of 180 mM KCl Ca^{2+} capacity was minimal 38.7 ± 5.1 nmol/mg and its further decrease was not observed even in the presence of diazoxide (33.12 ± 3.4 nmol/mg). The introduction of diazoxide into the standard incubation medium sharply reduces the initial value of Ca^{2+} capacity to 60.7 ± 6.5 nmol/mg (Fig. 2c).

Therefore, the reverse relationship between changes in Ca^{2+}-transport characteristics (initial velocity of Ca^{2+} accumulation (V_0) and Ca^{2+} capacity) and K^+ maintenance in the incubation medium was observed: an

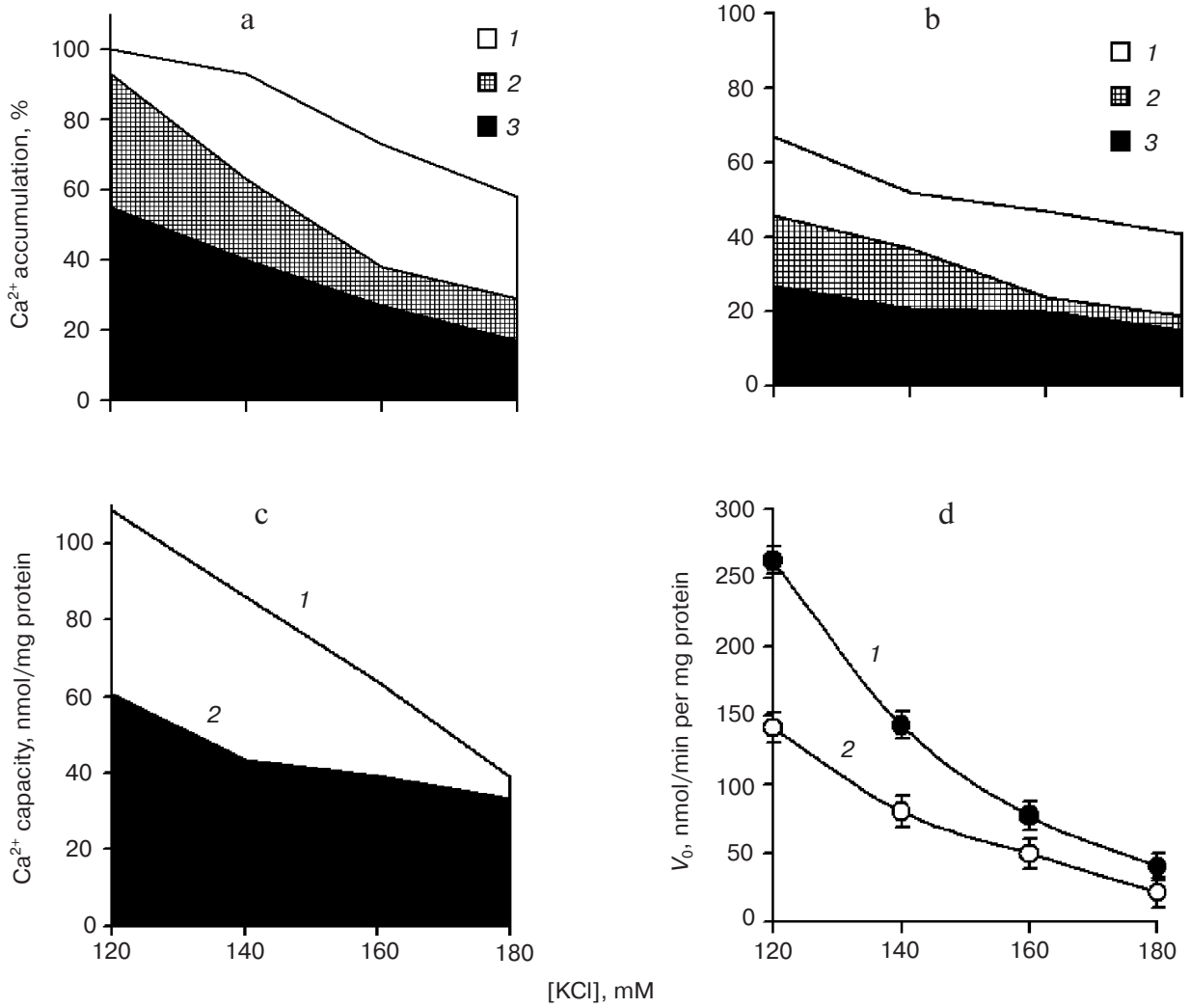

Fig. 2. Influence of K^+ on Ca^{2+} accumulation by mitochondria. Incubation medium: 10 mM Tris-HCl buffer, pH 7.4, 4 mM sodium glutamate, 1 mM KH_2PO_4, 1 μM cyclosporin A. Ordinate axis: Ca^{2+} accumulation as percent of the added Ca^{2+} in absence (a) and in presence of $5 \cdot 10^{-4}$ M diazoxide (b), Ca^{2+}-capacity of mitochondria in nmol Ca^{2+} per mg protein (c), and initial velocity of Ca^{2+} accumulation in nmol/min per mg (d) in absence (*1*) and in presence (*2*) of diazoxide. Final concentration of added Ca^{2+}: a, b) 30 (*1*), 60 (*2*), 70 μM (*3*); d) 60 μM.

increase in K^+ concentration sharply inhibits calcium capture by mitochondria. At the same time the data on swelling caused by K^+ influx in the matrix of energized mitochondria [19] show that an increase in K^+ concentration causes an increase in its transport from the medium to the matrix, especially in the presence of diazoxide (Fig. 1a, column *2*). Thus, an increase in the concentration of K^+ leads to an increase in both the velocity of K^+ accumulation and in the amplitude of mitochondrial swelling (Figs. 1a and 1b).

Uncoupling action on the respiratory chain [11, 14, 17] and the dissipation of membrane potential [13-15], causing decrease in energy-dependent accumulation of calcium, is often cited as one of the reasons for the suppression of Ca^{2+}-accumulating ability of mitochondria upon the action of K^+_{ATP}-channel openers observed also by other authors [11, 13, 17]. Therefore, we decided to compare the influence of K^+_{ATP}-channel opener (diazoxide) on basic characteristics of oxygen consumption by the mitochondrial respiratory chain using the "classical" uncoupler, the protonophore CCCP.

Figure 3 shows typical dependences of diazoxide influence on oxygen consumption by liver mitochondria. The results of the experiments demonstrate that introduction of $5 \cdot 10^{-4}$ M diazoxide into the suspension of coupled mitochondria, responsible for the oxidation of the substrate (glutamate), leads to the significant acceleration of respiration in comparison with the control (Fig. 3, curves *2* and *3*). A similar effect in Chance's state 2 is even more pronounced after the addition of 10^{-7} M K^+-ionophore valinomycin (Fig. 4), which is known to cause fast K^+ influx into energized mitochondria. In the presence of

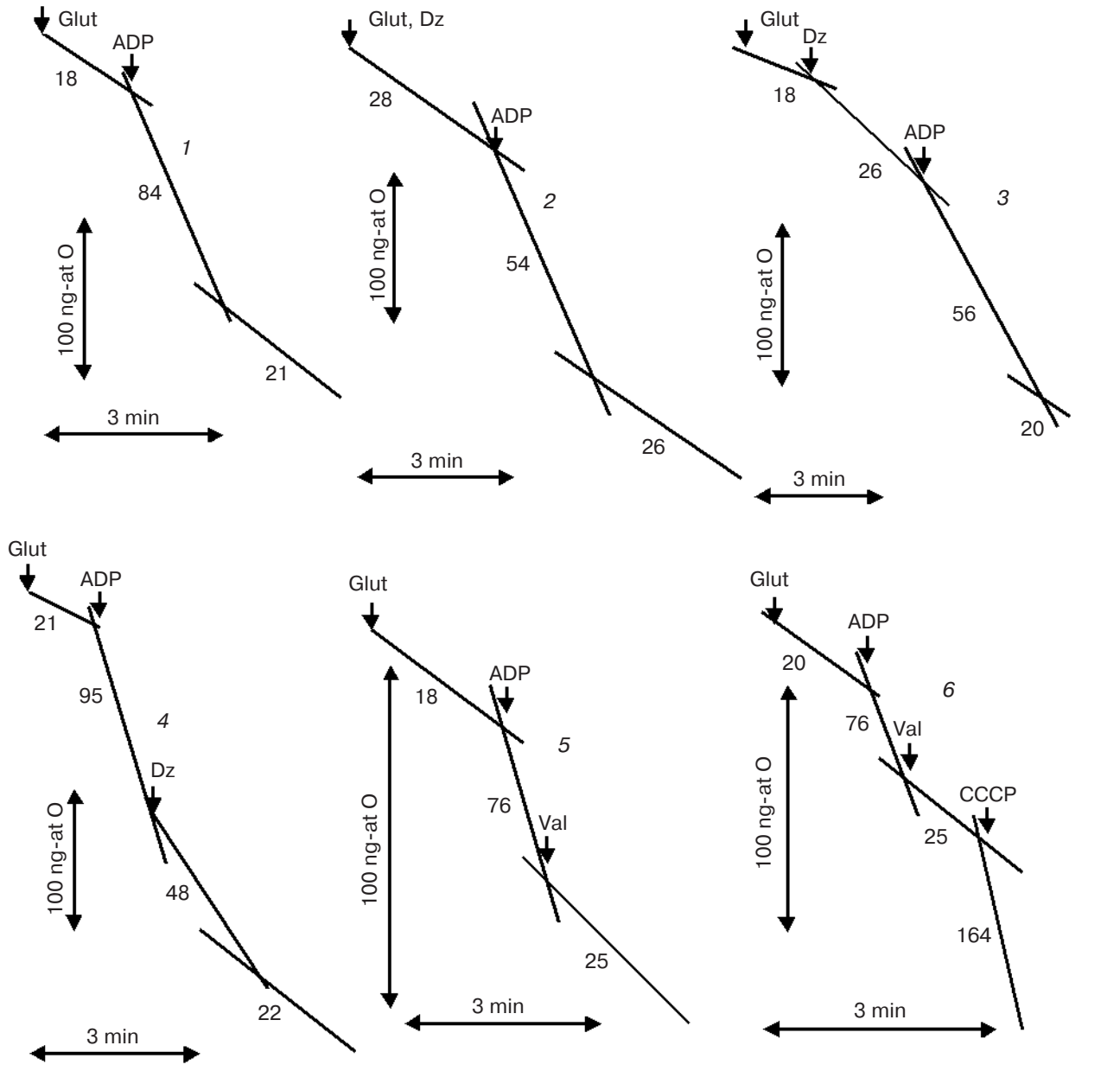

Fig. 3. Oxygen consumption by liver mitochondria during K^+_{ATP}-channel activation. Incubation medium: 120 mM KCl, 10 mM Tris-HCl buffer, pH 7.4, 4 mM sodium glutamate (Glut), 1 mM KH_2PO_4, 1 μM cyclosporin A. Reagents: $5 \cdot 10^{-4}$ M diazoxide (Dz), 10^{-7} M valinomycin (Val), 10^{-6} M CCCP, and 0.2 mM ADP. The additions are indicated by arrows. Time (min) is indicated on the abscissa axis, and oxygen consumption (ng-at O) is indicated on the ordinate axis.

valinomycin the velocity of oxygen consumption in state 2 increased sharply to 106.6 ± 8.7 ng-at O/min per mg and reached the maximal registered value of respiration velocity for CCCP protonophore uncoupling, 179.4 ± 10.2 ng-at O/min per mg.

However, in Chance's state 3 diazoxide addition leads to an obvious decrease in velocities of oxygen consumption and oxidative phosphorylation revealed by the decrease in consumption velocity ADP, ADP/Δ*t*, that also corresponds to earlier data [14]. The effect of diazoxide is unidirectional with the action of valinomycin (Figs. 3 (curves *4-6*) and 4) in this case also.

It should be noted that respiration acceleration in Chance's state 2 and its delay in state 3 upon the action of diazoxide (as well as that of valinomycin) occurs independently of the time of addition of K^+_{ATP}-channel opener in the suspension (Fig. 3). Thus, the immediate addition of diazoxide at the stage of substrate oxidation leads to acceleration (Fig. 3, curves *2* and *3*), while the addition at the stage of oxidative phosphorylation leads to signifi-

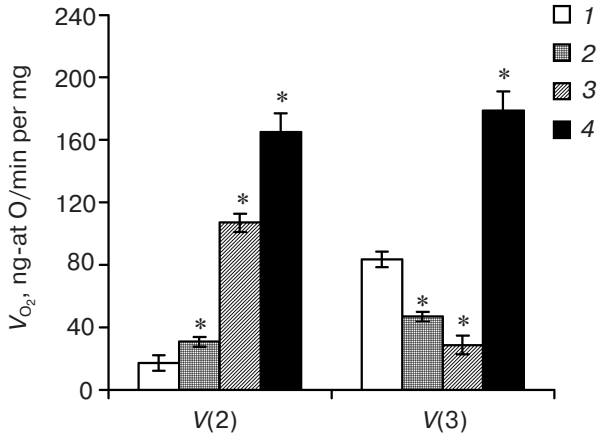

Fig. 4. Influence of K^+_{ATP}-channel activation on oxygen consumption, V_{O_2}, in Chance's states 2 and 3. Incubation medium: 120 mM KCl, 10 mM Tris-HCl buffer, pH 7.4, 4 mM sodium glutamate, 1 mM KH_2PO_4, 1 μM cyclosporin A. The final concentration of ADP was 0.2 mM. *1*) Control; *2*) diazoxide ($5 \cdot 10^{-4}$ M); *3*) valinomycin (10^{-7} M); *4*) CCCP (10^{-6} M). In state (3), diazoxide and valinomycin were added after ADP. (Effect of diazoxide did not depend on the time of its addition to the medium.) Protein concentration, 1 mg/ml. $p < 0.05$ (6); * significant compared to control.

cant decrease in respiration (Fig. 3, curves *2-4*). However, the ratio between V_{O_2} parameter in the control and in the presence of diazoxide remains the same (Fig. 4). These results provide the basis for the unequivocal connection between the change in functional characteristics of the respiratory chain in presence of K^+_{ATP}-channel opener with the absorption of K^+ by mitochondria.

Therefore, the mechanism of diazoxide action is different from the action of "classical" uncoupler, CCCP, at first by the observable sharp decrease in oxygen consumption in state 3. As it is known, in all cases the uncoupling effect of protonophore is revealed by acceleration of respiration due to the dissipation of the transmembrane proton gradient, which is in agreement with our data (Figs. 3 (curve *5*) and 4). Since the addition of CCCP at the stage of phosphorylating respiration both before and after the addition of diazoxide or valinomycin leads to the identical acceleration of oxygen consumption independently from the presence of diazoxide, the data indicate that the effect of respiration decrease in state 3 upon the action of K^+_{ATP}-channel opener is not a consequence of the inhibition of the respiratory chain by diazoxide itself.

It should be noted that despite the obvious connection between the effect of both diazoxide and valinomycin on the respiratory chain and K^+ transport into the mitochondrial matrix, the nature of the observed effect is not quite clear. In the literature [11, 14, 17] the uncoupling action of K^+_{ATP}-channel openers is often assumed as a possible reason for changes in functional parameters of the respiratory chain [14] and the decrease in Ca^{2+} capacity of mitochondria [13, 17]. In our opinion this assumption is insufficient for the explanation of differences between significant decrease in oxygen consumption in state 3 due to the activation of K^+ transport in the presence of diazoxide and sharp acceleration of O_2 consumption caused by the dissipation of proton gradient upon uncoupler action, as well as for the explanation of observed increase in the quantity of K^+ captured by mitochondria along with the increase of cation concentration when it is expected to decrease during the uncoupling and decrease of membrane potential (as observed in the presence of CCCP). The data of the present work indicate that observed changes in functional characteristics of the respiratory chain under the action of K^+_{ATP}-channel opener (respiration acceleration in state 2, slow-down of phosphorylating respiration, and, respectively, decrease in the velocity of ATP synthesis) are caused at first by K^+ influx in the mitochondrial matrix, which is dependent on the value of membrane potential ($\Delta\Psi$) and K^+ transmembrane gradient as shown above (Fig. 1b).

Thus despite that, according to the literature, some pharmacological substances—K^+_{ATP}-channel openers—also possess uncoupling and depolarizing effects [15], in our opinion the uncoupling of the respiratory chain and membrane depolarization are not the main causes of the slow-down of calcium transport under conditions of K^+_{ATP}-channel activation. Based on the results of the present study it is possible to conclude that the decrease of Ca^{2+} capacity and the slowing of Ca^{2+} transport are not caused by the depolarization of the mitochondrial membrane, but by an increase in K^+ diffusion into the matrix (which is dependent on concentration gradient of K^+ and value of $\Delta\Psi$), that is amplified by the activation of K^+_{ATP}-channel upon the action of diazoxide. The analysis of the experimental data shows that an increase in K^+ influx takes place simultaneously with the suppression of Ca^{2+} transport and is not accompanied by uncoupling of the respiratory chain.

It should be noted that the observed relationship was not satisfactorily explained in the literature. It is known that cation transport (K^+ and Ca^{2+}) in matrix is coupled with oxygen consumption and proton efflux from mitochondria in certain stoichiometric quantities. In our opinion it is possible to cite the limited ability of mitochondria to absorb cations from the medium, which is defined by the velocities of oxygen consumption and generation of proton gradient, responsible for the accumulation of mono- and bivalent cations by the organelles, as a main reason for the decrease in Ca^{2+} capacity. It is possible to assume that the activation of energy-dependent transport of K^+ amplified by K^+_{ATP}-channel opening and accompanied by the acceleration of proton extrusion from matrix, which is providing the accumulation of cations, should inevitably lead to simultaneous suppression of other energy-dependent processes, first of all ATP synthesis, and, respectively, to changes in all mitochondrial functions observed in the experiment.

Therefore, the results of the present study show that K^+ transport is an important regulator of the respiratory chain and Ca^{2+}-accumulating system of mitochondria, and its activation provides the protective mechanism from calcium overload of the organelles which represents a molecular basis for cytoprotective and cardioprotective effects of K^+_{ATP}-channel openers under physiological conditions. We believe that further studies will bring the necessary improvements in our conclusions and will allow deeper understanding of regulatory mechanisms of calcium transport and also the role of K^+ in the regulation of energy coupling in mitochondria.

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