Isolated kidney proximal tubules subjected to hypoxia/reoxygenation (H/R) have incomplete recovery of mitochondrial membrane potential ($\Delta \Psi_m$) that can be improved, but not normalized, by ATP in permeabilized cells as measured by safranin O uptake. In these studies, the mechanisms for the decreased $\Delta \Psi_m$ in the tubules after H/R are further investigated and impairment of the function of the mitochondrial $F_1F_0$-ATPase is assessed. Normoxic control tubules had a small ATP-dependent component to $\Delta \Psi_m$, but it required low micromolar levels of ATP, not the millimolar levels needed to support $\Delta \Psi_m$ in tubules de-energized with rotenone or after H/R. Micromolar levels of ATP did not improve $\Delta \Psi_m$ after either mild or severe H/R injury. The dependence of $\Delta \Psi_m$ on millimolar levels of ATP after H/R decreased over time during reoxygenation. ATP hydrolysis by the oligomycin-sensitive, mitochondrial $F_1F_0$-ATPase was well preserved after H/R as long as Mg$^{2+}$ was available, indicating that function of both the $F_1F_0$-ATPase and of the adenine nucleotide translocase, which delivers nucleotides to it, are largely intact. However, ATP hydrolysis by the ATPase did not restore $\Delta \Psi_m$ as much as expected from the rate of ATP utilization. These findings, taken together with the observation that substrate-supported generation of $\Delta \Psi_m$ is impaired despite intact electron transport, make it likely that uncoupling plays a major role in the mitochondrial dysfunction in proximal tubules during H/R.

Materials and Methods

Materials

Female New Zealand white rabbits (1.5 to 2.0 kg) were obtained from Harlan (Indianapolis, IN). Type I collagenase was from Worthington Biochemical Corporation (Lakewood, NJ). Percoll was purchased from Amersham Biosciences (Piscataway, NJ). HPLC-grade acetonitrile was from Fisher Scientific (Pittsburgh, PA). JC-1 was supplied by Molecular Probes (Eugene, OR). High-purity digitonin was purchased from Cal-
biochem (catalog #300411, San Diego, CA). All other reagents and chemicals were of the highest grade available from Sigma-Aldrich (St. Louis, MO). Aqueous stock solutions of experimental reagents were all pH adjusted so as not to alter the final pH levels of the experimental medium. Regents that required solubilization in ethanol or DMSO were delivered from stock solutions.

Isolation of Tubules
Proximal tubules were prepared from kidney cortex of female New Zealand white rabbits by collagenase digestion and centrifugation on self-forming Percoll gradients as described (1–4,11).

Experimental Procedures
Incubation conditions were similar to our previous studies (1–5,12). Tubules were suspended at 3.0 to 5.0 mg tubule protein/ml in a 95% air/5% CO2–gassed medium containing (in mM) 110 NaCl, 2.6 KCl, 25 NaHCO3, 2.4 KH2PO4, 1.25 CaCl2, 1.2 MgCl2, 1.2 MgSO4, 5 glucose, 4 sodium lactate, 0.3 alanine, 5 sodium butyrate, 2 glycine, and 0.5 mg/ml bovine gelatin (75 bloom) (Solution A). After 15 to 30 min of preincubation at 37°C, tubules were resuspended in fresh Solution A with experimental agents and regassed with either 95% air/5% CO2 (normoxic controls) or 95% N2/5% CO2 (hypoxia). Solution A was kept at pH 6.9 to simulate tissue acidosis during ischemia in vivo (12) and omitted glucose, lactate, alanine, and butyrate (with adjustment of medium NaCl to maintain constant osmolality). These incubation conditions result in near anoxic conditions for the tubules, but it is not possible to confirm the presence of complete anoxia in the flasks, so we use the term hypoxia to describe it. The energetic deficit is evident within 15 to 30 min of hypoxia and becomes progressively more severe as the duration of hypoxia increases (12).

Studies in this manuscript as in most of our previous work (1–5,12) used 60 min of hypoxia to produce severe expression of the deficit in unprotected tubules to facilitate investigation of its mechanisms while also allowing strong protection by supplemental substrates. After 60 min, samples were removed for analysis. The remaining tubules were pelleted and then resuspended in fresh 95% air/5% CO2-gassed, pH 7.4 Solution A with experimental agents as needed. Sodium butyrate was replaced with 2 mM heptanoic acid and, to assure availability of purine precursors for ATP resynthesis, 250 mM AMP was added at the start of reoxygenation (2,12). After either 7.5 or 60 min of reoxygenation, samples were removed again for analysis. α-Ketoglutarate and malate (αKG/MAL, 4 mM each) were added from concentrated stock solutions of neutralized sodium salts of the two substrates during either reoxygenation alone or during hypoxia and reoxygenation to vary the severity of injury and allow assessment of the behavior of substrate-protected tubules (1,2,4,5).

Measurement of ATP Levels
Samples of tubule cell suspension were immediately deproteinized in trichloroacetic acid, neutralized with triethylamine-CFC 113 and, stored at −20°C as described previously (12). Purine nucleotides and their metabolites in 20 μl aliquots of the neutralized extracts were separated and quantified using a reverse-phase, ion-pairing, gradient HPLC method as described previously (4).

JC-1 Fluorescence
JC-1 was added to the suspensions for 15 min at the end of reoxygenation; suspensions were then sampled for measurement of fluorescence at 488 nm excitation and at 535 and 595 nm emission as described previously (5).
Measurement of ΔΨm with Safranin O

At the end of the experimental period, tubules were pelleted, washed three times in an ice-cold solution containing (in mM) 110 NaCl, 25 NaHEPES, 1.25 CaCl2, 1.0 MgCl2, 1.0 KH2PO4, 3.5 KCl, 5.0 glycine, pH 7.2, and 5% polyethylene glycol (average mol wt, 8000), and held in solution at 4°C until use. For the safranin O uptake measurements (5–8), the tubules were resuspended at a final concentration of 0.10 to 0.15 mg/ml in an intracellular buffer-type solution containing 120 mM KCl, 1 mM KH2PO4, 2 mM EGTA, 5 μM safranin O, 100 μg digitonin/mg protein, 10 mM K-HEPES, pH 7.2 at 37°C (Solution B), supplemented as needed for specific experiments with either αKG/MAL (4 mM each) or succinate (4 mM) and other experimental reagents that are described with the data. Succinate was used as the substrate during safranin O uptake in most studies because it supports slightly better energization of mitochondria in both permeabilized normoxic control tubules and after H/R than αKG/MAL (5). Fluorescence was followed at 485 nm excitation, 586 nm emission using Photon Technology International (Lawrenceville, NJ) Deltascan and Alphascan fluorometers, equipped with temperature-controlled, magnetically stirred cuvette holders, as illustrated by the tracings shown in Results. Uptake of safranin O into the matrix of energized mitochondria results in quenching of its fluorescence, so the measured signal decreases. To make it easier to follow the tracings relative to high and low ΔΨm, they are inverted in the figures shown. For studies done on normoxic, control tubules, all experiments used tubules from the same suspension, so variability between cuvettes was limited to pipetting differences and was <1 to 2%. For studies comparing tubules subjected to different experimental conditions in separate flasks before sampling for safranin O, protein concentrations were targeted to be the same as for the normoxic control and were always within 10% of each other. For studies where changes in fluorescence between groups are compared, fluorescence changes were factored by protein. Safranin O uptake by isolated mitochondria is well documented to be a linear function of ΔΨm (6–8). Using valinomycin to induce K+ diffusion potentials, we confirmed this to be true for mitochondria in permeabilized tubules (data not shown). However, K+ diffusion calibrations were not available for every experiment, so data are reported in terms of safranin O uptake only.

Figure 2. Effects of inhibitors on ATP-supported mitochondrial energization. Representative tracing of permeabilized tubules incubated initially with no substrate, 5 μM rotenone, and 2.0 mM ATP. At 600 s, either 15 μM oligomycin or 500 μM atracyloside was added during the indicated tracings.

Figure 3. Increased stability of substrate-supported mitochondrial energization with low concentrations of ATP. Representative tracings of safranin O uptake by mitochondria in permeabilized tubules energized with succinate alone or succinate plus the indicated concentrations of nucleotides. The order of the tracings from strongest to weakest uptake is the same as the order of listed conditions in the figure.
Table 1. Distribution of nucleotide metabolites after addition of nucleotides during safranin O uptake

<table>
<thead>
<tr>
<th>Condition</th>
<th>AMP</th>
<th>ADP</th>
<th>ATP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No addition</td>
<td>1.02 ± 0.11</td>
<td>0.35 ± 0.05</td>
<td>0.43 ± 0.13</td>
<td>1.81 ± 0.26</td>
</tr>
<tr>
<td>10 μM ATP</td>
<td>1.47 ± 0.15</td>
<td>2.23 ± 0.57</td>
<td>7.88 ± 0.09</td>
<td>11.59 ± 0.73</td>
</tr>
<tr>
<td>25 μM ATP</td>
<td>1.63 ± 0.20</td>
<td>5.26 ± 0.49</td>
<td>19.11 ± 1.38</td>
<td>26.0 ± 1.27</td>
</tr>
<tr>
<td>25 μM ADP</td>
<td>2.41 ± 0.24</td>
<td>5.78 ± 0.39</td>
<td>15.25 ± 1.34</td>
<td>23.44 ± 0.97</td>
</tr>
<tr>
<td>25 μM AMP</td>
<td>8.27 ± 1.24</td>
<td>6.97 ± 0.70</td>
<td>9.78 ± 1.04</td>
<td>25.02 ± 1.02</td>
</tr>
</tbody>
</table>

*aCondition indicates the nucleotide added at the start of safranin O uptake. Values are means ± SEM μM concentrations in the medium of the indicated nucleotide or the total of all three at the end of the 10-min period of safranin O uptake for 4 to 5 experiments. Adenosine, inosine, hypoxanthine, and xanthine were not detectable.

Measurement of F_{1}F_{0}-ATPase Activity

**Activity during Safranin O Uptake.** Safranin O uptake was followed fluorometrically as usual, except Solution B was substrate-free and was supplemented with 1 mM ATP, 1 mM ouabain, 5 μM rotenone, and, in some studies, 1 mM MgCl₂. Separate assays were run for each sample with and without 15 μM of the F₁,F₀-ATPase inhibitor, oligomycin (10). After 20 min of uptake, samples were taken for ATP and immediately processed to extract nucleotides in trichloroacetic acid exactly as with tubule suspension samples. ATP, ADP, AMP, and all their nucleoside metabolites were then separated and quantified by reverse-phase HPLC as with tubule suspension samples. Under these conditions, most of the metabolized ATP appeared as ADP, with some AMP, but no further degradation beyond AMP. It was possible to more accurately quantify the appearance of ADP and AMP than the disappearance of the ATP, so ATP hydrolysis was calculated as the oligomycin-sensitive AMP+ADP formed in nmol/mg tubule protein/min with correction for the small amounts of contaminating AMP and ADP in the ATP before the start of incubation.

**Activity of Frozen-Thawed Samples Using an NADH-Coupled Assay.** After the desired experimental maneuvers, tubules were pelleted, immediately resuspended and snap-frozen in 120 mM KCl, 1 mM KH₂PO₄, 2 mM EGTA, 10 mM K-HEPES, pH 7.2, and stored at −80°C. Samples were assayed within 4 wk during which activity was stable. For assays, tubule samples were thawed and briefly sonicated on ice. An aliquot of tubule sample was added at a final protein concentration of 0.04 mg/ml to a reaction medium containing 100 mM KCl, 50 mM Tris-HCl, 5 mM phosphoethanolpyruvate, 0.075 mM NADH, 6 mM MgCl₂, 10 μM rotenone, 1.0 mM ouabain, 25 U/ml lactate dehydrogenase (rabbit muscle in glycerol), 25 U/ml pyruvate kinase (rabbit muscle in glycerol), pH 8.0 at 37°C. The reaction was started by addition of 0.5 mM ATP and conversion of NADH to NAD was followed fluorimetrically at 346 nm excitation/458 nm emission. After the initial rate was obtained, 15 μM oligomycin was added to measure the oligomycin-insensitive rate. Oligomycin-sensitive activity was calculated as the difference between the initial and oligomycin-insensitive rates of NADH oxidation in nmol/min per mg protein (13,14).

**Statistical Analyses.** Paired and unpaired t-test were used as appropriate. Where experiments consisted of multiple groups, they were analyzed statistically by ANOVA for repeated measure or independent group designs as needed. Individual group comparisons for the multigroup studies were then made using the Holm-Sidak test for multiple comparisons (SigmaStat 3, SPSS, Chicago, IL). P < 0.05 was considered to be statistically significant. Data shown are either means ± SEM of no less than 3 to 5 experiments or are tracings representative of the behavior in that many experiments.
**Results**

**Substrate versus ATP-Dependent Support of Energization**

Figure 1A illustrates mitochondrial safranin O uptake in permeabilized, normoxic, control tubules initially incubated with αKG/MAL as the substrate supporting safranin O uptake, followed by addition of rotenone, then by various concentrations of ATP. As described previously (5), safranin O uptake by the mitochondria begins after a brief lag and is maximal after 9 to 10 min. Addition of rotenone at this point to block respiration at complex I and, therefore, utilization of the αKG/MAL to support electron transport, fully de-energizes the mitochondria. Subsequent addition of ATP with hydrolysis by the F$_1$F$_0$-ATPase (10,15–17) then re-energizes them in a concentration-dependent fashion. Minimal transient energization was seen with 10 and 25 μM ATP, and 100 μM ATP supported 67% of the effect of 2 mM (Figure 1A). In studies of additional concentrations (not shown), full energization was reached at 1 mM ATP. If ATP was not added, no re-energization occurred (Figure 1B). If the uncoupler carbonyl cyanide p-(trifluoromethoxyphenyl)-hydrazone (FCCP), was added along with rotenone, de-energization was more rapid and the re-energization with ATP was prevented (Figure 1B). In the Figure 2 studies, permeabilized tubules were initially incubated with rotenone and no substrate plus 2 mM ATP. After energization produced by the ATP was maximal, addition of either the F$_1$F$_0$-ATPase inhibitor, oligomycin (10), or the adenine nucleotide translocase inhibitor, atracyloside (10), rapidly de-energized the mitochondria.

**Effect of Micromolar Levels of ATP on Energization in the Presence of Substrate**

In studies where substrate-dependent energization was followed for longer periods after peak safranin O uptake without additional experimental maneuvers, we observed that energization then decreased over time (tracings labeled “No ATP” in Figures 3, A and B). If ATP was present, the decay of energization was prevented. As little as 10 μM ATP was sufficient (Figure 3A). Similar behavior was seen with AMP and ADP (Figure 3B). The effects of these low concentrations of nucleotides were not accompanied by substantial net ATP utilization. After addition of both 10 and 25 μM ATP, ATP levels in the medium at the end of the safranin O uptake period were 80% of the amount added (Table 1). Large fractions of the added ADP and AMP were converted to ATP, so it can account for their effects. In view of these actions of ATP to prevent spontaneous de-energization, we tested whether this de-energization was mediated by mitochondrial ATP-sensitive K$^+$ channels using channel inhibitors (18). Neither glibenclamide nor 5-hydroxydecanoate reproduced the effect of ATP to prevent the mitochondrial de-energization.

**Effects of ATP on Energization after H/R**

As previously reported (1–4), tubules subjected to H/R without protective substrates had poor recovery of ATP, decreased formation of high ΔΨm-dependent, 595-nm-emitting, JC-1 aggregates, and decreased 595/535-nm JC-1 emission ratios (Figure 4, left panel). All these parameters were improved in tubules protected by inclusion of αKG/MAL during reoxygenation. After H/R, mitochondrial safranin O uptake measured without supplemental ATP in the medium was substantially decreased. Uptake was not improved by 10 μM ATP, but was substantially increased by 2 mM ATP. However, energization was not restored to normoxic levels (Figures 4, right panel, and 5). Mitochondria in tubules that were protected by
**aKG/MAL during reoxygenation** had improved safranin O uptake.

Figures 6 and 7 further explore this behavior. For these studies, tubules received aKG/MAL during both hypoxia and reoxygenation, so they showed strong recovery of ATP levels and mitochondrial energization during the reoxygenation period (Figure 6). However, recovery was time-dependent, which allowed studies of progressive changes in the same flasks. At 7.5 min of reoxygenation, when recovery of mitochondrial energization was incomplete based on both JC-1 (Figure 7, left panel) and safranin O uptake (Figures 6, right panel, and 7), there was a large increment of safranin O uptake in the presence of 2 mM ATP as compared with no supplemental ATP or 10μM ATP. By 60 min of reoxygenation, this increment was no longer present, primarily because of increases of energization of mitochondria in the tubules studied with either no added ATP or 10μM ATP. As in the studies in Figures 4 and 5, maximal recovery of ΔΨm, although strong at 60 min, still fell short of normoxic control levels.

**Figures 6. Mitochondrial energization and ATP levels of tubules after H/R as a function of the duration of reoxygenation. Tubules were subjected to normoxic incubation or to 60 min hypoxia with aKG/MAL followed by reoxygenation with aKG/MAL and were sampled for measurement of suspension ATP levels and JC-1 fluorescence (left panel) after 7.5 min (7.5' REOX) and 60 min (60' REOX) of reoxygenation. Other aliquots of tubules from the same flasks were permeabilized and succinate-supported mitochondrial safranin O uptake (Figures 6, right panel, and 7) was measured concomitantly. Figure 7 illus- trates safranin O uptake tracings from these studies and Figure 8 summarizes the group data for ATP hydrolysis and safranin O uptake. Tubules were tested under the full range of injury conditions that had been used in the studies in Figures 4 to 7.**

**Figure 7. J C-1 fluorescence and safranin O uptake.** Tubules were tested under the full range of injury conditions that had been used in the studies in Figures 4 to 7. Safranin O uptake after H/R never recovered to normoxic control levels and was always more impaired than ATP hydrolysis measured in the same cuvettes.

**Normalization of F1F0-ATPase Activity by Mg2+.** To test whether the inhibition of ATPase activity after H/R (Figure 9) was secondary to damage to the enzyme per se or to alterations in its regulation or in the delivery of ATP to it, further studies were done using a standard NADH-coupled assay for oligomycin-sensitive ATPase activity and frozen-thawed, sonicated tubule samples (Figure 10, left panel). With this assay, which also permitted assessment of F1F0-ATPase activity immediately at the end of hypoxia, moderate inhibition of ATP hydrolysis was seen at the end of hypoxia, but normal activity was measured in all groups at the end of reoxygenation. Mg2+ is present in the NADH-coupled assay but was not used in the measurements of oligomycin-sensitive ATP hydrolysis during safranin O uptake (Figure 9) and could have been depleted in the mitochondrial matrix of the most severely affected H/R samples during the uptake period by loss to the Mg2+-free medium (19). The experiments in the right panel of Figure 10 show that inclusion of 1 mM Mg2+ in the medium during safranin O uptake restored most oligomycin-sensitive ATPase activity after H/R. Adding Mg2+ did not increase safranin O uptake supported by ATP alone in these studies or in other experiments with substrate alone or substrate+ATP to support energization (data not shown).

**Discussion.** We have recently reported on the use of safranin O to study the energetic deficit and failure of recovery of ΔΨm that develops in proximal tubules after H/R (5). Mitochondrial safranin O uptake in digitonin-permeabilized tubules supported by respiratory substrates was impaired after H/R relative to normoxic controls and was improved when tubules were protected.
by αKG/MAL during reoxygenation before measurement of safranin O uptake. ATP-supported energization of the mitochondria, like substrate-supported energization, was also impaired after H/R. The combination of substrate and ATP improved energization relative to substrate or ATP alone, although ∆Ψm was not normalized (5). The studies reported here have substantially extended these observations on the behavior of ∆Ψm in tubules after H/R and have assessed whether decreased activity of the mitochondrial F1F0-ATPase contributes to the energetic deficit.

Normally, the proton extrusion from the mitochondrial matrix that generates ∆Ψm results from substrate-supported electron transport. Movement of protons back into the matrix via the F1F0-ATPase then drives phosphorylation of ATP (9,10,20,21) (Figure 11). When electron transport is inhibited, ∆Ψm is low, and ATP is available, the F1F0-ATPase works in reverse to hydrolyze ATP to extrude protons and restore ∆Ψm (16,22,23) (Figure 11). In these studies, we have used these processes as expressed in the permeabilized tubules to further define the factors contributing to the impaired recovery of ∆Ψm after H/R and to assess F1F0-ATPase function.

Stability of substrate-supported energization in normoxic controls was improved by the addition of low micromolar levels of ATP to the system (Figure 3). After H/R, tubules showed different behavior with large effects of added ATP on ∆Ψm, seen with millimolar ATP, but no effect of 10 μM ATP (Figures 4 to 7). The concentration dependence for support of ∆Ψm by ATP in H/R tubules is similar to that of mitochondria from normoxic tubules deliberately de-energized with rotenone (Figure 1A) and is consistent with the presence of mitochondria that are de-energized despite the availability of substrate, but capable of generating and maintaining better ∆Ψm by utilizing the F1F0-ATPase to hydrolyze ATP and extrude protons. This may not, however, be the only explanation for the benefit of the added ATP because, in our previous studies, millimolar concentrations of ATP provided without substrate did not energize any better than substrate alone (5). ATP was only beneficial when added with substrate, which suggests that, rather than only energizing an additional population of mitochondria that is not effectively using substrate-supported electron transport, the ATP in combination with substrate could also be improving other as yet unidentified functional abnormalities. Interestingly, in this regard these studies demonstrate that the dependence on millimolar ATP for energization decreases substantially as reoxygenation progresses (Figures 6, right panel, and 7) in tubules that are recovering and restoring their own ATP levels (Figure 6, left panel) before sampling for measurement of safranin O uptake. These time course studies also importantly indicate that the processes set in motion that account for the energetic deficit occur primarily during hypoxia and are not induced by reoxygenation.

In both our earlier work (5) and the studies reported here, addition of ATP to the permeabilized tubules did not fully restore energization of mitochondria in tubules subjected to H/R compared with mitochondria in normoxic control tubules. This raises the question of whether inhibition of the F1F0-ATPase or decreased delivery of nucleotides into the mitochondrial matrix by the adenine nucleotide translocase contributes to the impaired oxidative phosphorylation and ATP depletion present in the unprotected tubules after H/R and limits the effect of ATP on mitochondrial energization in the tubules when they are subsequently permeabilized for the safranin O uptake studies. Decreases of both F1F0-ATPase and adenine nucleotide translocase activity have been reported in isolated mitochondria after ischemia and related insults (23–28). Although it preserves ∆Ψm, ATP hydrolysis by the F1F0-ATPase...
is energetically unfavorable for the cell, because it will tend to further deplete ATP generated by glycolysis or less damaged mitochondria (16,23). It is regulated by reversible, low-pH–induced binding to the $F_{1}F_{0}$-ATPase under de-energized conditions of an $F_{1}F_{0}$-ATPase inhibitory subunit, IF1, which inhibits activity during ischemia and thereby conserves ATP (23,24). The content of IF1 and degree of inhibition of the $F_{1}F_{0}$-ATPase during ischemia varies between tissues and species (23). The NADH-coupled assay for oligomycin-sensitive ATPase using frozen/thawed tubules demonstrated moderate inhibition at the end of hypoxia, which recovered during reoxygenation (Figure 10, left panel). Using a similar assay, a recent study has described increases of $F_{1}F_{0}$-ATPase activity, but decreases of mitochondrial $F_{1}$ protein expression, after ischemia/reperfusion in the kidney (29).

In our initial measurements of oligomycin-sensitive ATPase activity during safranin O uptake (Figure 9), activity at the end of reoxygenation was substantially decreased as a function of the severity of injury (Figure 9). However, this was mostly corrected by addition of Mg$^{2+}$/H$^{+}$ (Figure 10, right panel). Preserved oligomycin-sensitive ATPase activity in digitonin-permeabilized tubules after H/R, where mitochondria are intact as in the safranin O uptake assay, indicates that neither activity of the ATPase nor delivery of ATP to it by the adenine nucleotide translocase is limiting.

We have previously shown that inhibition of electron transport cannot explain the energetic deficit in the tubules after H/R (4). The studies reported here demonstrate that, despite retention of $F_{1}F_{0}$-ATPase and adenine nucleotide translocase activity, ATP hydrolysis by mitochondria maintains a much lower $\Delta V_{m}$ in tubules that have been subjected to H/R than in normoxic control tubules. This implicates an increased leak rate across the inner mitochondrial membrane after H/R that limits energization and uncouples the mitochondria (30). Such a leak may involve movement of protons themselves and other cations back across an inner mitochondrial membrane altered by H/R or shuffling of protons associated with nonesterified fatty acids (19,31–34) and could significantly contribute to the failure of the tubules to restore their ATP levels during reoxygenation.

Being freshly isolated tubules, this preparation retains the sensitivity of proximal tubules to oxygen deprivation as seen...
The average rate for the normoxic controls was 437.4 ± 52.6 nmol/min per mg protein. (Right panel) Oligomycin-sensitive ATP hydrolysis measured using digitonin-permeabilized tubules during safranin O uptake without and with 1 mM Mg\(^{2+}\) at the end of normoxic control (NC) incubation or 60 min hypoxia followed by 60 min reoxygenation (R) without or with αKG/MAL. Values are means ± SEM for 6 to 7 experiments. *Significantly different from the corresponding normoxic control. #Significantly different from the corresponding Mg\(^{2+}\) condition. The average oligomycin-sensitive ATP hydrolysis rate for the normoxic controls was 27.0 ± 4.0 nmol/min per mg protein.

in vivo. The conditions of study, including the presence of glycine and pH 6.9 during hypoxia, reproduce major protective factors present in vivo (35) and allow for the energetic deficit to be cleanly studied. Although this system accurately models the intrinsic behavior of the tubules, the impact of these tubule effects on organ function and the effect of hemodynamic changes in the tissue to promote tubule injury over prolonged periods after defined insults are complex and not simply predictable from the behavior of the tubules themselves (35). Nonetheless, given the major role of tubule cell injury as an endpoint in the organ pathology and the renewed appreciation of the critical role played by mitochondrial cell death, defining tubule behavior in this context is of relevance to understanding acute renal failure and approaches to modify it.

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