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Inhibition of the Mitochondrial K_{ATP} Channel by Long-chain Acyl-CoA Esters and Activation by Guanine Nucleotides*

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Petr Paucek, Vladimir Yarov-Yarovoy‡, Xiaocheng Sun, and Keith D. Garlid§

From the Department of Chemistry, Biochemistry, and Molecular Biology, Oregon Graduate Institute of Science & Technology, Portland, Oregon 97291-1000

The mitochondrial K_{ATP} channel (mitoK_{ATP}) is highly sensitive to ATP, which inhibits K^+ flux with $K_{½}$ values of 20–40 μM. This raises the question, how can mitoK_{ATP} be opened in the presence of physiological concentrations of ATP? We measured K^+ flux in liposomes reconstituted with purified mitoK_{ATP} and found that guanine nucleotides are potent activators of this channel. ATP-inhibited K^+ flux was completely reactivated by both GTP ($K_{½}$ = 7 μM) and GDP ($K_{½}$ = 140 μM). These ligands had no effect in the absence of ATP. The $K_{½}$ for ATP inhibition exhibited quadratic dependence on [GTP] and [GDP], consistent with two binding sites for guanine nucleotides. We also found that palmitoyl-CoA and oleoyl-CoA inhibited K^+ flux through reconstituted mitoK_{ATP} with $K_{½}$ values of 260 nM and 80 nM, respectively. This inhibition was reversed by GTP ($K_{½}$ = 232 μM) as well as by the K^+ channel openers cromakalim (20 μM) and diazoxide (10 μM). Inhibition of mitoK_{ATP} by long-chain acyl-CoA esters, like that of ATP, exhibited an absolute requirement for Mg^{2+} ions. We propose that the open-closed state of the mitochondrial K_{ATP} channel is determined by the relative cytosolic concentrations of GTP and long-chain acyl-CoA esters.

Mitochondrial K_{ATP} channels (mitoK_{ATP})[1] were first discovered in 1991 (1–3). Inoue *et al.* (1) reported electrophysiological evidence from patch clamp studies of fused mitoplasts, and we described reconstitution of a highly purified mitoK_{ATP} (2, 3). In our protocols, which have the advantage of being free of the complexities of intact mitochondria, K^+ flux is measured using steady-state spectroscopy of the fluorescent probe PBFI. These measurements have permitted initial characterization of the kinetics and regulation of mitoK_{ATP} (4). The K_m for K^+ is 32 mM, and the channel is highly selective for K^+ (Na^+ and TEA^+ are neither transported nor do they affect K^+ flux through mitoK_{ATP}). MitoK_{ATP} is inhibited with high affinity by ATP and ADP, and this inhibition exhibits an absolute requirement for divalent cations. We have recently shown that ATP inhibition of K^+ flux through mitoK_{ATP} is reversed by submicromolar levels of K^+ channel openers (5).

We also demonstrated mitoK_{ATP} activity in respiring rat liver mitochondria (6). The confounding and unavoidable coexistence of K^+ diffusion (leak) was controlled by comparing K^+ flux to TEA^+ flux, for which there are no endogenous pathways other than diffusive leak. We made the simple demonstration that ATP inhibited K^+ uptake to rates similar to those of TEA^+ uptake and had no effect on TEA^+ uptake itself.

These studies left us with a conundrum: given the high affinity for ATP, how can mitoK_{ATP} ever be opened under normal physiological conditions? We hypothesized (4) that endogenous activators of mitoK_{ATP} must exist to overcome the high affinity for ATP, and we now present support for this hypothesis. K^+ flux through the MgATP-inhibited channel is restored to full activity by GTP and GDP, neither of which has any effect in the absence of MgATP. GTP and GDP are competitive with ATP, and their reversal of ATP inhibition exhibits hyperlinear kinetics consistent with two guanine nucleotide binding sites. We also report that palmitoyl-CoA and oleoyl-CoA inhibit mitoK_{ATP} with high potency, and this inhibition is also reversed by GTP and by the potassium channel openers, cromakalim and diazoxide. Inhibition by long-chain acyl-CoA esters, like inhibition by ATP, exhibits an absolute requirement for Mg^{2+} ions and is immediately reversed upon chelation of Mg^{2+}. From these findings, we infer that GTP and long-chain acyl-CoA esters may be the physiological regulators of mitoK_{ATP} and that this channel may play a role *in vivo* in regulating fatty acid oxidation.

EXPERIMENTAL PROCEDURES

Extraction, Purification, and Reconstitution of MitoK_{ATP} from Rat Liver Mitochondria—Purification and reconstitution generally followed protocols previously described (4, 7). Rat liver mitochondria were purified on a linear sucrose density gradient and used to prepare inner membrane vesicles according to the procedure of McEnery *et al.* (8). Mitochondria were suspended in 220 mM D-mannitol, 70 mM sucrose, 0.5 mg/ml bovine serum albumin, 20 mM HEPES (K^+), pH 7.4, sonicated on ice, and centrifuged for 10 min at 10,000 × *g*. The supernatant was recentrifuged at 210,000 × *g* for 30 min, and the resulting membrane pellet was washed three times in PA buffer (0.15 M potassium phosphate, 1 mM ATP, 25 mM EDTA, 0.5 mM dithiothreitol, 5% ethylene glycol, pH 7.9). The final membrane pellet was suspended to 15 mg/ml in PA buffer and stored at −70 °C until needed. Prior to use, the vesicles were incubated in PA buffer containing 3 M guanidine HCl to remove F1-ATPase and bound chaperonins. Treated vesicles were then washed three times in PA buffer (30 min at 138,000 × *g*) and finally solubilized (2 mg of protein/ml) in 3% Triton X-100, 20% glycerol, 0.1% β-mercaptoethanol, 0.2 mM EGTA, 1 mM $MgCl_2$, and 50 mM Tris-HCl, pH 7.2.

After incubation on ice for 20 min, the mixture was centrifuged at 120,000 × *g* for 35 min. Ten ml of the supernatant, typically containing 50–80 mg of extracted proteins, were loaded onto a DEAE-cellulose column (10-ml bed volume) that had been equilibrated with a buffer containing 1% Triton X-100, 0.1% β-mercaptoethanol, 1 mM EDTA, and 50 mM Tris-HCl, pH 7.2. The active fraction, eluting at 250 mM KCl, was

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§ To whom correspondence and reprint requests should be addressed: Dept. of Chemistry, Biochemistry, and Molecular Biology, Oregon Graduate Institute of Science & Technology, P.O. Box 91000, Portland, OR 97291-1000. Tel.: 503-690-1680; Fax: 503-690-1464; E-mail: garlid@admin.ogi.edu.

[1] The abbreviations used are: mitoK_{ATP}, mitochondrial K_{ATP} channel; FCCP, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone; PBFI, potassium-binding benzofuran isophthalate; TEA^+, tetraethylammonium cation.

desalted and concentrated by filtration.

The purified mitoK_{ATP} fraction was added to a 10:1 mixture of L-α-lecithin (Avanti) and cardiolipin in 10% octylpentaoxyethylene. The buffer composition at this stage defines the *internal medium*, which contained 300 μM PBFI, 100 mM TEA-SO_4, 0.14 mM KCl, 1 mM TEA-EDTA, and 25 mM TEA-HEPES, pH 6.8. This mixture was loaded onto a 2-ml Bio-Beads SM-2 column (Bio-Rad) to remove detergent and form proteoliposomes. After incubation for 90 min at 0–4 °C, the column was centrifuged at 400 × *g* for 2 min to collect the proteoliposomes. To remove extravesicular PBFI, 200-μl aliquots of the proteoliposome suspension were passed twice through 4-ml Sephadex G-25–300 columns. The final stock vesicle suspension (nominally 50 mg of lipid/ml) was stored on ice during the experiment. Protein content, measured by the amido black method (9), was normally 10 ng of protein per mg of lipid. Intraliposomal volume of each preparation was estimated from the volume of distribution of PBFI and was normally found to be 1 μl per mg of starting lipid.

Assay of K^+ Flux through Reconstituted MitoK_{ATP}—15 μl of stock vesicles were added to 2 ml of *external medium* containing 150 mM KCl, 1 mM TEA-EDTA, and 25 mM TEA-HEPES, pH 7.4. Electrophoretic K^+ flux was initiated by 1 μM FCCP to provide charge compensation via H^+ flux. K^+ flux was quantitated from the fluorescence of intraliposomal PBFI, which increases with increasing $[K^+]_{in}$. Fluorescence was followed with an SLM/Aminco 8000C spectrofluorometer. The K^+ response of intravesicular PBFI was calibrated by stepwise additions of KCl to proteoliposomes in internal medium in the presence of nigericin and tributyltin (7).

Chemicals and Reagents—Tris salts of adenine and guanine nucleotides were titrated to pH 7.2 with Tris base. PBFI was from Molecular Probes Inc. (Eugene, OR). All other chemicals were obtained from Sigma unless otherwise indicated.

RESULTS

Activation of the ATP-inhibited K_{ATP} Channel by GTP and GDP—The results in Fig. 1 demonstrate activation of ATP- and ADP-sensitive K^+ flux by GTP and GDP. The following observations can be made from these data: (i) K^+ flux was completely restored by both GTP and GDP, and (ii) GTP was 20–30 times more potent than GDP, irrespective of whether ATP or ADP was used to inhibit K^+ flux. In 0.5 mM ATP, the $K_{½}$ values for GTP and GDP activation were 6.9 μM and 143 μM, respectively. In 0.5 mM ADP, the $K_{½}$ values for GTP and GDP activation were 0.12 μM and 3.4 μM, respectively.

Additional experiments (not shown) further characterize guanine nucleotide reversal of ATP inhibition of K^+ flux through mitoK_{ATP}. (i) Guanine nucleotides had no effect on K^+ flux through the open channel, measured in the absence of MgATP. (ii) Activation required that guanine nucleotides be added to the same side as MgATP. Thus, external GTP had no effect on K^+ flux when it was inhibited by internal MgATP. (iii) GTP or GDP activated K^+ flux when added 30 s after inhibition by MgATP had already been established.

Kinetics of Guanine Nucleotide Activation of the K_{ATP} Channel—To examine the kinetics of activation, we measured ATP inhibition of K^+ flux in the presence of 3 mM Mg^{2+} and different concentrations of GTP or GDP. Fig. 2 contains representative dose-response curves.

In the *absence* of GTP, ATP inhibited K^+ flux through reconstituted mitoK_{ATP} with a $K_{½}$ of 21 μM in this experiment (*solid circles*, Fig. 2). We observed $K_{½}$ values for ATP ranging between 20 and 30 μM in 4 independent experiments, and the Hill coefficient was always 1.0 ± 0.1. These $K_{½}$ values are lower than our previously reported value of 39 μM (4) because they are calculated from ATP-sensitive, rather than total, K^+ flux. We have recently established that 10–15% of channels are reconstituted with their regulatory sites facing inward and are, therefore, inaccessible to external ATP (10, 11).

In the *presence* of increasing doses of GTP, the $K_{½}$ value for ATP inhibition was shifted sharply higher (see Fig. 2). It is striking that 20 μM GTP increased the $K_{½}$ for ATP inhibition from 21 μM to 6 mM. ATP was ineffective in the presence of 3 mM GTP (not shown).

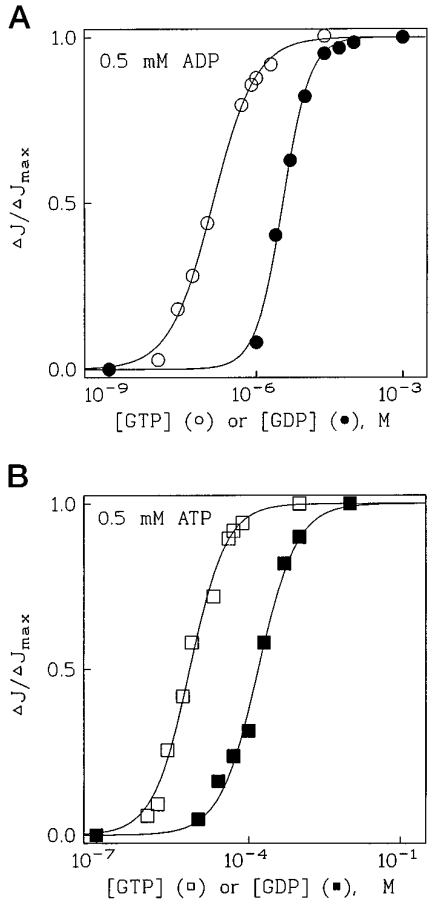

FIG. 1. **Activation of ATP- and ADP-inhibited mitoK_{ATP} by GTP and GDP.** The relative ATP-sensitive K^+ uptake into liposomes reconstituted with mitoK_{ATP}, $\Delta J/\Delta J_{max}$, is plotted *versus* concentration of GTP or GDP. All assay media contained 3 mM Mg^{2+}, and K^+ influx was initiated by adding 1 μM FCCP to assay medium at 10 s. Nucleotides were added to assay medium. *A*, activation of ADP-inhibited K^+ flux. GTP or GDP was added to assay medium containing 0.5 mM ADP. ΔJ_{max} is the difference between control fluxes in the absence or presence of 0.5 mM ADP, which inhibited *total* K^+ flux by 65% (4). ΔJ is the difference between fluxes in the presence or absence of guanine nucleotide measured in the presence of 0.5 mM ADP. The $K_{½}$ values and Hill slopes (in *parentheses*) for activation were 0.12 μM (1.0) for GTP and 3.4 μM (1.6) for GDP. *B*, activation of ATP-inhibited K^+ flux. GTP or GDP was added to assay medium containing 0.5 mM ATP. ΔJ_{max} is the difference between control fluxes in the absence or presence of 0.5 mM ATP, which inhibited *total* K^+ flux by 85% to 90% (4). ΔJ is the difference between fluxes in the presence or absence of guanine nucleotide with both fluxes measured in the presence of 0.5 mM ATP. The $K_{½}$ values and Hill slopes (in *parentheses*) for activation were 6.9 μM (1.2) for GTP and 140 μM (1.2) for GDP.

Fig. 3 contains a summary of the results of five experiments in which the $K_{½}$ for ATP inhibition of mitoK_{ATP} was measured at various concentrations of GTP (●) or GDP (○). These data show that the apparent affinity of mitoK_{ATP} for ATP decreases ($K_{½}$ increases) in a quadratic manner with guanine nucleotides. In order to extract parameters from the data in Fig. 3, we constructed a simple model for nucleotide interaction with the mitoK_{ATP} receptor, *R*:

$$\mathrm{ATP}\cdot R \overset{K_i}{\rightleftharpoons} R \overset{k_1}{\rightleftharpoons} \mathrm{GTP}\cdot R \overset{k_2}{\rightleftharpoons} \mathrm{GTP_2}\cdot R \qquad \text{(Eq. 1)}$$

This model is consistent with available data. For example, if ATP binds to a second binding site, its affinity is too low to be detected. Solving the kinetic equations for $K_{½}$(ATP),

$$K_{½}/K_i = 1 + [\mathrm{G}]/k_1 + [\mathrm{G}]^2/k_1k_2 \qquad \text{(Eq. 2)}$$

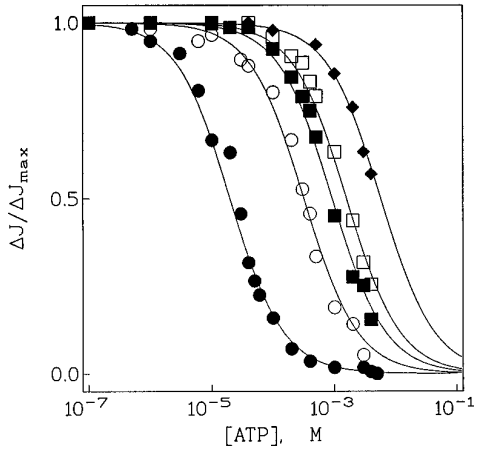

FIG. 2. **Effect of GTP on the kinetics of ATP inhibition of mitoK$_{ATP}$.** Figure contains dose-response curves for ATP inhibition of K^+ flux through reconstituted mitoK$_{ATP}$. ATP titrations were done in the presence of GTP added to assay medium in concentrations of 0 μM (●), 2 μM (○), 4 μM (■), 8 μM (□), and 20 μM (♦). ΔJ_{max} is the difference between control fluxes in the absence or presence of 0.5 mM ATP measured in the absence of GTP. ΔJ is the difference between fluxes in the presence or absence of GTP measured in the presence of 0.5 mM ATP (90% inhibition of *total* K^+ flux). In four separate experiments carried out in the *absence* of GTP, $K_{½}$ values for ATP inhibition ranged between 20 μM and 30 μM, and the Hill slope was 1.0 ± 0.1.

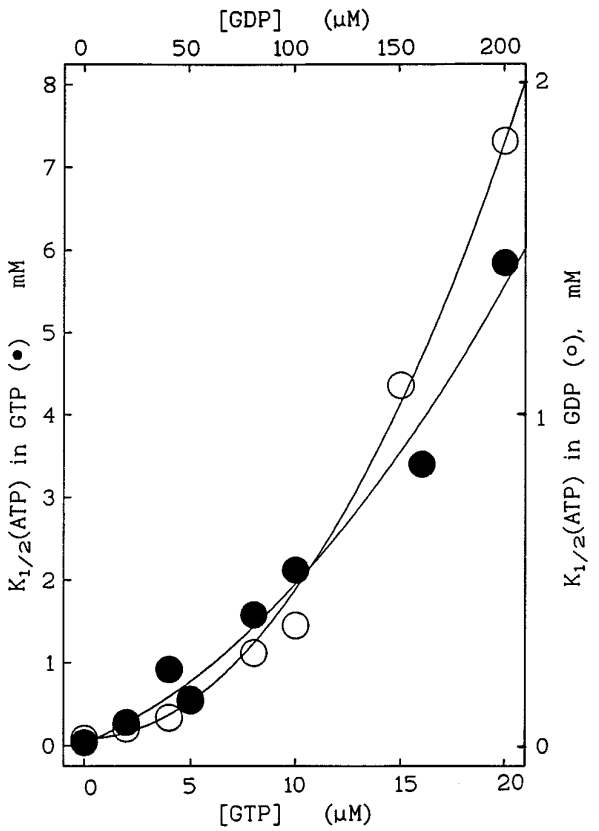

FIG. 3. **Quadratic competitive opening of the ATP-inhibited mitoK$_{ATP}$ by GTP and GDP.** Observed $K_{½}$ values for ATP inhibition of K^+ flux through the reconstituted mitoK$_{ATP}$ channel are plotted *versus* [GTP] (●) and [GDP] (○). The $K_{½}$ values were obtained from nonlinear regression of dose-response curves ($\Delta J/\Delta J_{max}$ *versus* log[ATP]) for ATP inhibition in the presence of indicated concentrations of GTP or GDP. The data plotted were from three separate experiments, each with GTP and GDP. The *solid lines* were fitted to the second-order polynomial, $K_{½}/K_1 = 1 + [G]/k_1 + [G]/k_1k_2$, as described under "Results."

where [G] refers to GTP or GDP concentrations. The data were fit to this equation (*solid lines* in Fig. 3), using K_i(ATP) ≈ 21 μM. The derived dissociation constants for GTP were $k_1 \approx 0.18$ μM and $k_2 \approx 14$ μM. For GDP, the values were $k_1 \approx 21$ μM and $k_2 \approx 25$ μM. A simple qualitative interpretation of these results is that GTP reacts at a high-affinity and a low-affinity site, whereas GDP reacts with two low-affinity sites. The low-affinity sites appear to have similar affinities for ATP, GTP, and GDP.

Inhibition of the K_{ATP} Channel by Long-chain Acyl-CoA Esters—Fig. 4 contains the results of experiments on the effects of oleoyl-CoA and palmitoyl-CoA on K^+ flux through reconstituted mitoK$_{ATP}$. Long-chain acyl-CoA esters are known to inhibit other ATP-binding transport proteins in mitochondria (12), and they are potent inhibitors of K^+ flux through mitoK$_{ATP}$. Oleoyl-CoA inhibited with $K_{½} \approx 80$ nM and Hill coefficient of 1.7. Palmitoyl-CoA inhibited with $K_{½} \approx 260$ nM and Hill coefficient of 2. These experiments were carried out in the presence of 3 mM Mg^{2+}. Strikingly, acyl-CoA esters had no effect on K^+ flux in the absence of Mg^{2+} (0.5 mM EDTA).

Activation of the Palmitoyl-CoA-inhibited K_{ATP} Channel by GTP and K^+ Channel Openers—Fig. 5*A* contains fluorescence traces from experiments designed to determine whether palmitoyl-CoA inhibition of K^+ flux is reversed by K^+ channel openers. Control flux (*trace a*) was inhibited by 1 μM palmitoyl-CoA (*trace b*), and this inhibition was prevented in the presence of 10 μM diazoxide (*trace c*) or 20 μM cromakalim (*trace d*). Fig. 5*B* contains fluorescence traces from experiments designed to determine whether palmitoyl-CoA inhibition of K^+ flux is reversed by GTP. Control flux (*trace a*) was inhibited by 1 μM palmitoyl-CoA (*trace b*), and this inhibition was prevented by the inclusion of 1 mM GTP in the assay medium (*trace c*). GTP and K^+ channel openers also activated K^+ flux when added after flux was inhibited by palmitoyl-CoA (not shown).

The dose-response curves in Fig. 6 demonstrate GTP activation of K^+ flux inhibited by ATP (●), palmitoyl-CoA (○), or a combination of ATP and palmitoyl-CoA (▲). In these experiments, the $K_{½}$ values were 4 μM (in 0.5 mM ATP), 232 μM (in 1 μM palmitoyl-CoA), and 283 μM (ATP plus palmitoyl-CoA). The two important features of these results are that palmitoyl-CoA moved the $K_{½}$ for GTP activation toward the physiological range of GTP concentration and that ATP had no effect on the $K_{½}$ for GTP in the presence of palmitoyl-CoA.

DISCUSSION

Regulation of the Mitochondrial K_{ATP} Channel—The purpose of these experiments was to explore regulation of mitoK$_{ATP}$ by physiological ligands. MitoK$_{ATP}$ is inhibited by ATP, ADP (4), and long-chain acyl-CoA esters. Inhibition of mitoK$_{ATP}$ by long-chain acyl-CoA esters with high affinity is consistent with a proposed signaling role of this channel in regulating β-oxidation of fatty acids (13). Inhibition by these ligands exhibits an absolute requirement for Mg^{2+} ions, and Mg^{2+} reduces the apparent affinity for glibenclamide in inhibiting K^+ flux through mitoK$_{ATP}$ (4). These findings suggest that Mg^{2+} interacts separately with the mitoK$_{ATP}$ complex, because acyl-CoA esters and glibenclamide are not Mg^{2+} chelators. It is noteworthy that ADP and acyl-CoA esters, which are chemical analogues, exert opposite effects on K$_{ATP}$ channels from mitochondria and plasma membranes. They inhibit mitoK$_{ATP}$ (4, this paper), but they activate the plasma membrane K$_{ATP}$ channels of pancreatic β cells (14).

Inhibition by adenine nucleotides or acyl-CoA esters can be fully overcome by GTP and GDP, and by the pharmacological agents known as K^+ channel openers (5, this paper). Guanine nucleotide activation is competitive with ATP, with kinetics indicating two nucleotide binding sites. The effects on the $K_{½}$

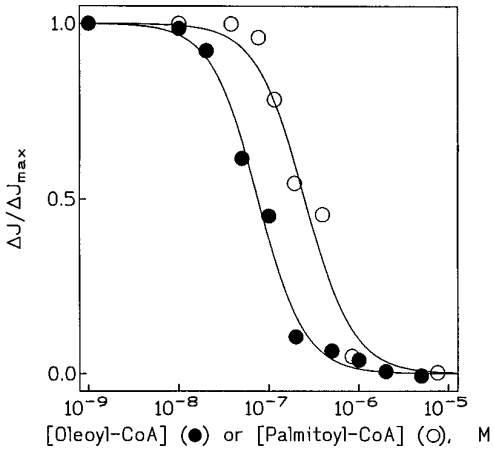

FIG. 4. **Oleoyl-CoA and palmitoyl-CoA inhibit K^+ flux through mitoK_{ATP}.** The relative ATP-sensitive K^+ uptake, $\Delta J/\Delta J_{max}$, into liposomes reconstituted with mitoK_{ATP} is plotted *versus* concentrations of oleoyl-CoA and palmitoyl-CoA. ΔJ_{max} is the difference between control fluxes in the absence or presence of 0.5 mM ATP, which inhibited *total* K^+ flux by 90%. ΔJ is the difference between fluxes in the presence or absence of acyl-CoA ester measured in the absence of ATP. $K_{½}$ values for oleoyl-CoA and palmitoyl-CoA inhibition were 260 nM and 80 nM, respectively (averages of three independent experiments). Assay medium contained 3 mM Mg^{2+}. Oleoyl-CoA and palmitoyl-CoA had no effect on K^+ flux in the absence of Mg^{2+}.

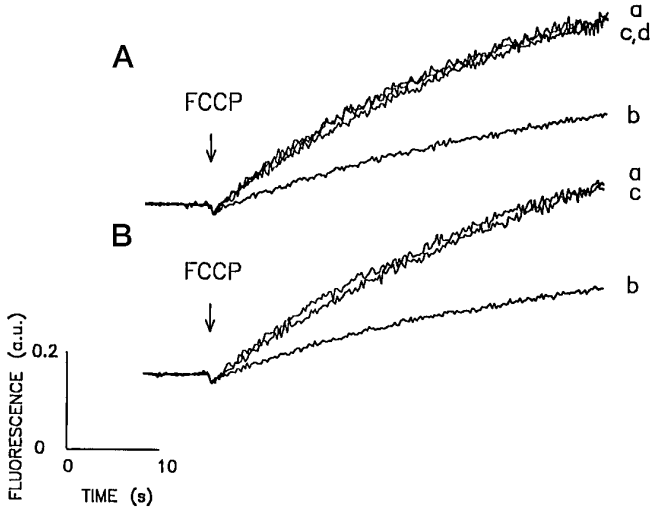

FIG. 5. **Activation of the palmitoyl-CoA-inhibited mitoK_{ATP} by GTP, cromakalim, and diazoxide.** Shown are PBFI fluorescence traces from proteoliposomes reconstituted with purified mitoK_{ATP}. Increasing fluorescence reflects increasing intraliposomal $[K^+]$ due to K^+ transport. Electrophoretic K^+ influx was initiated by adding 1 μM FCCP to KCl assay medium at 10 s. *A*, reversal of palmitoyl-CoA inhibition by cromakalim and diazoxide. *Trace a*, assay medium contained 1 mM Mg^{2+} and no palmitoyl-CoA; *trace b*, assay medium contained 1 mM Mg^{2+} and 1 μM palmitoyl-CoA; *traces c* and *d,* assay medium contained Mg^{2+} and palmitoyl-CoA as in *trace b* and 20 μM cromakalim (*c*) or 10 μM diazoxide (*d*). *B*, reversal of palmitoyl-CoA inhibition by GTP. *Trace a*, assay medium contained 1 mM Mg^{2+} and no palmitoyl-CoA; *trace b*, assay medium contained 1 mM Mg^{2+} and 1 μM palmitoyl-CoA; *trace c*, assay medium contained Mg^{2+} and palmitoyl-CoA as in *trace b* and 1 mM GTP.

for ATP inhibition (Fig. 3) suggest both high-affinity and low-affinity GTP sites.

It is characteristic of all K_{ATP} channels that the $K_{½}$ values for ATP inhibition are roughly 2 orders of magnitude lower than normal cytosolic [ATP]. We now show that the $K_{½}$ values for GTP reversal of ATP inhibition of mitoK_{ATP} are 2 orders of magnitude less than normal cytosolic [GTP]. These results can, however, be rationalized by the simple consideration that the

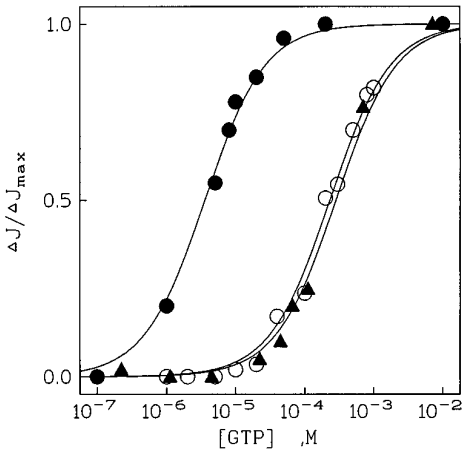

FIG. 6. **GTP activation of K^+ flux in the presence of ATP and/or palmitoyl-CoA.** The relative ATP- or palmitoyl-CoA-sensitive K^+ uptake, $\Delta J/\Delta J_{max}$, into liposomes reconstituted with mitoK_{ATP} is plotted *versus* concentration of GTP. Assay medium contained 1 mM Mg^{2+} and either 0.5 mM ATP or 1 μM palmitoyl-CoA. The channel's open state, ΔJ_{max}, was obtained as the difference between control fluxes in the absence or presence of inhibitors. ΔJ is the difference between fluxes in the presence or absence of GTP. The $K_{½}$ values for GTP activation were 4 μM (●) in ATP, 232 μM (○) in palmitoyl-CoA, and 283 μM (▲) in 0.5 mM ATP and 1 μM palmitoyl-CoA.

nucleotide binding sites will be occupied *in situ* by high-affinity ligands other than ATP. The data suggest that ATP cannot inhibit mitoK_{ATP} in the presence of physiological [GTP], raising the possibility that ATP is *not* a physiological regulator of mitoK_{ATP}. On the other hand, when long-chain acyl-CoA esters and GTP are present together, as in the experiments of Fig. 6, their $K_{½}$ values fall within their respective physiological ranges. We infer from our results that the nucleotide binding sites on mitoK_{ATP} are fully occupied by GTP or long-chain acyl-CoA esters under physiological conditions, and that the fraction of open channels is determined by the balance between these regulators.

Mitochondrial Volume Is Controlled by the Potassium Cycle—The mitochondrial K^+ cycle consists of electrophoretic K^+ uptake and electroneutral K^+ efflux across the inner membrane. Any *net* K^+ flux will be accompanied by electroneutral flux of anions and osmotically obligated water (15). Because matrix $[K^+]$ is about 180 mM, net K^+ transport will have little effect on the matrix *concentration* of K^+, but it will have a profound effect on matrix volume. Thus, the redox energy consumed by the K^+ cycle is the cost of regulating matrix volume (15). The K^+ cycle is mediated by two highly regulated processes. Efflux is mediated by the K^+/H^+ antiporter, whose existence was predicted by Mitchell (16) and first demonstrated by Garlid (17) nearly 20 years later. Influx is mediated by the mitochondrial K_{ATP} channel (mitoK_{ATP}), which was described by Inoue *et al.* (1) and Paucek *et al.* (4).

A primary role of regulated K^+/H^+ antiport is to compensate for unregulated K^+ leak into the matrix, driven by the high voltages required for oxidative phosphorylation. *Uncompensated* K^+ uptake amounting to as little as 10% of proton pumping would double matrix volume within 1–2 min (18). The K^+/H^+ antiporter is inhibited by matrix Mg^{2+} ($K_i \approx 300$ μM) as well as by matrix protons, and the concentrations of these inhibitors decrease with uptake of K^+ salts, causing compensatory activation of K^+ efflux (15). Thus, the K^+/H^+ antiporter is responsible for *volume homeostasis* and is essential for maintaining vesicular integrity in the face of high ionic traffic across the inner membrane.

The discovery of mitoK_{ATP} has profound new implications for mitochondrial physiology, because the existence of a regulated

K^+ influx pathway permits *volume regulation*. For example, opening mitoK_{ATP} will transiently shift the balance between K^+ uniport and K^+/H^+ antiport until the antiport catches up with the higher rate of K^+ influx. This will cause transient swelling to a higher steady-state volume that will persist for as long as mitoK_{ATP} remains open. Such a "regulated interplay" between K^+ uniport and K^+/H^+ antiport was correctly postulated many years ago by Brierley (19).

Matrix Volume Regulates Electron Transport—Fatty acids are the fuel for thermogenesis by brown adipose tissue mitochondria, and their rate of oxidation is strictly controlled by matrix volume (20). A thorough characterization of this phenomenon by Halestrap (21) has demonstrated that increasing matrix volume, over the narrow range thought to obtain *in vivo*, greatly stimulates activity of the respiratory chain in both heart and liver mitochondria. β-oxidation of fatty acids is particularly sensitive to matrix volume. The site of activation has been localized to membrane enzymes that feed electrons to ubiquinone. The molecular mechanism is not known, but may involve a stretch receptor. Matrix volume changes have been observed *in vivo* during respiratory stimulation secondary to hormonal activation of liver (21) and brown adipose tissue (22).

A role for mitoK_{ATP} in regulating cellular bioenergetics has been suggested by Halestrap (23), Szewczyk *et al.* (24), and Garlid (13), and the exquisite sensitivity of mitoK_{ATP} to long-chain acyl-CoA esters dovetails nicely with this hypothesis. A plausible scenario is that mitoK_{ATP} will open in the glucose-depleted state, where long-chain acyl-CoA esters are low. The resulting matrix expansion will activate β-oxidation and direct energy to support gluconeogenesis in liver, increased mechanical work in heart and skeletal muscle, and thermogenesis in brown adipose tissue. Conversely, elevated long-chain acyl-CoA esters in the fed state may inhibit mitoK_{ATP}, and, together with inhibition of carnitine palmitoyltransferase I (25), promote diversion of energy to fatty acid esterification in hepatocytes, adipocytes, and pancreatic β-cells, and to glycolysis in heart and skeletal muscle.

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