On the Regulation of K⁺ Uniport in Intact Mitochondria by Adenine Nucleotides and Nucleotide Analogs*

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Respiring mitochondria drive the electrophoretic uptake of K⁺ and other cations. In the presence of permeant acids this transport leads to mitochondrial swelling if it is not compensated by electroneutral K⁺/H⁺ exchange mediated by the K⁺/H⁺ antiporter. The mechanism of influx has yet to be established; however, evidence is accumulating that in addition to leak pathways, a specific K⁺ channel or uniporter may be involved. We examine some of the properties of K⁺ uniport which are consistent with the existence of a specific ATP-regulated K⁺ channel. In contrast to the K⁺/H⁺ antiporter, K⁺ uniport shows little dependence on pH. K⁺ uniport is, however, very sensitive to inhibition by adenine nucleotides. The maximum percent inhibition is increased from 40 to 60% by treatment of mitochondria with N-ethylmaleimide (30 nmol/mg) which stimulates K⁺ uniport 3.6-fold. N-Ethylmaleimide, however, has no effect on the IC₅₀ values which are 0.5, 2.3, and 8 μM for ADP, ATP, and AMP, respectively. GDP has no effect, while carboxyatractyloside is found to inhibit. The nucleotide analogs Cibacron blue 3GA and erythrosin B exhibit three effects on K⁺ uniport. Low doses partially inhibit K⁺ uniport (IC₅₀ = 0.13 μM Cibacron Blue), while higher doses stimulate (EC₅₀ = 13 μM Cibacron Blue). Stimulation is especially apparent in N-ethylmaleimide-treated mitochondria. These analogs also antagonize inhibition by ATP. Since the EC₅₀ values for this antagonism for these two drugs are similar, while the IC₅₀ values for inhibition of ATP transport differ by a factor of five, we suggest that inhibition of K⁺ uniport by ATP is not mediated via the adenine nucleotide translocase. These data are consistent with the existence of an ATP-regulated K⁺ channel in the inner mitochondrial membrane.

It has been long established that the inner mitochondrial membrane has a finite permeability to K⁺ (1, 2). Moreover, as a consequence of the high membrane potential generated by respiration, there is a large driving force for the electrophoretic influx of K⁺, which is the most abundant cation of the cytosol and mitochondrial matrix. The inevitable influx of K⁺ is compensated by electroneutral efflux via K⁺/H⁺ antiport (see Ref. 3, for a review). It has been recognized for a number of years that a specific 82-kDa protein is responsible for K⁺/H⁺ antiport, however, the pathways responsible for K⁺ influx remain to be established. The two types of pathway which must be considered are diffusive leak and specific channel or uniporter proteins.

A number of properties of K⁺ uniport have been described. Diwan's (4) group has shown that K⁺ uniport in liver mitochondria is modulated by dicyclohexylcarbodiimide, Mg²⁺ (5), pH (6), mercurials (7, 8), and drugs such as quinine (9), while Brierley's (10–14) group has demonstrated similar properties in heart. More recently, evidence has accumulated for the existence of a protein which can mediate K⁺ uniport. Mironova et al. (15) reported purification of a cation selective channel from mitochondria and more recently Diwan's (16, 17) group have partially purified a protein which mediates K⁺ transport in liposomes and which appears to have channel activity when the liposomes are patch-clamped. We have also presented preliminary reports of purification of a protein which mediates K⁺ uniport (18–20) and is inhibited by ATP (20). These proteins all appear to have molecular masses in the 50–60-kDa range. Halestrap's group (21–23) has suggested that the adenine nucleotide translocase may be responsible for K⁺ uniport and that cyclophilin is an important regulator. Recently, Bernardi's group (24, 25) has investigated K⁺ uniport in intact mitochondria and has come to the conclusion that it is regulated by matrix Mg²⁺.

In this paper, we examine some previously undescribed properties of K⁺ uniport in intact mitochondria. Most notably, we present evidence that K⁺ uniport is regulated by adenine nucleotides and nucleotide analogs. These data suggest that K⁺ uniport in mitochondria may be mediated by an ATP-sensitive K⁺ channel similar to that found in plasma membranes of heart, skeletal muscle, pancreatic β cells, and the central nervous system (for reviews, see Refs. 26–28). This conclusion is supported by patch-clamp studies of Inoue et al. (29) showing that mitochondria contain a K⁺ channel which is blocked by ATP and glibenclamide.

**EXPERIMENTAL PROCEDURES**

**Assay of Potassium Transport**—Potassium transport was assayed by following swelling, which accompanies net salt transport, using the light scattering technique as described in detail elsewhere (30, 31). Using this technique we generate a light scattering variable, β, which normalizes reciprocal absorbance for mitochondrial protein concentration, P (milligrams/ml), according to the following formula.

\[
\beta = \frac{P_i}{a} \left( A^{-1} - a \right)
\]

(Eq. 1)

where a is a machine constant and Pᵢ (equals 1 mg/ml) is a constant introduced to make β dimensionless.

The rate of salt transport is calculated from the rate of change of β according to the formula (30),...
where $\varphi$ is the medium osmolality (110 milliosmol in most studies reported here), $S_0$ the solute content of the stock preparation of mitochondria (190 mosmol/mg), $b$ (15 milliosmolal) is the slope of the equilibrium absorbance osmotic curve, and $n$ is the number of moles of osmotically active particles which make up 1 mol of the transported salt. At $\varphi = 110$ milliosmol $\Delta S_0/b$ is about 1400 nmol/mg.

To determine the rates of solute transport, we use a Brinkmann Probe Colorimeter (Model PC700) with a 1-cm probe (2 cm light path). For optimum sensitivity we normally use mitochondria at a concentration between 0.1 and 0.2 mg/ml.

Assay of Uniport—Mitochondrial swelling is observed when there is a net influx of salt into the mitochondrial matrix. The light scattering traces contained in Fig. 1 show that respiring mitochondria swell when suspended in a medium containing K+ and acetate assay medium are shown. Trace a, control. Trace b, plus nigericin (2 nmol/mg). Trace c, plus CCCP (8 $\mu$m). Trace d, no respiratory substrates present. The composition of the assay medium is described under "Experimental Procedures"; traces a-c contained ascorbate and TMPD as respiratory substrates.

FIG 1. Respiration drives K+ uniport in mitochondria. Light scattering kinetics of mitochondria (0.1 mg/ml) suspended in K+ acetate assay medium are shown. Trace a, control. Trace b, plus nigericin (2 nmol/mg). Trace c, plus CCCP (8 $\mu$m). Trace d, no respiratory substrates present. The composition of the assay medium is described under "Experimental Procedures"; traces a-c contained ascorbate and TMPD as respiratory substrates.

in the absence of respiratory substrates (trace b), in the presence of an uncoupler (CCCP) (trace c), or in the presence of the exogenous K+/H+ antiporter nigericin (trace d). These findings indicate that the swelling must be secondary to the electrophoretic influx of K+ driven by the membrane potential generated by respiration.

In order to use this procedure to assay K+ uniport, it is important for swelling to be limited by the rate of K+ uniport and not by the rate of respiration. When the acetate concentration increased from 0 to 60 mM while keeping the K+ concentration constant with KCl, the rate of swelling was found to increase with increasing acetate concentration. In a parallel experiment, rates of respiration were determined and found to increase in parallel with the increase in the rate of swelling. In fact, at 50–60 mM acetate they approached the uncoupled rate. That this increase is secondary to an increase in K+ uniport was demonstrated by the finding that acetate concentration had a negligible effect on the rate of uncoupled respiration measured in the same trace. Since acetic acid transport is extremely rapid, the increased rate of K+ uniport probably results from an increase in membrane potential secondary to a decrease in the $\Delta$PH which accompanies the increase in acetate concentration.

We have also examined the effect of medium osmolality on the rate of K+ uniport. For this experiment, the acetate concentration was maintained constant (29 mM) while the osmolality was varied with KCl. As shown by the data contained in Fig. 2, between 1/4 = 4 and 12 mosmol−1 the rate of K+ uniport increases as the osmolality decreases despite decreasing K+ concentration. Again, there is a parallel increase in the rate of respiration and, again, there is no increase in the uncoupled rate measured after the swelling has taken place. In fact, the uncoupled rate decreases somewhat after swelling. Thus, the stimulation of respiration appears to be secondary to an increase in the rate of K+ uniport.

On the basis of these data, a medium containing the K+ salts of OAc− (25.4 mM) and Cl− (45 mM), which has an osmolality of 137 mosM (1/4 = 7.3), was selected for subsequent studies.

pH Dependence of K+ Uniport—The pH dependence of K+ uniport is controversial. Thus, we have examined the effect of pH on K+ uniport using our assay. The data contained in Fig. 3 show that the rate of swelling declines as the pH is raised from 6 to 8.5. Since this decrease could be due to direct effects on K+ transport or respiration or could be due to uncoupling, we also examined the rate of respiration under these conditions. As shown by the open circles, the rate of respiration was found to increase over this pH range, which
The rates of K+ influx that both mercurials and NEM are able to stimulate K+ consumption to allow the maximum respiration rate. Since the LS and respiration assays, respectively. To allow the maximum respiratory rate to be determined under "Experimental Procedures." The data were obtained in parallel experiments using mitochondria at 0.14 and 1.6 mg/ml, respectively. To allow the maximum respiratory rate to be determined (■), CCCP (0.28 μM) was added after 50% of the oxygen had been consumed to allow the maximum respiration rate (□) to be determined.

sustains that uncoupling was the cause. Since it is well established that the activity of the K+/H+ antiporter increases as pH rises (3), an increased rate of K+ cycling could explain both respiratory stimulation and inhibition of swelling. The data contained in Fig. 6, show that ATP also inhibits K+ uniport in both control and NEM-treated mitochondria respiring on ascorbate/TMPD. Although the extent of inhibition is higher in NEM-treated mitochondria (66 versus 44%), the inhibited rate remains faster than the control rate, indicating that ATP does not completely block NEM-induced transport. The data in Fig. 5C show that TEA+ transport is also stimulated and becomes sensitive to ATP-sensitive pathway. The absence of any effect on TEA+ transport in normal mitochondria suggests that TEA+ transport is mediated by an ATP-sensitive channel. The absence of any effect on TEA+ transport when succinate is used as a substrate, no inhibition is observed in the absence of NEM. These data suggest that in both normal and NEM-treated mitochondria, K+ transport is mediated by an ATP-sensitive pathway. The finding that TEA+ transport is also stimulated and becomes sensitive to ATP suggests that NEM may decrease the cation selectivity of the ATP-sensitive pathway.

The increased percent inhibition found in NEM-treated mitochondria enabled dose-response curves to be examined. The data contained in Fig. 6, show that ATP, ADP, and AMP inhibit K+ uniport in mitochondria pretreated with NEM and oligomycin with IC50 values of 2.1, 0.4, and 8.4 μM, respectively, and with Hill coefficients very close to 1.0. Similar values were obtained in non-NEM-treated mitochondria, suggesting that NEM itself has no effect on the IC50 (not shown). In contrast to ADP, GDP at 0.2 mM had no effect on K+ uniport. Moreover, this concentration had no effect on inhibition by ATP. In beef heart mitochondria inhibition of K+ uniport by adenine nucleotides was found to be only about 10%.

Effect of Carboxyatractysloside on K+ Uniport—Although our
finding that ATP inhibits K⁺ uniport is in qualitative agreement with the findings of Inoue et al. (29) our IC₅₀ is more than 2 orders of magnitude lower than theirs. In fact, since all these drugs inhibit the translocase, not all stimulate K⁺ uniport. In fact, alizarin red is found to inhibit while eosin has no effect.

Do ATP and CAT Interfere with the Stimulation Induced by Cibacron Blue?—The data in Fig. 9 show that K⁺ uniport

The data were obtained with NEM- (37 nmol/mg) and oligomycin-pretreated mitochondria respiring on ascorbate/TMPD. The rates of K⁺ uniport were determined from the light scattering kinetics traces similar to those shown in Fig. 6B. The IC₅₀ values, Hill coefficients, and maximum extent of inhibition observed are for ATP, 2.1 µM, 54%; ADP, 0.4 µM, 101, 60%; AMP, 8.4 µM, 1.02, 48%, respectively. The composition of the assay medium and pretreatment procedure are described under "Experimental Procedures."

FIG. 7. The effect of carboxyatractyloside on K⁺ uniport. LS kinetics of NEM-treated (37 nmol/mg) mitochondria (0.11 mg/ml) suspended in the standard assay medium containing ascorbate/TMPD. The composition of the assay medium and pretreatment procedure are described under "Experimental Procedures." The TEA⁺ medium was identical to the K⁺ medium except for the replacement of K⁺ by TEA⁺.

Effect of Cibacron Blue on K⁺ Uniport—In order to determine whether the inhibition of K⁺ uniport by adenine nucleotides might be related to their binding to the adenine nucleotide translocase, we compared the effect of a number of nucleotide analogs on K⁺ uniport activity and the translocase. To facilitate comparison, we assayed the translocase activity using the same assay medium used to assay K⁺ uniport but with the respiratory substrates removed and ATP added. Under these conditions, valinomycin induces rapid swelling which is dependent on the activity of the adenine nucleotide translocase and the F₁F₀-ATPase (33). As shown in Fig. 8, Cibacron Blue inhibited ATP-dependent swelling with an EC₅₀ of 12.3 µM. Thus, the EC₅₀ values differ by a factor of about 3, suggesting that different sites are involved. The EC₅₀ values and IC₅₀ values for these transport pathways are compared for a variety of nucleotide analogs in Table I (columns C and D). Of particular interest is the finding that, although all these drugs inhibit the translocase, not all stimulate K⁺ uniport. In fact, alizarin red is found to inhibit while eosin has no effect.

Do ATP and CAT Interfere with the Stimulation Induced by Cibacron Blue?—The data in Fig. 9 show that K⁺ uniport

FIG. 6. Inhibition of K⁺ uniport by ATP, ADP, and AMP. Dose-response curves are shown for inhibition by ATP (●), ADP (▲), and AMP (■). The data were obtained with NEM- (37 nmol/mg) and oligomycin-pretreated mitochondria respiring on ascorbate/TMPD. The rates of K⁺ uniport were determined from the light scattering kinetics traces similar to those shown in Fig. 6B. The IC₅₀ values, Hill coefficients, and maximum extent of inhibition observed are for ATP, 2.1 µM, 54%; ADP, 0.4 µM, 101, 60%; AMP, 8.4 µM, 1.02, 48%, respectively. The composition of the assay medium and pretreatment procedure are described under "Experimental Procedures."
which has been inhibited by addition of 10 μM ATP can be stimulated by Cibacron Blue. In fact, the inhibition is completely reversed, and the EC₅₀ for stimulation appears to be lower than the control. Stimulation of K⁺ uniport is still observed when the ATP concentration is raised to 200 μM and also after inhibition by carboxyatractyloside. Under these conditions, the EC₅₀ is close to that of the control; however, the maximum extent of stimulation is much lower. These data suggest that Cibacron Blue has two effects, first it is able to stimulate K⁺ uniport and second it is able to displace ATP but not CAT from its inhibitory site. If the latter is true then Cibacron Blue should increase the IC₅₀ for ATP.

Does Cibacron Blue Interfere with the Inhibition by ATP—Fig. 10 contains dose-response curves for inhibition of K⁺ uniport by ATP in the presence and absence of Cibacron Blue. In the presence of 6.48, 20, and 30 μM Cibacron Blue the IC₅₀ is increased from 1.85 to 13.7 μM and 29.3 and 59.7 μM, respectively. From these values, one can calculate that Cibacron Blue must compete with ATP binding with Kᵢ values of 1.01, 1.3, and 1.1 μM, respectively. These values are about one-tenth the EC₅₀ for stimulation by Cibacron Blue, consequently, a different site must be involved.

Does ATP Inhibit K⁺ Uniport by Binding to the Adenine Nucleotide Translocase?—Although it is evident that stimulation of K⁺ uniport by Cibacron Blue and inhibition of the adenine nucleotide translocase by Cibacron Blue are mediated via action at different sites, it remains possible that inhibition of K⁺ uniport by ATP may be mediated via the adenine nucleotide translocase. Since this inhibition is antagonized by Cibacron Blue one can predict that the Kᵢ for this should equal the Kᵢ for inhibition of the translocase. It is, however, difficult to compare these values because the assays are carried out under different conditions especially with respect to the ATP concentration. Therefore, to investigate this relationship, we have compared the IC₅₀ values for inhibition of the translocase with the Kᵢ values for antagonizing ATP inhibition of K⁺ uniport for a number of nucleotide analogs. The data contained in Table I (column B) reveal that with the exception of bromcresol green all the dyes both inhibit ATP transport/hydrolysis and protect against inhibition of K⁺ uniport. It is also evident that the Kᵢ for protection by erythrosin B is lower than that for Cibacron Blue while the Kᵢ for inhibition of ATP transport/hydrolysis is 5-fold higher. This finding suggests that different sites are involved. However, since it is also possible that the low IC₅₀ for inhibition of ATP hydrolysis by Cibacron Blue could reflect inhibition of the F,Fₐ-ATPase and not the translocase, we also examined the IC₅₀ values for inhibition of oligomycin-sensitive ATP hydrolysis in frozen-thawed mitochondria in the presence of carboxyatractyloside. Under these conditions the IC₅₀ for Cibacron Blue was actually higher than that for erythrosin.

Nucleotide Analogs Can Inhibit K⁺ Uniport—In several experiments including those shown in Fig. 9, we observed that at the lowest doses of Cibacron Blue the rate of swelling was slightly lower than the control. This suggested that a Cibacron Blue inhibitory site may in fact exist. More convincing evidence was obtained when we examined the effect of Cibacron Blue on mitochondria which had not been treated with NEM. As shown in Fig. 11, in these mitochondria low doses of Cibacron Blue induce pronounced inhibition of both swelling (Fig. 11A) and respiration (Fig. 11B). This inhibition is most evident in the later rates. As shown in Fig. 11C further increase in dose of Cibacron Blue stimulated both swelling and respiration with an EC₅₀ value similar to that observed in NEM-treated mitochondria. The net stimulation above the

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**Fig. 8. The effect of Cibacron Blue on K⁺ uniport and the adenine nucleotide translocator.** Rates of K⁺ influx into NEM-treated (39 nmol/mg) mitochondria are plotted versus the concentration of Cibacron Blue in the assay medium. , rates limited by endogenous K⁺ uniport in mitochondria respiring on ascorbate/TMPE (left-hand scale). , rates of valinomycin-mediated K⁺ influx in respiration inhibited mitochondria limited by the entry of ATP (0.2 mM) into the mitochondria as a substrate for the F,Fₐ-ATPase (right-hand scale). Except for the presence or absence of respiratory substrates, ATP and valinomycin, the media were identical and as described under "Experimental Procedures." The curves are drawn on the basis of EC₅₀ values and Hill coefficients of 2.3, 1.3, and 1.1 μM, respectively.

**Table I**

<table>
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<th>Drug</th>
<th>IC₅₀ inhibition</th>
<th>IC₅₀ protection</th>
<th>EC₅₀ stimulation</th>
<th>D, IC₅₀ ATP hydrolysis</th>
<th>E, IC₅₀ ATP hydrolysis</th>
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<tr>
<td>Cibacron blue 3GA</td>
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<td>1.35</td>
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<td>3.95</td>
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<tr>
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<td>47.0</td>
<td>None</td>
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<tr>
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<td>Yes*</td>
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<td>84.8</td>
</tr>
<tr>
<td>Alizarin red S</td>
<td>1.3*</td>
<td>22.7</td>
<td>None</td>
<td>55.3</td>
<td>29</td>
</tr>
</tbody>
</table>

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*a Determined in the absence of NEM.
*b Inhibition was evident up to about 3 μM. Above this, stimulation was observed.
* No protection was observed in presence of 68 μM bromcresol green.
K⁺ Uniport in Mitochondria

**Fig. 9.** Effect of ATP and CAT on stimulation of K⁺ uniport by Cibacron Blue 3GA. Dose-response curves for stimulation of K⁺ uniport by Cibacron Blue are shown. The experiment was carried out essentially as described in the legend to Fig. 8. ○, control (no ATP); △, +10 μM ATP; □, +200 μM ATP; ○, +CAT (0.5 nmol/mg added to the pretreatment). The mitochondria were pretreated with NEM (33 nmol/mg) and oligomycin.

**Fig. 10.** Effect of Cibacron Blue on inhibition of K⁺ uniport by ATP. Dose-response curves for inhibition of K⁺ uniport were obtained as described in the legend to Fig. 7 in the absence (●) and presence of Cibacron Blue (●, 6.48 μM; ■, 20 μM; ○, 30 μM). IC₅₀ values are 1.86, 13.7, 29.3, and 59.7 μM ATP, respectively, based on a maximum absolute inhibition of 63, 72, 62, and 65%, respectively, and a Hill slope of 1.0 in each case. The mitochondria were pretreated with NEM (40 nmol/mg).

The data contained in Fig. 12 show the results of an experiment in which we examined the control rate is, however, very small. The data contained in Fig. 12 show the results of an experiment in which we examined the dose-response relationship for inhibition. The IC₅₀ obtained from both the swelling traces and the oxygen electrode traces was 0.13 μM with maximum inhibition of 39% for the respiration and 51% for K⁺ uniport. Since this IC₅₀ is only one-tenth of the value estimated for the binding constant of Cibacron Blue at the ATP-inhibitory site, this inhibitory effect appears to reflect the existence of a third Cibacron Blue-binding site. The inhibitory effect is also evident with the other dyes tested except for eosin Y (see Table I, column A).

**The Effect of Glibenclamide on K⁺ Uniport.—**The possibility that the ATP-sensitive K⁺ uniport pathway in mitochondria may be related to the K⁺ ATP channels of plasma membranes led us to investigate the effect of glibenclamide, a potent inhibitor of K⁺ ATP channels (26–28). Using ascorbate/TMPD as substrate we found 10% inhibition with 70 μM glibenclamide and no inhibition was observed with 10 μM. With succinate as substrate, inhibition was about 40%; however, valinomycin-dependent K⁺ uptake was also inhibited, suggesting that glibenclamide inhibits succinate oxidation. Thus, potent inhibition of K⁺ uniport in intact mitochondria was not evident.

**DISCUSSION**

In this paper, we have presented evidence that K⁺ uniport in intact mitochondria is regulated in a complex manner by adenine nucleotides and nucleotide analogs. K⁺ uniport is an electrophoretic process by which K⁺ enters the mitochondrion down its electrochemical gradient. In respiring mitochondria this represents a huge driving force and, consequently, leakage probably accounts for a significant proportion of K⁺ uniport. As discussed in the Introduction, considerable evidence has now accumulated which suggests that a specific protein, a channel or uniporter, may also be involved. This parallel pathway, however, must be regulated to avoid excessive swelling and uncoupling. The mechanism by which this is achieved has yet to be established.

In order to establish appropriate assay conditions for K⁺ uniport, we examined the effect of acetate concentration, osmolality, and pH on K⁺ uniport. Some workers have em-
K⁺ Uniport in Mitochondria

Fig. 12. Cibacron Blue inhibits K⁺ uniport at low doses. Rates of K⁺ uptake (●) and oxygen consumption (▲) are plotted versus the concentration of Cibacron Blue. The experiment was carried out as described in the legend to Fig. 11. The curves were both drawn on the basis of a Hill analysis which gave an IC₅₀ of 0.126 μM, and a Hill coefficient of 1.15 in both experiments. The concentrations of mitochondria used were 0.11 and 0.32 mg/ml, respectively.

Employed passive swelling in KSCN as an assay for K⁺ uniport (22); however, under our conditions transport occurs at a very low rate in this medium. In contrast, respiring mitochondria swell rapidly in KOAc, even when the concentration of acetate is decreased to the point where swelling induced by the addition of nigericin is negligible (Fig. 1). In fact, addition of nigericin to respiring mitochondria inhibits swelling demonstrating that K⁺ transport under these conditions must be occurring via a uniport mechanism. The acetate concentration and osmolality were selected to obtain conditions where the respiration rate was not limiting but which gave rapid rates of K⁺ transport. The effect of acetate concentration on the rate of K⁺ uniport can best be explained on the basis of an increase in the membrane potential driving K⁺ uniport. As the acetate concentration is raised the transmembrane ΔpH will decline and a concomitant increase in Δψ is expected. This explanation is consistent with the lack of passive swelling in KSCN. The effect of osmolality/matrix volume on K⁺ uniport rates in which the transport rate increases as the K⁺ concentration decreases requires further investigation.

The most interesting finding of the present study is that in liver mitochondria adenine nucleotides are potent inhibitors of K⁺ transport. This finding is consistent with the observations of Webster and Bronk (34) that 5 mM ATP blocks the energy-dependent transition from the condensed to orthodox conformation which reflects uptake of K⁺ from the medium. Our finding that ATP does not affect TEA⁺ transport suggests that inhibition of a specific pathway rather than a leak is being observed. The mechanism of the effect of NEM and mercurials has yet to be established. A change in gradient may be involved; however, the finding that NEM increases the percent inhibition by ATP while mercurials eliminate inhibition by ATP suggests that interaction with a specific K⁺ channel may be responsible. The finding that NEM also stimulates TEA⁺ transport and that this transport becomes sensitive to ATP suggests that NEM may stimulate transport by decreasing the selectivity of the K⁺ transport pathway and thereby increasing its conductance.

Since the IC₅₀ values obtained for ADP and ATP are similar to the Kᵣ values reported for the translocase (35) it seemed possible that the adenine nucleotide translocase may be involved. Halestrap's (22) group has suggested that under certain conditions the adenine nucleotide translocase is in fact a K⁺ uniporter. They have reported that this process is blocked by ATP and ADP and that this inhibition is blocked by CAT. In our experiment, however, we have very clearly demonstrated that like ATP, CAT inhibits K⁺ uniport. Opposing effects of ADP and CAT have also been observed by others for other processes not related to adenine nucleotide transport. For example, the Ca²⁺ induced permeability transition (36, 37) is facilitated by CAT and inhibited by ADP. Similarly, Panov et al. (38) have reported that K⁺ and H⁺ uniport are inhibited by ADP and that this inhibition is reversed by CAT. More recently, Halestrap and Davidson (22) have reported a similar finding with regard to K⁺ uniport.

These findings are all dependent on the presence of Ca²⁺ and are probably related to the involvement of the translocase in the permeability transition. In another study, Rottenberg and Marbach (39) have reported that Ca²⁺ uniport is stimulated by ADP and that this is reversed by CAT. They have attributed these effects to a change in conformation of the adenine nucleotide translocase from the c-state to the m-state. In view of the abundance of this protein in the inner membrane, they suggest that this can change the membrane surface charge and, hence, affect other transport processes. It is quite possible that such a conformational change could affect the interaction between the translocase and other membrane components and that this could be the mechanism by which the translocase modulates the Ca²⁺-induced permeability transition (37).

If this were the mechanism by which adenine nucleotides and CAT affect K⁺ uniport, one would expect CAT and ATP to have opposite effects, and one would not expect CAT alone to cause inhibition. These predictions were not confirmed by our studies. To our knowledge, the only other report of adenine nucleotides and CAT producing similar effects is that by Skulachev's group (40) which reported that both CAT and ADP partially block uncoupling induced by palmitate. Since these experiments were carried out in the presence of EGTA to chelate Ca²⁺, they conclude that this effect is mediated via the translocase. Another difference between our findings and other putative "translocase-mediated" effects is that AMP is effective in our experiment while it does not modulate the effects reported by others (38, 39).

Thus, while it is quite likely that the observed inhibition by CAT is mediated via the translocase, inhibition by adenine nucleotides is probably mediated via a different site. The most direct evidence for this comes from the 7-fold difference in the ratio of potencies for inhibition of the translocase and protection of inhibition of K⁺ uniport by the drugs Cibacron Blue and erythrosin B.

In this study we have also identified several direct effects of nucleotide analogs on K⁺ uniport. At high concentrations in NEM-treated mitochondria, the predominant effect is stimulation of K⁺ uniport, while at low concentrations inhibition is observed. Inhibition is most evident in non-NEM-treated mitochondria; however, the EC₅₀ values are sufficiently different that both effects can be detected in a dose-response curve. Inhibition by Cibacron Blue does not appear to be related to inhibition by ATP since it can be observed even in the presence of high doses of ATP. Cibacron Blue and erythrosin B do, however, appear to be able to antagonize the inhibition by ATP since they increase the IC₅₀ for ATP.

Thus, the activity of the K⁺ uniport appears to be regulated by at least three nucleotide analog-binding sites. A high affinity binding site which inhibits transport, a low affinity site which stimulates transport even when the high affinity site is occupied, and an intermediate affinity site which mediates inhibition of transport when occupied by adenine nu-
cleotides but not when occupied by the nucleotide analogs. The location of these sites has yet to be established; however, our data suggest that the ATP-inhibitory site is probably closely associated with the K⁺ channel. This is supported by our finding that ATP also inhibits K⁺ transport in a partially purified preparation containing a 53-kDa protein (20).

The physiological function of K⁺ uniport has not been established. It does, however, provide a pathway for entry of K⁺ into the mitochondrial matrix and, therefore, may be important during growth of mitochondria. K⁺ uniport is usually viewed as an undesired transport which should be minimized and compensated for by electroneutral K⁺/H⁺ antiport important during growth of mitochondria. K⁺ uniport is usually viewed as an undesired transport which should be minimized and compensated for by electroneutral K⁺/H⁺ antiport.

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REFERENCES