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Influence of ATP-Dependent K^+-Channel Opener on K^+-Cycle and Oxygen Consumption in Rat Liver Mitochondria

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Abstract—The influence of the K^+_{ATP}-channel opener diazoxide on the K^+ cycle and oxygen consumption has been studied in rat liver mitochondria. It was found that diazoxide activates the K^+_{ATP}-channel in the range of nanomolar concentrations (50-300 nM, $K_{1/2}$ ~ 140 nM), which results in activation of K^+/H^+ exchange in mitochondria. The latter, in turn, accelerates mitochondrial respiration in respiratory state 2. The contribution of K^+_{ATP}-channel to the mitochondrial potassium cycle was estimated using the selective K^+_{ATP}-channel blocker glibenclamide. The data show that the relative contribution of K^+_{ATP}-channel in the potassium cycle of mitochondria is variable and increases only with the decrease in the ATP-independent component of K^+ uptake. Possible mechanisms underlying the observed phenomena are discussed. The experimental results more fully elucidate the role of K^+_{ATP}-channel in the regulation of mitochondrial functions, especially under pathological conditions accompanied by impairment of the mitochondrial energy state.

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

In the search for means for efficient protection of myocardium and other tissues from ischemic lesions and the development of cell apoptosis, researchers increasingly turn to pharmacological activators of the mitochondrial ATP-dependent K^+-channel (K^+_{ATP}-channel) [1-3] found by Inoue [4] in the inner mitochondrial membrane nearly 20 years ago. The cardioprotective effect of pharmacological activators of the K^+_{ATP}-channel revealed in clinical study was the primary stimulus for its further intensive investigation [1-3, 5]. At present, the mitochondrial K^+_{ATP}-channel calls for the attention of a rather wide circle of researchers. However, even though the cardio- and cytoprotective effects of K^+_{ATP}-channel activators are undisputed, the question of the mechanisms of their protective action is still open.

The system of K^+ transport in mitochondria includes K^+-channels and a K^+/H^+-exchanger [6-8]. In the early work of Inoue [4], numerous potential-dependent K^+-channels were revealed in the mitochondrial membrane in addition to the K^+_{ATP}-channel; most of them have not yet been identified. The most studied type of K^+-channels in mitochondria is the K^+_{ATP}-channel blocked by ATP in the presence of Mg^{2+} [2, 4, 9]. Potassium ions can enter the matrix both via the K^+-channels and via nonspecific diffusion, "K^+-leak" [8, 9], the nature of which is still unclear. Potassium ion is released from the matrix under physiological conditions through K^+/H^+-exchange [6, 8]. The influx of K^+ through the K^+-channels and its release through the K^+/H^+-exchanger make up the mitochondrial potassium cycle [8].

According to the estimates in the literature, the scale of bioenergetic effects determined by K^+_{ATP}-channel activation is low [1, 10]. However, although depolarization due to K^+ influx through the K^+_{ATP}-channel must be only 1-2 mV [1], which in itself cannot affect the energy-dependent processes in mitochondria, results of many experiments show that activation of the K^+_{ATP}-channel influences the whole complex of the main mitochondrial functions: oxygen consumption [10, 11], ATP synthesis and hydrolysis [3, 11, 12], Ca^{2+} transport [12, 13], matrix volume [1, 8, 14], and production of reactive oxygen species [1, 15]. Probably just this complex variation in the functional state of mitochondria underlies the reliably confirmed cytoprotective effects.

It is known that K^+ accumulation through K^+-channels occurs until the rate of K^+ uptake through these

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channels is balanced by the rate of its efflux through the K^+/H^+-exchanger [8] and the rate of K^+ transport in the K^+-cycle reaches certain steady-state values corresponding to establishment of a steady-state respiration rate under stationary equilibrium between K^+ accumulation and release. Because of a certain stoichiometric relationship existing between oxygen consumption and transmembrane transport of positive charges [16], it is obvious that acceleration of K^+ transport in the K^+-cycle results in acceleration of oxygen consumption by the mitochondria oxidizing substrate in respiratory state 2. In the literature, they rather seldom draw a parallel between the activities of K^+_{ATP}-channel and K^+/H^+-exchange and, accordingly, between the rate of K^+ transport in the K^+-cycle and the basic parameters characterizing the functional state of mitochondria. Besides, it should be noted that the experimental conditions revealing bioenergetic effects determined by K^+_{ATP}-channel activation (the presence of blockers, Mg^{2+}, and ATP, followed by channel reactivation with the high concentrations of openers [2, 9, 11, 14, 17]) do not show the true sensitivity of native K^+_{ATP}-channel of isolated mitochondria to its pharmacological (diazoxide, pinacidil, cromacalim [2, 9, 17]) and endogenous activators (guanosine diphosphate, uridine diphosphate [18]).

In energized mitochondria, K^+ enters the matrix not only through the K^+_{ATP}-channel but also through other K^+-conducting structures of the mitochondrial membrane (potential-dependent K^+-channels) and through K^+ leak [8]. In the absence of magnesium ions [6] and other blockers, these systems of K^+ transport are open and functionally active [8, 9, 14].

The goal of the present work was to study the influence of diazoxide, a K^+_{ATP}-channel opener, on the cyclic transport of K^+ provided simultaneous function of this channel with other, ATP-independent K^+-conducting systems of mitochondria, and to estimate the share of participation of the K^+_{ATP}-channel in the K^+-cycle of isolated rat liver mitochondria.

MATERIALS AND METHODS

Wistar white rats with a body weight of 200-250 g were used in the experiments. Liver was washed with cooled 0.9% KCl solution (4°C), ground, and homogenized in a 5-fold volume of the medium (250 mM sucrose, 20 mM Tris-HCl, 1 mM EDTA, pH 7.4). Mitochondria were isolated by centrifugation of the homogenate for 7 min at 700*g* (4°C). Then the supernatant was centrifuged for 15 min at 11,000*g* (4°C). The precipitate was suspended in a small volume of the medium without EDTA and kept on ice at 4°C. Protein content was assayed by the Lowry method.

Light absorption was recorded at 520 nm beginning from the introduction of mitochondria into the incubation medium (120 mM KCl, 5 mM Tris-HCl buffer, pH 7.4, 1 mM EDTA, 4 mM Na glutamate, 1 mM KH_2PO_4; final protein concentration 0.3 mg/ml). Oxygen consumption was studied under standard conditions by the polarographic method in a closed cell with a platinum electrode at 26°C in the same medium (final protein concentration, 1.5 mg/ml).

The effect of diazoxide on the oxygen consumption rate in state 2 (J_{O_2}) was estimated from the dependence of its relative change on diazoxide concentration (J_{rel}) expressed as a difference ratio between the value measured in the presence of diazoxide (J_{O_2}) and the control value (J_c) to the maximal experimentally recorded increment of respiration rate induced by the activator: $J_{rel} = (J_{O_2} - J_c)/(J_{max} - J_c)$. Also, the effect of diazoxide on the initial velocity (V_0) of K^+ accumulation and the change in light absorption of the suspension (ΔA_{520}) due to reduction of mitochondrial volume after addition of the nonselective blocker of K^+-channels 4-aminopyridine (4-AP) were assessed.

The K^+_{ATP}-channel opener diazoxide was added to the medium in prescribed concentrations; the K^+-channel blockers glibenclamide and 4-AP were added at the concentrations of $5 \cdot 10^{-6}$ M and 5 mM, respectively, as indicated in Fig. 1 (a and b). Introduction of dimethylsulfoxide (5 μl per ml of medium) had no effect on the measured parameters.

The following reagents were used in the work: Na-glutamate and Tris (base) (Fluka, Switzerland); diazoxide, 4-AP, glibenclamide, and EDTA (Sigma, USA); and other reagents of "especially pure" and "analytically pure" grades. Solutions were prepared using bidistilled water. Reliability of results was estimated by Student's *t*-criterion, $p < 0.05$ being considered as a statistically significant value.

RESULTS AND DISCUSSION

In the presence of penetrating anions, K^+ transport into the matrix is coupled to water uptake, and K^+ accumulation in mitochondria is accompanied by their swelling [9, 14]. Accordingly, the release of K^+ through the K^+/H^+-exchanger results in mitochondrial volume reduction due to removal of water from the matrix [8]. The changes in mitochondrial volume, in turn, are accompanied by changes in light absorption of the suspension of isolated mitochondria: it increases as the volume is reduced and decreases upon swelling [14, 19]. Thus, significant changes in the volume of organelles create a methodical possibility of recording the differently directed stages of the K^+-cycle by the changes in light absorption of the mitochondrial suspension [8].

Diazoxide is a relatively selective opener of mitochondrial K^+_{ATP}-channel [2, 17]. This fact is indicated by comparison of the activation constants ($K_{1/2}$) of K^+_{ATP}-channels of the mitochondrial and plasma membranes by

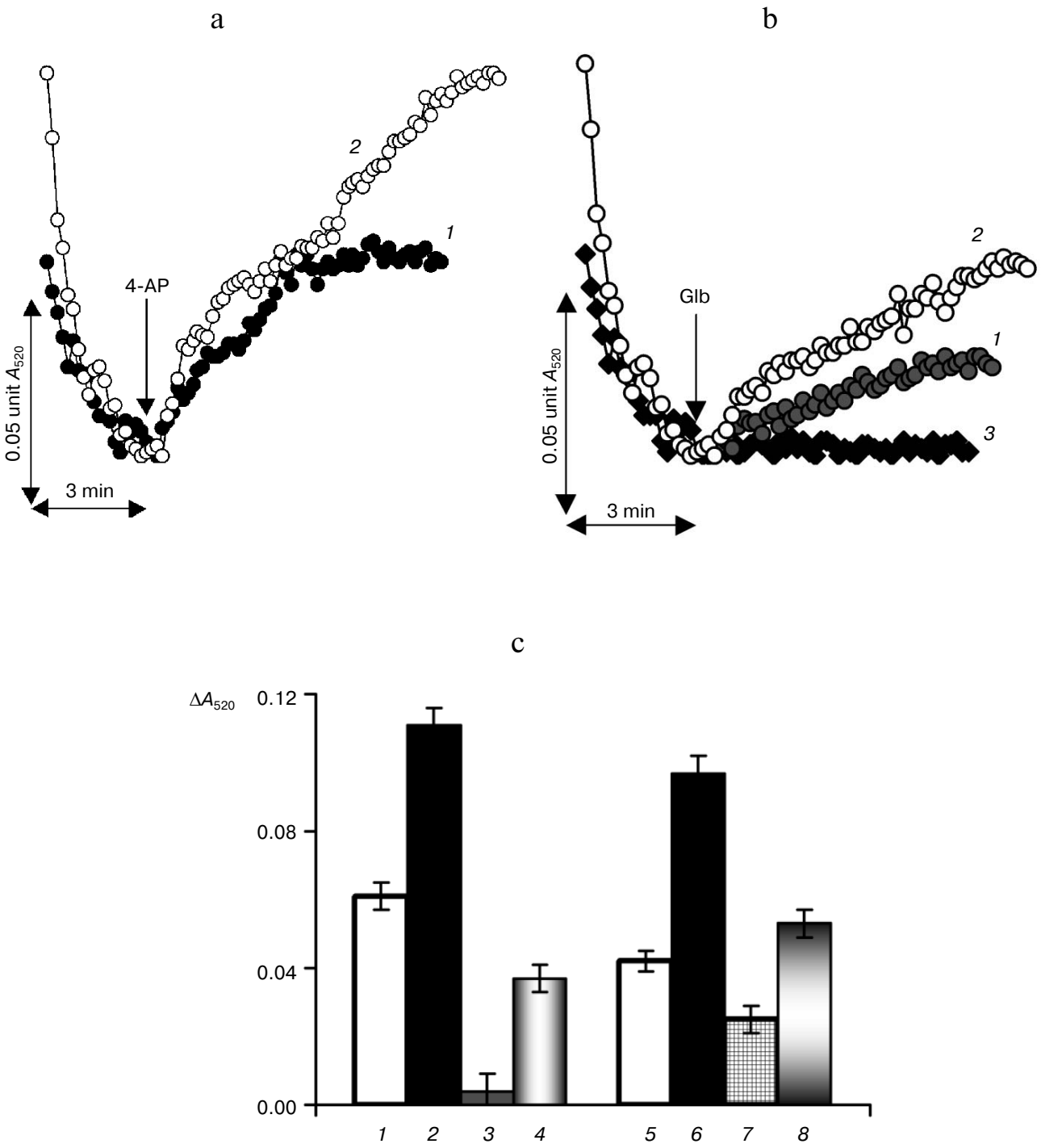

Fig. 1. Effects of diazoxide and K^+-channel blockers on light absorption of mitochondrial suspension. a, b) Blockers 4-AP and glibenclamide (Glb) were added on completion of swelling as indicated by arrows in the absence (*1*) and presence of 500 nM diazoxide (*2*). Curve *3* corresponds to light absorption change in the absence of the blockers. c) Difference in light absorption of the suspension (ΔA_{520}) during swelling (*1-4*) and mitochondrial volume reduction (*5-8*); the absolute value of difference between the initial and final absorption values was calculated. Mitochondria (0.3 mg/ml) were introduced into the medium in the presence of the following additives: 500 nM diazoxide (*2*, *6*, *8*); 1 mM $MgCl_2$, 1 mM ATP (*3*); 1 mM $MgCl_2$, 1 mM ATP, 50 μM diazoxide (*4*); 4-AP (*5*, *6*); glibenclamide (*7*, *8*); incubation medium in the absence of additives – control (*1*). $M \pm m$, $n = 6$; $p < 0.05$. In the presence of $MgCl_2$ EDTA was replaced by EGTA. Incubation medium: 120 mM KCl, 5 mM Tris-HCl buffer (pH 7.4), 4 mM Na glutamate, 1 mM KH_2PO_4, and 0.5 mM EDTA.

diazoxide (~370 nM and ~900 μM, respectively [17]). However, in the presence of blockers (Mg^{2+} and ATP), reactivation of mitochondrial K^+_{ATP}-channel requires high concentrations of activators; therefore, diazoxide concentrations used for the activation of ATP-dependent K^+ transport in isolated mitochondria differ by the order of magnitude according to the published data [3, 9, 12, 17]. Previously we showed that diazoxide increases K^+ accumulation in rat liver mitochondria in the absence of Mg^{2+} and ATP [20]. It would be logical to suppose that its preliminary block with Mg^{2+} and ATP is unnecessary for activation of the K^+_{ATP}-channel of isolated mitochondria, and the absence of blockers reveals the true sensitivity of native mitochondrial K^+_{ATP}-channel to diazoxide (just as to other openers). Hence, as consistent with the goal of this work, the concentration dependence of diazoxide effects on both stages of the K^+-cycle (K^+ accumulation and efflux through K^+/H^+-exchange) was studied in the absence of Mg^{2+} and ATP. The same conditions were used for estimating the contribution of K^+_{ATP}-channel proper to the cyclic K^+ transport and the rate of oxygen consumption in state 2, which is directly proportional to the rate of energy-dependent K^+ accumulation in mitochondria [16, 21].

The activity of K^+_{ATP}-channel in mitochondrial preparations was assessed at the beginning of K^+ uptake by the change in light absorption through blocking K^+-channels with Mg·ATP (1 mM Mg^{2+}, 1 mM ATP) followed by selective reactivation of K^+_{ATP}-channel with 50 μM diazoxide (Fig. 1c). Previously we showed [20] considerable decrease in the light absorption of suspension in the absence of Mg·ATP intensified by diazoxide (Fig. 1, a and b, curves *1* and *2*; Fig. 1c, columns *1* and *2*), which indicates the increase of K^+ influx into the matrix from the medium containing 120 mM K^+. Reactivation of the K^+_{ATP}-channel with diazoxide in the presence of Mg^{2+} and ATP blocking K^+ uptake (Fig. 1c, column *3*), according to the literature data [9, 11, 14], results in partial restoration of swelling, which does not increase during further enhancement of the activator concentration to 100-150 μM (Fig. 1c, column *4*) and is evidence of maximum activation of the K^+_{ATP}-channel. Comparison of the change in light absorption as a result of swelling under maximum activation of the K^+_{ATP}-channel before and after its reactivation with diazoxide (Fig. 1c, columns *2* and *4*) in the presence of Mg·ATP shows that ~33% of K^+ accumulation by energized liver mitochondria is accounted for the activated K^+_{ATP}-channel.

However, for revealing the role of the ATP-dependent component of K^+ transport in the mitochondrial K^+-cycle, it would be interesting to estimate the contribution of the K^+_{ATP}-channel to transmembrane K^+ exchange not at the initial times of K^+ accumulation, but under steady-state conditions established after K^+ accumulation in the matrix [8] and corresponding (in time as well) to the establishment of steady-state rate of cyclic K^+ transport and constant respiration rate in state 2.

The typical results of recording the light absorption of a mitochondrial suspension show that the addition of K^+-channel blockers after K^+ accumulation in mitochondria (Fig. 1, a and b) leads to an increase in light absorption due to K^+ release and mitochondrial volume reduction. Such effects of the same blockers are not observed in the absence of K^+ accumulation (e.g. in de-energized mitochondria). Since the blockers of K^+-channels under the experimental conditions did not cause membrane depolarization, the only route of K^+ release from the mitochondria is K^+/H^+-exchange, which is known to comprise, together with the potential-dependent K^+ uptake, the mitochondrial K^+-cycle [8]. In turn, the absence of changes in mitochondrial volume without K^+-channel blockers (Fig. 1b, curve *3*) is evidence that the completion of K^+ accumulation is followed by a state of stationary equilibrium, when the rate of K^+ accumulation is equal to the rate of its release through the K^+/H^+-exchanger [8]. Taking into account the equality of the rates of K^+ uptake and efflux in the moment of K^+-channel blocking, it can be assumed that the initial rate of light absorption increase after the block of K^+ uptake by a nonselective blocker (4-AP) corresponds to the rate of K^+ efflux through the K^+/H^+-exchanger (Fig. 1a). Therefore, for estimation of the effect of K^+_{ATP}-channel opener diazoxide on mitochondrial K^+/H^+-exchange, the uptake of K^+ into the matrix after K^+ accumulation by mitochondria was blocked by the nonselective blocker of K^+-channels 4-AP [4], followed by the recording of the initial rate of light absorption increase corresponding to mitochondrial volume reduction due to K^+ efflux through the K^+/H^+-antiporter [8].

The dependence of experimentally found relative rates of K^+ accumulation, oxygen consumption, and K^+ release through the K^+/H^+-exchange on diazoxide concentration is shown in Fig. 2. As consistent with these data, diazoxide activates both components of the K^+-cycle (energy-dependent K^+ accumulation and K^+/H^+-exchange) and, accordingly, increases the oxygen consumption rate in state 2 (Fig. 2). Maximum variations in all of the recorded parameters, i.e. the initial rate of K^+ accumulation (Fig. 2, curve *1*), respiration rate (Fig. 2, curve *2*), and the initial rate of mitochondrial volume reduction after addition of 4-AP (Fig. 2, curve *3*), take place in the nanomolar range of diazoxide concentrations (50-300 nM). Increasing the opener concentration to 100 μM causes no further changes in these values. The observed half-maximum activation of K^+ transport and mitochondrial respiration, $K_{1/2}$, is 140 nM ($K_{1/2}$ is determined by linearization of the found dependences in Hill coordinates (Fig. 2b)) and shows the high affinity of diazoxide to the mitochondrial K^+_{ATP}-channel exhibited, according to the experimental results, not only in the reconstituted channels [17] but also in the suspension of native isolated mitochondria.

However, it should be noted that the found $K_{1/2}$ values differ from published data ($K_{1/2}$ ~ 370 nM for the

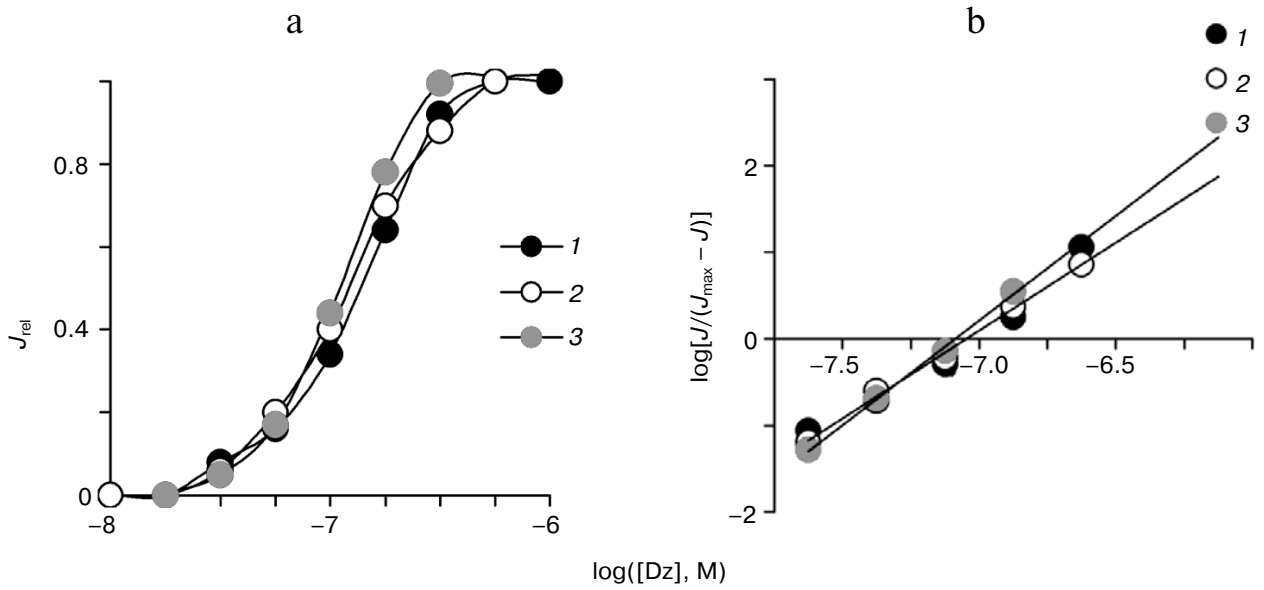

Fig. 2. a) Effect of diazoxide (Dz) on oxygen consumption rate (*1*) and initial rate of change in light absorption of suspension during swelling (*2*) and mitochondrial volume reduction (*3*) induced by 4-AP. The parameters are given in relative units, J_{rel}, as described in "Materials and Methods". b) Linearization of concentration dependences in Hill coordinates (reliability of approximation $R^2 = 0.9978$). $K_{1/2} = 134 \pm 8$ nM ($n = 9$) ($K_{1/2}$ values were found from the plot).

K^+_{ATP}-channel of rat liver mitochondria reconstituted in lipid bilayer membranes [17]) and, for a number of reasons, might be underestimated in comparison with the true constants of K^+_{ATP}-channel activation by diazoxide. Such reasons may include the limited values of maximum rates of oxygen consumption and K^+/H^+-exchange, which similarly limit both the initial rate of K^+ accumulation and the rate of K^+ transport in the mitochondrial K^+-cycle. In turn, it restrains the possibility of activation of mitochondrial respiration and swelling under the action of diazoxide. Also, understated $K_{1/2}$ values can be explained by a comparatively low distribution density of K^+_{ATP}-channel in the native membrane of isolated mitochondria. Another probable reason of low K^+_{ATP}-channel activation constants is higher affinity of the native channel to diazoxide compared to the reconstituted one.

Thus, the K^+-cycle acceleration observed under the action of diazoxide and enhancement of respiration rate in state 2 correspond to the concept of "mild uncoupling" of the respiratory chain supposedly underlying the cytoprotective effects of K^+_{ATP}-channel openers [1-3]. Therefore, it is interesting to reveal the relative share of diazoxide-activated K^+_{ATP}-channel in the K^+-cycle and oxygen consumption by mitochondria. The selective (glibenclamide) and nonselective (4-AP) blockers of K^+-channels were used for this purpose (Fig. 3). As mentioned above, under stationary equilibrium between the K^+ uptake and efflux, the initial rate of mitochondrial volume reduction and the change in light absorption after nonselective block of the K^+ uptake (Fig. 1a) are equal (with opposite sign) to the contribution of all K^+-channels to K^+ accumulation; hence, evaluation of the contribution of K^+_{ATP}-channel to the K^+-cycle is based on the assumption that K^+ release and organelle volume reduction after the selective block of K^+_{ATP}-channel with gliben-

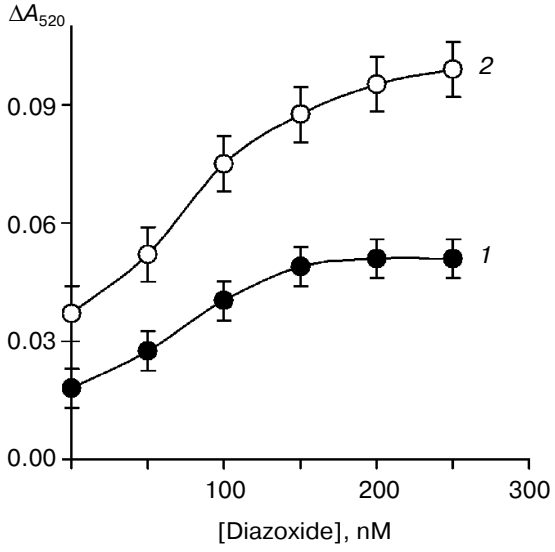

Fig. 3. Effect of diazoxide on mitochondrial volume reduction induced by K^+-channel blockers. Glibenclamide (*1*) and 4-AP (*2*) were added upon completion of swelling in the presence of the above diazoxide concentrations. Light absorption was recorded at 520 nm after addition of the blockers. The difference between the final and initial values during 10 min of measurements was found ($M \pm m$, $n = 6$).

clamide are equal (also with opposite sign) to the contribution of K^+_{ATP}-channel to K^+ accumulation and mitochondrial swelling (Fig. 1b, curves *1* and *2*; Fig. 1c, columns *7* and *8*; Fig. 3, curve *1*).

Although diazoxide is a selective opener of mitochondrial K^+_{ATP}-channel, it should be noted that experimental results demonstrate not only the activation of K^+_{ATP}-channel proper but also the activation of ATP-independent components of K^+ transport, resulting in the increase in its contribution to the mitochondrial K^+-cycle (Fig. 3, curve *2*). Probably, that is why the portion of diazoxide-activated K^+_{ATP}-channel in the K^+-cycle does not increase compared to the control, in spite of acceleration of K^+ transport through the K^+_{ATP}-channel induced by the opener (Fig. 3, curve *1*), being no more than 40-50% even under the maximum activation of K^+_{ATP}-channel. To ascertain the validity of our conclusions, we attempted to determine the relative contribution of K^+_{ATP}-channel to oxygen consumption, because it is known that respiration rate in state 2 is proportional to the rate of energy-dependent accumulation of K^+ in the mitochondrial K^+-cycle [21]. For this purpose, the K^+_{ATP}-channel blocker glibenclamide was used. Mitochondrial respiration was recorded in state 2 in the absence and presence of glibenclamide, which was added into the medium after establishment of a constant respiration rate. The contribution of K^+_{ATP}-channel to the K^+-cycle was defined as the glibenclamide-sensitive component of oxygen consumption and found by the difference between respiration rates in the absence and presence of the blocker.

The experimental results presented in Fig. 4 demonstrate the change in time of the contribution of separate components of the K^+-cycle, ATP-dependent (K^+_{ATP}-channel), and ATP-independent K^+ transport to the oxygen uptake by mitochondria in the absence (a) and presence (b) of diazoxide. Estimation of the rate of ATP-dependent K^+ uptake by the difference between oxygen consumption rates in the absence and presence of glibenclamide (Fig. 4, a and b, curves *1* and *2*), with allowance for the stoichiometric ratio between oxygen uptake and transport of monovalent cations given in the literature, which is 1 : 11 for NADH-dependent substrates according to some data [21], shows that the rate of K^+ transport through the K^+_{ATP}-channel under the action of diazoxide increases to 45 nmol K^+/min per mg protein and almost twofold exceeds the rate of ATP-dependent K^+ transport in the absence of the opener (25 nmol K^+/min per mg protein), which is in good agreement with the results of light absorption recording (Fig. 3, curve *1*). However, in spite of the higher rate of K^+ transport through the K^+_{ATP}-channel under the action of diazoxide, the share of its involvement in oxygen consumption and, consequently, in transmembrane K^+ exchange irrespective of the presence of activator is actually unchanged. This fact, as mentioned above (Fig. 3), demonstrates activation of not only K^+_{ATP}-channel but also the ATP-insensitive component of cyclic K^+ transport not suppressed by glibenclamide. Besides, the non-additivity of contributions of K^+_{ATP}-channel and ATP-independent component to oxygen consumption shows itself as the time dependence of the observed effect of K^+_{ATP}-channel block (Fig. 4, a and b) and is expressed in the fact that the relative share of K^+_{ATP}-channel increases only as the contribution of ATP-independent K^+ transport to the K^+-cycle decreases to 45-55% in the absence and presence of diazoxide (Figs. 3 and 4).

The same non-additivity of the involvement of different types of K^+-channels in the K^+-cycle is revealed in the series of experiments where the K^+_{ATP}-channel is reactivated by diazoxide after being blocked by glibenclamide (Fig. 4c). For this purpose, glibenclamide was added to the medium, as before (Fig. 4b, curve *2*), on completion of K^+ accumulation in the presence of 500 nM diazoxide and establishment of a constant respiration rate in state 2 (Fig. 4c, column *1*). The activity of K^+_{ATP}-channel blocked by glibenclamide was restored by diazoxide (final concentration, 20 μM), with addition of the opener at different time intervals after channel blocking (Fig. 4c, column *2*). Respiration rate at zero time before glibenclamide addition was taken as 100%. Introduction of diazoxide in the presence of the blocker recovers respiration rate to the initial value corresponding to the rate of mitochondrial respiration under conditions of the maximum activation of K^+_{ATP}-channel (Fig. 4c, columns *2* and *3*).

The recovery of K^+_{ATP}-channel activity confirms selectivity of the effects of glibenclamide and diazoxide as a blocker and opener of the channel, respectively. The estimate of K^+_{ATP}-channel contribution to the K^+-cycle by the difference between respiration rates after its blocking and subsequent reactivation (Fig. 4c, columns *1* and *2*) coincides with the above estimate obtained under the block of channel pre-activated by diazoxide (Fig. 4b). It confirms the conclusion that, in spite of activation of the K^+_{ATP}-channel by diazoxide (Fig. 3, curve *1*), its share in the K^+-cycle does not increase, due to simultaneous activation of the K^+_{ATP}-channel and ATP-independent component of K^+ transport (Fig. 3, curve *2*). It also confirms the conclusion about non-additivity of the contributions of different types of K^+-channels to the K^+-cycle, due to which the effects of K^+_{ATP}-channel block and reactivation (Fig. 4) are found only as the share of ATP-independent K^+-channels in the K^+ transport decreases.

The revealed patterns can be explained by some suggestions that need further experimental verification. In contrast to the conclusions in a number of works [9, 14, 17], our data indicate the high sensitivity of native K^+_{ATP}-channel of isolated mitochondria not only to its opener, diazoxide, but also to the selective blocker of this channel, glibenclamide. Addition of glibenclamide in the final concentration of 5 μM ($K_{1/2}$ ~ 250 nM for the reconstituted K^+_{ATP}-channel of liver mitochondria [9]) completely blocks the K^+_{ATP}-channel under the conditions of our experiment,

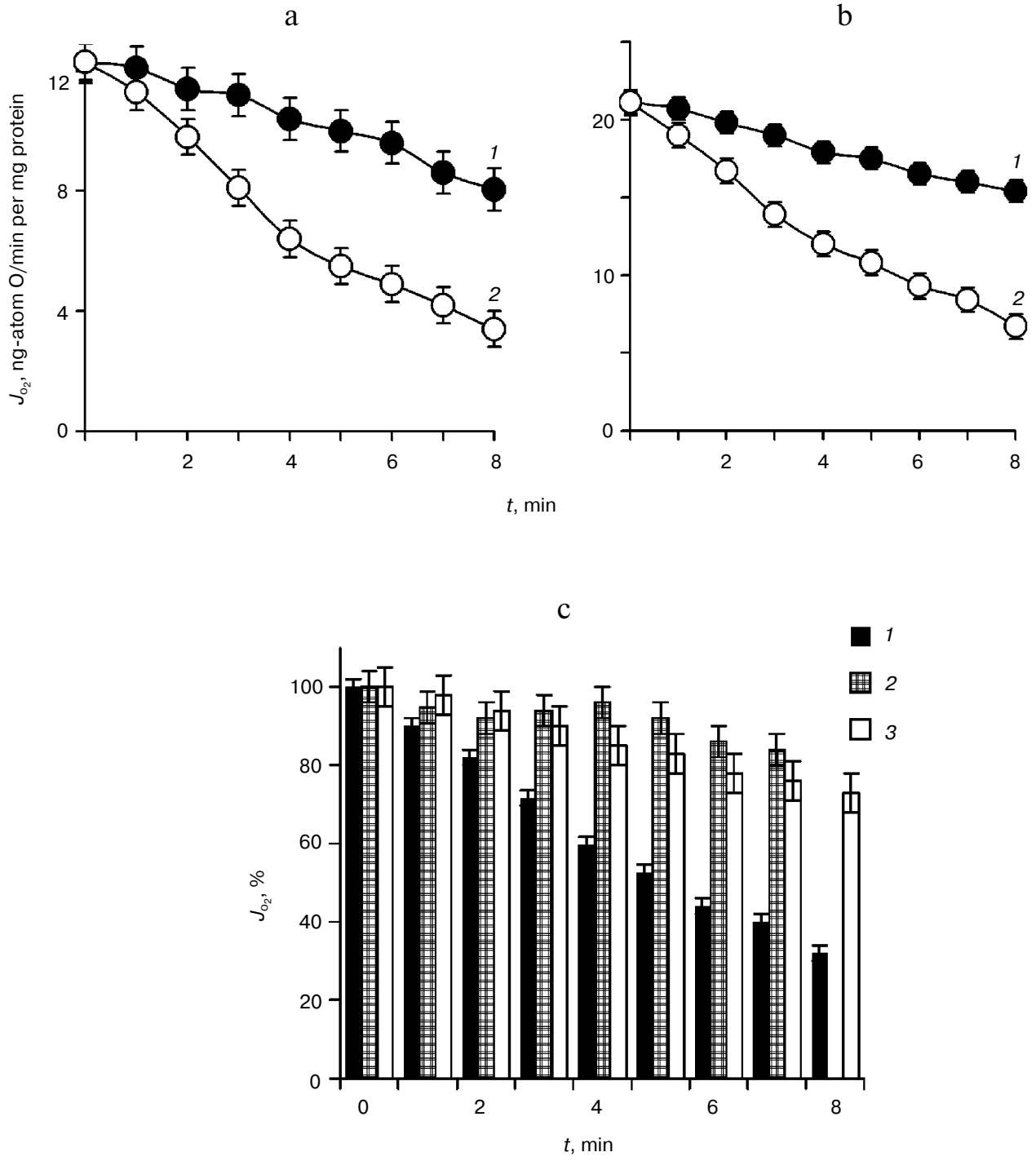

Fig. 4. Effects of K^+_{ATP}-channel opener and blocker on oxygen consumption rate in state 2. Mitochondria (1.5 mg/ml) were added to the incubation medium in the absence (a) or presence (b and c) of diazoxide, 500 nM. Glibenclamide, 5 μM (a and b, curves *2*; c, column *1*), was added after the establishment of stationary respiration rate (the beginning of measurements, $t = 0$). c) The sequence of additions: glibenclamide, $t = 0$ (column *1*); glibenclamide, diazoxide; diazoxide was added after glibenclamide in the intervals indicated on the abscissa axis (column *2*); respiration in the presence of 500 nM diazoxide (column *3*, control). Respiration rate at zero time in the absence of the blocker was taken as 100% ($M \pm m$, $n = 7$).

and increasing the blocker concentration to 20 μM does not intensify the observed effects. At the same time, glibenclamide blocks the K^+_{ATP}-channel without its preliminary block by Mg^{2+} and ATP followed by reactivation with diazoxide, which also corresponds to the results of Inoue obtained on mitoplasts from rat liver mitochondria [4].

However, consistent with our data, the contribution of K^+_{ATP}-channel to the cyclic K^+ transport is revealed

only in the course of partial inactivation of ATP-independent K^+-conductance providing, along with the K^+_{ATP}-channel, K^+ accumulation in the mitochondria. It should be noted that the nature of ATP-insensitive K^+-conductance of the inner mitochondrial membrane is still little studied. As is known, a considerable part of it is nonspecific K^+-conductance, the so-called potassium leak (K^+-leak [8]), which, just as the proton leak, depends on membrane potential and nonlinearly increases at high $\Delta\Psi_m$ values [22]. The mitochondrial membrane also contains quite many potential-dependent K^+-channels [4], which are probably homologs of the respective channels in the plasma membrane; however, most of them have not been identified yet [7].

Taking into consideration our results, it would be reasonable to assume that the efficiency of K^+_{ATP}-channel block in the native isolated mitochondria depends not as much on the conformational state of the channel [9] as on the functional activity of ATP-independent K^+-channels, and the relative share of K^+_{ATP}-channel in the K^+-cycle is determined mainly by the contribution of ATP-insensitive K^+-conductance of the mitochondrial membrane to K^+ accumulation by mitochondria, increasing only with inactivation of the latter. However, the amount of K^+ transported through the K^+_{ATP}-channel and ATP-insensitive K^+-channels, based on the ratio of initial rates of mitochondrial volume reduction in the presence of selective and nonselective blockers (Fig. 1, a and b), differs manifold, this being evidence of high activity of the ATP-insensitive component of K^+ transport in energized mitochondria.

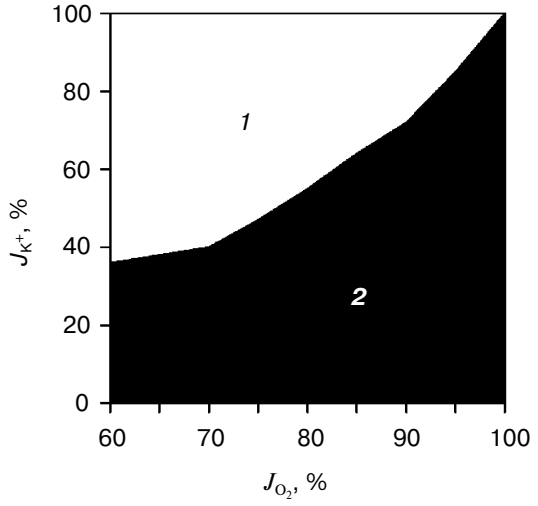

Fig. 5. Relative contribution of K^+_{ATP}-channel to energy-dependent K^+ transport under state 2 respiration. For assessing the contribution of K^+_{ATP}-channel to the K^+-cycle, the rate of energy-dependent K^+ transport, J_{K+}, was taken as 100% at all J_{O_2} values. The share of K^+_{ATP}-channel in the K^+-cycle (*1*) was calculated as the glibenclamide-sensitive component of oxygen consumption, by the difference between respiration rates before and after addition of the blocker, and expressed as percentage of J_{K+}. Region *2* corresponds to the contribution of glibenclamide-insensitive component to the K^+-cycle. The mean values of six measurements are given ($n = 6$, $p < 0.05$).

It is possible that just the differences in the kinetic characteristics of K^+ transport through the K^+_{ATP}-channel and other types of K^+-channels explain the distinguished fact that, besides the activation of K^+_{ATP}-channel by diazoxide (Fig. 3, curve *1*), the energized mitochondria also show the activation of the ATP-insensitive component of the K^+-cycle; as a result, the relative share of K^+_{ATP}-channel remains as it is, irrespective of the presence of activator (Fig. 3). So, it can be assumed that, although the increased K^+ accumulation is initially determined by the activation of K^+_{ATP}-channel, the activation of K^+/H^+-exchange following the increase in K^+ concentration in the mitochondrial matrix, in turn, results in the enhancement of K^+ accumulation also by the ATP-independent pathway under the steady-state conditions of the mitochondrial K^+-cycle.

The causes of non-additivity of the contributions of K^+_{ATP}-channel and ATP-insensitive K^+-channels to the mitochondrial K^+-cycle observed in the experiments, due to which the share of K^+_{ATP}-channel in the K^+-cycle increases only with the decrease in the ATP-independent component of K^+ transport, also needs further investigation. One should note the circumstance that the rate of oxygen consumption by the energized mitochondria in state 2 does not remain strictly constant during the experiment but gradually decreases (Fig. 4, a and b), evidencing the decrease in the amount of K^+ participating in the mitochondrial K^+-cycle. Therefore, the share of K^+_{ATP}-channel in the cyclic K^+ transport (the rate of K^+ cyclic transport, J_{K+}, was taken as 100%) could be presented as dependence of the share of glibenclamide-sensitive component (in %) on mitochondrial respiration rate in state 2 (respiration rate J_{O_2} at the beginning of measurements before addition of the blocker was taken as 100% (Fig. 5)). The increase in respiration rate was shown to result in nonlinear growth of ATP-independent component not suppressed by the K^+_{ATP}-channel blocker (Fig. 5, region *2*); as mentioned above, this could be a consequence of differences in the kinetic characteristics of K^+ transport by different K^+-conducting systems of mitochondria. However, one of the probable reasons of the observed decrease in respiration rate is partial release of cytochrome *c* from the intermembrane space, which intensifies during accumulation of K^+ from the medium even without the opening of the mitochondrial pore [23]. Partial suppression of mitochondrial respiration (Fig. 4, a and b, curves *1*) and, accordingly, the mechanism of $\Delta\Psi_m$ generation can also explain the decrease in ATP-insensitive K^+-conductance if it is determined by potential-dependent K^+-conducting structures of the mitochondrial membrane (potential-dependent K^+-channels or K^+ leak [8, 22]) and relative increase of the share of K^+_{ATP}-channel in the K^+-cycle (Fig. 5, region *1*). Thus, the

results of our experiments demonstrate that ATP-dependent K^+ transport is not a constant but a variable component of the mitochondrial K^+-cycle, the contribution of which increases in the course of inactivation of the ATP-independent component of transmembrane K^+ exchange.

The conclusions, in our opinion, provide deeper understanding of the role of K^+_{ATP}-channel in the regulation of basic mitochondrial functions, which is probably manifested not as much under the normal physiological conditions as under pathological states accompanied by the lowered energy status of mitochondria and, consequently, partial reduction of potential-dependent uptake of K^+ due to respiration suppression or mitochondrial depolarization. The revealed non-additivity of the contributions of ATP-dependent and ATP-independent K^+-channels to the mitochondrial K^+-cycle points to the pathways of directed modulation of the open state of different types of K^+-channels using pharmacological preparations with the goal of effective regulation of energy exchange in mitochondria.

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