Levosimendan protects against experimental endotoxemic acute renal failure

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LPS completely blocked ANG II-induced MC contraction, an action that may specifically offset sepsis-induced ARF, without any apparent reduction in the sepsis-initiated inflammatory cascade, e.g., TNF-α and monocyte chemoattractant protein-1 (MCP-1), can amplify acute tubular injury and intrarenal inflammation (28, 43, 44), potentially exacerbating ischemic ARF.

Despite a growing understanding of its pathophysiology, no proven pharmacological means for preventing endotoxemic ARF currently exist. Thus treatment remains largely supportive in nature, consisting of antibiotic administration and attempts to maintain systemic hemodynamics (e.g., volume resuscitation, vasoprotector agents). However, when vasoconstrictors are administered, renal ischemia and tubular injury may result. Recently, a new cardiac ionotrope was introduced for treating acute and chronic congestive heart failure: levosimendan (LS) (22, 23, 26, 36). A number of LS actions raise the theoretical possibility that this agent also may have value for endotoxemic ARF. These include the following: 1) LS is a myocardial calcium sensitizer, an action that may specifically offset sepsis-induced reductions in myocardial function and thus improve systemic hemodynamics (35, 40); 2) LS is an ATP-sensitive K+ (KATP) channel agonist and, as such, may augment renal perfusion, given that this class of agents has potent vasodilator effects (24, 25); and 3) LS may have anti-inflammatory properties (21, 22), suggesting that it may mitigate an LPS-induced inflammatory state.

In addition to the above properties, KATP channel agonists may directly impact the expression of tissue injury. For example, these agents have been purported to exert cytoprotective actions in the myocardium (4, 7, 18, 59). Conversely, in the kidney, KATP channel opening has been reported to exacerbate ischemic/hypoxic tubular necrosis and postischemic ARF (10, 27, 29). This raises a therapeutic concern: if LS, acting as a KATP channel opener, were to augment ischemic tubular injury, this could offset its potential benefits in both sepsis-induced ARF and severe congestive heart failure (i.e., conditions in which renal ischemia may develop).
Given these considerations, the present study was undertaken in an attempt to answer the following questions: 1) Does LS have potential utility in combating experimental endotoxic ARF? 2) If so, is this benefit mediated via reductions in LPS-induced inflammation? 3) Might the potential benefits of LS administration in sepsis syndrome be overshadowed by a purported K\textsubscript{ATP} channel-mediated "injury-provoking" effect (10, 27, 29)?

METHODS

Assessment of LS Effects on Endotoxic ARF

Male CD-1 mice (25–35 g; Charles River Laboratories, Wilmington, MA), maintained under standard vivarium conditions with free food and water access throughout, were used for all in vivo experiments. The employed protocols were submitted and approved by the institution's Institutional Animal Care and Use Committee. Twenty mice were individually placed into cylindrical restraining tubes. Each was given a 2 mg/kg intraperitoneal injection of Escherichia coli endotoxin [E. coli 0111:B4 (L-2630) in ~800 μl of saline; Sigma, St. Louis, MO]. Concomitantly with the LPS injection, half of this group of mice received a loading dose of LS (50 μg/kg, administered via the tail vein). For point of reference, the human LS dosage (per U.S. patent filing 6,399,610; Orion Pharmaceuticals, Helsinki, Finland) is \( \leq 150 \mu g\) kg\(^{-1}\) day\(^{-1}\). The remaining half of the LPS-injected group received LS vehicle injection. LS and its vehicle were a gift from Abbott Laboratories (Abbott Park, IL). After injections, the mice were returned to their cages. Approximately 6 h later, a second LS dose (25 μg/kg) or its vehicle was administered subcutaneously in a hindlimb. At 24 h after LPS injection, the mice were deeply anesthetized with pentobarbital sodium (–40 mg/kg ip), the abdominal cavities were opened through midline abdominal incisions, and the mice were killed by phlebotomy (from the inferior vena cava using heparinized syringes). The severity of ARF was gauged by blood urea nitrogen (BUN) and plasma creatinine (P\textsubscript{cr}) concentrations (using specific microtiter assays from Bioassay Systems, Hayward, CA).

To determine whether LS injections altered renal histology, we removed the left kidneys from four LPS/placebo-injected mice at the time of death and processed full-length sections using light microscopic analysis (10% formalin fixation, paraffin embedding; 4-μm section staining with hematoxylin and eosin). Histological appearances were compared with those of kidneys obtained from four LS/LS-treated mice and from four normal mice (obtained from the experiment described below).

LS Effects on BUN and PCr Concentrations in Normal Mice

The following experiment assessed whether the employed LS treatment protocol independently altered BUN and P\textsubscript{cr} concentrations in normal (nonendotoxemic) mice. Eight mice were divided into two equal groups and subjected to the above-described LS or LS placebo injection protocol. BUN and P\textsubscript{cr} concentrations were determined 24 h later.

Potential LS Effects on LPS-Induced Cytokine Response

The following experiment assessed whether LS might decrease the severity of endotoxic ARF by attenuating the LPS-induced inflammatory response. Plasma concentrations of TNF-α and MCP-1, and their corresponding cardiac and renal cortical mRNAs, were selected as surrogate markers of the LPS-induced inflammation. Twelve mice were studied, all injected with LPS as detailed above. Half of the group received either LS (50 μg/kg iv) or its vehicle. Two hours later (at, or near, the height of LPS-induced TNF-α/MCP-1 generation; Zager R, unpublished observations), the mice were anesthetized, plasma samples were obtained from the inferior vena cava for cytokine assay, and then the heart and one kidney per mouse were resected and iced. Plasma TNF-α and MCP-1 were quantified using ELISA (TNF-α: R&D Systems, Minneapolis, MN; MCP-1: BD Biosciences, San Diego, CA) (48, 52–54). Renal cortices and cardiac apical tissues were resected from their respective organs with the use of a sterile razor blade, followed by total RNA extraction via the TRIzol method (52). TNF-α, MCP-1, and GAPDH mRNA expression were assessed using RT-PCR (48, 52–54). The TNF-α and MCP-1 products were factored by simultaneously obtained GAPDH results. Control plasma TNF-α and MCP-1 concentrations and control cardiac and renal mRNA levels were assessed using tissue samples previously obtained from normal mice (53).

Potential LS Effects on LPS-Initiated NO Generation

Eight mice were injected with LPS, with half of the group receiving concomitant LS or placebo injection. Four hours later, the mice were anesthetized and rapidly killed via phlebotomy. Serum samples were assayed for NO with a commercially available kit based on the Greiss reaction (nitrates converted to nitrite for total NO assessment; Promega, Madison, WI). Serum samples obtained in the same fashion from four normal mice provided control NO concentrations.

Potential LS Effects on Hypoxic/Ischemic Renal Tubular Injury

Cultured proximal tubular cell experiments. Cultured human proximal tubular (HK-2) cells were maintained in keratinocyte serum-free medium (K-SFM; in T75 Costar flasks) as previously described (31). For experimentation, the flasks were trypsinized and the recovered cells were seeded into Costar 24-well cluster plates. After overnight recovery from reseeding, the following protocols were undertaken: 1) control incubation; K-SFM ± LS (500 or 1,000 ng/ml) or LS vehicle; 2) mitochondrial inhibition with antimycin A (AA; 7.5 μM) ± 500 ng/ml LS or vehicle; 3) glycolytic blockade with 2-deoxyglucose (DG; 20 mM) ± 500 ng/ml LS or vehicle; and 4) AA + DG treatment (to induce profound ATP depletion) ± 500 ng/ml LS or vehicle.

The incubations were maintained under routine culture conditions (20% O\textsubscript{2}, 5% CO\textsubscript{2}, 37°C). After 18 h, viable cell numbers were quantified by trytophane dye [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)] cell uptake assay (with the degree of uptake directly correlating with viable cell numbers; Ref. 31). Each of the above incubations was conducted in quadruplicate. Results were expressed as %MTT uptake observed in simultaneously incubated cells maintained in the absence of the experimental additions.

Isolated tubule experiments: Effects of K\textsubscript{ATP} channel agonists and antagonists of cell viability and responses to hypoxia-reoxygenation injury. LS STUDIES. Mouse proximal tubules (PTs) were isolated from six normal mice as previously described (collagenase digestion, sieving, differential centrifugation through Percoll; Ref. 49). After isolation (at 4°C), the tubules were rewarmed to 37°C over 15 min, and each preparation was diluted in experiment buffer (56) to a protein concentration of ~2–3 mg/ml and divided into five equal 1.25-ml aliquots (in 10-ml Erlenmeyer flasks). This created five experimental groups: 1) control incubation for 25 min at 37°C; 2) control incubation with LS vehicle for 25 min; 3) incubation with LS (500 ng/ml) for 25 min; 4) hypoxic incubation (12.5 min at 95% N\textsubscript{2}-5% CO\textsubscript{2}) in the presence of LS vehicle; and 5) H/R in the presence of 500 ng/ml LS. After these incubations were completed, cell injury was assessed as percent lactate dehydrogenase (LDH) release (56).

DIAZOXIDE STUDIES. To further assess the potential impact of K\textsubscript{ATP} channel agonists on H/R injury, we repeated the LS experiment described above using four additional sets of PTs, substituting 50 μM diazoxide (Sigma) or its vehicle (DMSO) for LS/LS vehicle addition.

GLIBENCAMIDE STUDIES. To test the impact of glibenclamide (GCM; Sigma), a K\textsubscript{ATP} channel antagonist, on mouse tubule viability under control and hypoxic conditions, we performed both high- and low-dose GCM incubations. For high-dose GCM incubations, we
repeated the LS experiment in four additional sets of PTs, substituting 500 μM GCM or its vehicle (DMSO; 10 μl) for LS/LS vehicle addition. For low-dose incubations, we repeated the experiment in four additional sets of PTs, utilizing 250 μM GCM or its vehicle. Thus, in total, eight independent sets of tubules were used to evaluate GCM effects.

A corollary of tubular cell injury can be an increase in nonesterified free fatty acids (NEFA), which then confer potent cytoprotective effects against hypoxic cell injury (56–59). Therefore, we also assessed whether GCM toxicity (see RESULTS) was associated with NEFA increases. Four sets of tubules were incubated for 25 min under control conditions (GCM vehicle) or with 500 μM GCM. After the incubations were completed, tubule lipids were extracted in chloroform-methanol and the recovered lipid was assayed for NEFA as previously described (56). Results were expressed as nanomoles of fatty acid per milligram of tubule protein.

TETRAETHYLAMMONIUM CHLORIDE STUDIES. To assess the impact of a second K$_{\text{ATP}}$ antagonist, tetraethylammonium (TEA; Sigma), on hypoxic tubule injury, we prepared four additional sets of PTs, and each was divided into six experimental aliquots: 1) control incubation for 25 min; 2) incubation with 5 mM TEA for 25 min; 3) incubation with 10 mM TEA for 25 min; 4) H/R as described in LS STUDIES; 5) H/R + 5 mM TEA; or 6) H/R + 10 mM TEA. After the 25-min incubations were completed, cell injury was assessed as %LDH release.

In vivo ischemia-reperfusion injury. MILD ISCHEMIC INJURY PROTOCOL. Sixteen mice were deeply anesthetized with pentobarbital sodium and injected via the tail vein with either LS (50 μg/kg) or vehicle (n = 8 each). A midline laparotomy was performed, followed by right nephrectomy and induction of 12.5 min of left renal pedicle occlusion via a microvascular clamp. After the vascular clamp was removed, reperfusion was confirmed by reversal of organ cyanosis, and then the abdominal incisions were sutured in two layers. Body temperature was maintained at 36–37°C throughout until recovery from anesthesia. Postoperatively, free food and water access was provided. Approximately 4 h postsurgery, a second dose of LS (25 μg/kg) or vehicle was administered. At 18 h postsurgery, the mice were reanesthetized, the abdominal incisions were opened, and they were killed by phlebotomy from the vena cava. The severity of ARF was gauged using BUN and PCr concentrations.

SEVERE ISCHEMIC INJURY PROTOCOL. The above-described protocol was repeated in nine mice (n = 5 with LS; n = 4 with vehicle), except that a 20-min left renal ischemia protocol was employed.

LS Effects on Angiotensin II-Mediated Mesangial Cell Contraction

The following experiment was undertaken to ascertain whether LS might impact mesangial cell (MC) contraction in response to vasoconstrictors generated during endotoxemia. Polydimethylsiloxane (Sigma)-coated sterile coverslips were prepared and seeded with ~20,000 rat MCs, prepared as previously described (3). The cells were placed into 20% FCS-RPMI 1640 medium and incubated at 37°C in 5% CO2 for 24 h. After 24 h, the medium was changed to 2% FCS-RPMI 1640 to induce growth arrest. After an additional 24 h, contraction experiments were conducted as previously described (3). In brief, 5 min before the addition of angiotensin II (ANG II, 100 μM; Sigma), cells were treated with LS (100 or 500 ng/ml) or LS vehicle or were left under control conditions. Cell contraction was demonstrated after ANG II addition (ascertained by wrinkling of the silicone membrane). The percentage of contracted cells was determined by counting. Contraction was documented by digital photography, using a Zeiss Axiomat microscope equipped with an Axiophot camera. The entire experiment was repeated on three separate occasions. In addition, cell morphology under conditions of LS addition vs. vehicle addition was documented using routine photomicroscopy.

Calculations and Statistics

Values are presented as means ± SE. Comparisons were made using unpaired (whole animal, HK-2 cell experiments) or paired (isolated tubule experiments) Student’s t-test. Significance was judged using a P value < 0.05.

RESULTS

LS Effects on Severity of LPS-Induced ARF

As shown in Fig. 1, LPS induced significant renal insufficiency, as denoted by an approximate doubling of BUN and PCr concentrations, compared with normal mouse levels (P < 0.01). LS administration largely blocked these LPS-mediated

![Fig. 1. Levosimendan (LS) treatment abrogates lipopolysaccharide (LPS)-induced azotemia. LPS injection induced significant azotemia, raising blood urea nitrogen (BUN) and plasma creatinine (PCr) concentrations approximately two- to threefold over control (Cont) values. LS treatment almost completely prevented LPS-induced azotemia (with values approximating those in normal animals). LPS injection into control (non-LS treated) mice did not lower BUN or PCr concentrations, implying that the LS activity in LPS-treated mice did not simply represent a nonspecific glomerular filtration rate (GFR) enhancing effect. NS, no significant difference.](http://ajprenal.physiology.org/)

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BUN and P\text{Cr} increases ($P < 0.025$ vs. LPS alone). LS administered to control (non-LPS treated) mice failed to alter either 24-h BUN or P\text{Cr} concentrations.

Renal histological sections obtained from LPS-treated mice demonstrated no morphological abnormalities (appearing identical to those obtained from non-LPS controls). Specifically, no tubular necrosis, microthrombi, or interstitial infiltrates were observed. This is in agreement with prior observations that endotoxemic/gram-negative sepsis-induced ARF in mice is devoid of overt histological damage (51).

**LPS-Induced Cytokine Generation With or Without LS Treatment**

Plasma TNF-\alpha and MCP-1 levels. TNF-\alpha was undetectable ($<20$ pg/ml) in plasma samples obtained from control mice (Fig. 2). By 2 h after LPS administration, massive plasma TNF-\alpha increases were observed. LS administration did not alter this LPS-induced TNF-\alpha response.

Plasma MCP-1 levels in normal mice were $\sim 10$ ng/ml. LPS induced $\sim 10$-fold MCP-1 increases. LS treatment did not alter these results.

Renal and cardiac mRNA levels. The 95% upper confidence limits for renal and cardiac TNF-\alpha mRNA and MCP-1 mRNA are depicted by the horizontal line in Fig. 3. LPS induced marked TNF-\alpha mRNA and MCP-1 mRNA increases in both tissues. LS did not diminish any of these LPS responses.

NO levels. By 4 h after LPS injection, $\sim 10$-fold increases in plasma NO levels were observed (Fig. 4). LS administration caused a slight, albeit nonsignificant, increase in these LPS-induced NO levels.

Assessment of potential LS effects on HK-2 cell injury. LS treatment alone. LS (500 ng/ml) did not alter HK-2 cell MTT uptake (100% of control values; Fig. 5). In data not shown, LS, at a concentration of 1,000 ng/ml also failed to exert a cytotoxic HK-2 cell effect (i.e., decreased MTT uptake).

DG CHALLENGE. DG-induced glycolytic inhibition (which causes a approximate 30% ATP reduction in HK-2 cells; Ref. 15) approximately halved MTT uptake. A virtually identical degree of cell injury occurred when the DG challenge was conducted in the presence of 500 ng/ml LS.

AA CHALLENGE. Inhibiting mitochondrial respiration with AA (causing $\sim 30\%$ ATP depletion; Ref. 15) caused less cell injury ($\sim 25\%$ MTT reduction) than did DG. LS did not alter the extent of AA-induced cell death.

COMBINED AA AND DG CHALLENGE. AA + DG (which induces $\sim 90\%$ ATP depletion in HK-2 cells; Ref. 47) caused massive cell death, as denoted by $\sim 98\%$ reductions in MTT uptake. LS did not rescue these cells via a cytoprotective effect (as previously reported in cardiac tissue; Refs. 37–40).

Isolated mouse proximal tubule injury. LS AND DIAZOXIDE STUDIES. As shown in Fig. 6, addition of either LS or diazoxide to oxygenated tubules did not alter %LDH release (compared with vehicle-treated controls; indicating a lack of direct toxicity). H/R caused $\sim 40\%$ LDH release. Neither LS nor diazoxide altered this result (indicating the lack of either a direct cytoprotective- or injury-provoking effect).

GCM STUDIES. GCM induced dose-dependent cytotoxicity (Fig. 7) when added to oxygenated tubules (control tubules, 10% LDH release; 250 and 500 \mu M GCM, 17 and 33% LDH release, respectively; $P < 0.025$ vs. controls). At the 250 \mu M dosage, GCM decreased the extent of hypoxia-induced cell death. Despite the fact that 500 \mu M GCM induced marked cytotoxicity (33% LDH release), it still caused a statistically significant reduction in H/R-induced LDH release. Thus a corollary of GCM-mediated protection against H/R injury was an independent cytotoxic effect.

After control incubations were completed, tubule NEFA concentrations were $11 \pm 2$ nmol/mg protein. Incubating with 500 \mu M GCM nearly doubled tubule NEFA levels (19 $\pm$ 4 nmol/mg protein; $P < 0.025$). Hence, a corollary of GCM toxicity, assessed as %LDH release, was a marked increase in NEFA concentrations.

TEA STUDIES. Addition of TEA did not alter tubule viability under oxygenated conditions ($12 \pm 1, 11 \pm 2,$ and $10 \pm 2\%$).
LDH release with 0, 5, and 10 mM TEA, respectively). H/R induced 44 ± 3% LDH release. Inclusion of TEA did not confer a cytoprotective effect (43 ± 2 and 46 ± 1% LDH release with 5 and 10 mM TEA, respectively).

In vivo ischemic ARF. The 12.5-min ischemic challenge induced approximately twofold increases in BUN and PCr levels over normal values (Fig. 8). With 20 min of ischemia, more profound azotemia resulted (~5-fold increase). LS did not substantially alter the degree of azotemia with either the 12.5- or 20-min ischemic insults.

LS effects on MC contraction. Within 30 min of ANG II addition, 70 ± 4% MC contraction was apparent in control cells. In the presence of LS vehicle, 56 ± 7% contraction was observed. Conversely, 0 ± 0% of cells contracted when ANG II was added in the presence of either 100 or 500 ng/ml LS. As shown in Fig. 9, ANG II-induced MC contraction caused...
wringling of the silicone matrix. This did not occur in the presence of 100 or 500 ng/ml LS. As shown in Fig. 10, within 5 min of the LS addition, and before ANG II addition, LS induced a marked spread, or “relaxed” MC phenotype (compared with their normal “straplike” shape). Thus LS directly induced MC relaxation both under unchallenged conditions and in the presence of ANG II.

DISCUSSION

The results of the present studies indicate that LS can confer substantial protection against a rodent model of endotoxemic ARF. This conclusion is based on ~75% reductions in BUN and PCr concentrations in LS-treated endotoxemic mice compared with their placebo-matched, LPS-challenged controls. Notably, the employed LS treatment protocol when administered to normal mice failed to lower either BUN or PCr concentrations. Thus the functional benefit imparted against endotoxemia does not simply reflect a nonspecific “glomerular filtration rate (GFR) enhancing” effect.

Endotoxemic ARF is predominantly, if not exclusively, hemodynamic in nature (5, 19, 34). This conclusion is based on both prior (17, 51, 55) and present observations that endotoxic mice develop substantial azotemia in the absence of overt tubular injury or glomerular thromboses. Without discernible histological damage in the employed endotoxemia model, it is reasonable to conclude that LS-induced protection arose from either 1) a reduction in the LPS-mediated inflammatory response or 2) a blunting of its secondary renal hemodynamic consequences. To test for the first possibility, we measured plasma TNF-α and MCP-1 concentrations at 2 h after LPS injection, a time corresponding to the approximate apogee of LPS-induced cytokine increases. As predicted, LPS evoked massive plasma TNF-α and MCP-1 elevations; however, LS failed to decrease these levels. To more directly test for potential LS-mediated reductions in LPS activity in target organs, we assessed cardiac and renal TNF-α/MCP-1 mRNAs. However, LS failed to blunt the LPS-induced TNF-α/MCP-1 mRNA increases. Finally, given that NO is both a biomarker of LPS toxicity and a critical mediator of the resulting hemodynamic changes, we tested whether LS might reduce LPS-mediated NO production. However, this did not occur. Thus, when these sets of data are interpreted together, they strongly imply that LS did not protect against endotoxemic ARF by decreasing LPS-mediated inflammation; rather, LS appeared to
protect the kidney from the “downstream” consequences of that inflammatory response.

It is noteworthy that LPS administration to mice, even in doses of up to 10 mg/kg, has either no effect or a minimal effect on arterial blood pressure (5, 17, 19). Thus LPS-induced GFR decrements are not due to reductions in renal perfusion pressure; rather, intrarenal vasoconstriction (5, 19) and possible decreases in $K_f$ (approaching $\sim 50\%$ of control values; Ref. 19) are involved. Given that KATP channel openers are potent vasodilators, it seems reasonable to postulate that the LS-mediated protection against LPS-induced ARF likely arose from reductions in renal vascular resistance. Because of technical limitations within this laboratory, we were unable to make direct renal hemodynamic assessments in these experi-

Fig. 8. BUN and PCr concentrations at 24 h after moderate (12.5 min) or severe (20 min) ischemic ARF. LS treatment did not worsen the severity of postischemic ARF with either ischemic challenge. At 24 h after 12.5 min of ischemia, a trivial, albeit statistically significant ($P < 0.05$) reduction in PCr was noted with LS treatment.

Fig. 9. Digital images of rat mesangial cells (MCs) exposed to vehicle or LS (100 or 500 ng/ml) before (Pre) or after (25 min or 1 h) addition of angiotensin II (ANG II). In the presence of vehicle, ANG II caused dramatic MC contraction, as evidenced by wrinkling of the paraffin matrix upon which the cells were cultured. Both low- and high-dose LS completely blocked this contractile response.
ments. However, because renal vasoconstriction during sepsis is mediated, at least in part, by ANG II (7, 34), we did pursue an alternative hypothesis: LS might block ANG II-mediated MC contraction, an action that should increase glomerular capillary surface area and, hence, raise both Kf and GFR. Indeed, LS treatment completely blocked ANG II-mediated MC contraction at doses that are well within a pharmacologically relevant range (100 and 500 ng/ml). Furthermore, even in the absence of ANG II, LS exerted a MC “relaxant” effect (converting the MC phenotype from a typical straplike appearance to a more spread appearance). These observations suggest a novel potential action by which LS might confer functional protection against hemodynamic, or prerenal, forms of ARF: relaxation of a mesangial cell contractile state. Given that LS was able to prevent ANG II-mediated MC contraction, it seems plausible that it also can mitigate afferent and/or efferent arteriolar contractile states. By so doing, it could thus abrogate the major cause of sepsis-mediated renal insufficiency: a microvascular vasoconstrictor state. Finally, in additional experiments (not shown), we demonstrated that diazoxide, a second KATP channel opener, also blocked ANG II-mediated MC contraction. This indicates that it is, indeed, LS’s KATP channel opening action (compared with its calcium-sensitizing or phosphodiesterase-inhibiting effects; Ref. 22–24) that is responsible for its blockade of ANG II’s constrictor effects.

The final goal of this study was to test whether LS alters the expression of hypoxic or ischemic tubular cell death. As previously noted, although KATP channel agonists have been reported to protect the myocardium from ATP depletion injury (6, 18, 47, 59), the literature suggests that, in kidney, the opposite may be the case. For example, renal tubular KATP channels antagonists (GCM, TEA) have been reported to exert substantial protection against hypoxic and ischemic tubular cell death (10, 27, 29). That antagonists are protective suggests a possible corollary: agonists could exert an injury-promoting effect. However, the available data suggest that this is not the case: LS did not worsen either mild (AA or DG) or severe (AA + DG) ATP depletion-mediated HK-2 cell death. Furthermore, LS did not exacerbate either type of hypoxic injury in isolated mouse tubules or in two models of in vivo ischemic ARF. In composite, these in vitro and in vivo observations make a strong case for the concept that LS does not predispose renal tubular cells to ischemic damage, e.g., as might develop during hypoperfusion states.

To further explore potential KATP channel effects on proximal tubular cell injury, we assessed the impacts of a second channel agonist (diazoxide) and two channel antagonists (GCM, TEA) on hypoxic proximal tubular injury. These experiments are noteworthy in the following respects. First, diazoxide failed to worsen hypoxia-mediated proximal tubular cell death. This finding is consistent with the LS results reported, strengthening the concept that KATP channel blockers are not necessarily deleterious to the evolution of hypoxic renal damage. Second, TEA failed to protect against hypoxic cell injury, further arguing that KATP channels are not critical determinants of ATP depletion-induced tubular cell death. In contrast, GCM did mitigate hypoxic tubular injury, as previously reported in the literature (29). However, it is important to note that GCM also exerted an independent cytotoxic action, raising LDH release under oxygenated conditions from a baseline of 10% to ~17 and 37% with 250 and 500 μM exposure, respectively. At first glance, these results appear paradoxical: a drug caused independent cytotoxicity and yet still conferred protection against hypoxic death. However, this “paradox” may simply be explained by the recent observation that GCM is a potent inhibitor of proximal tubular mitochondrial respiration (9), a process that can increase tubular cell free fatty acid (FFA) content (50, 58). Indeed, the present study demonstrates that GCM approximately doubled tubule FFA levels. It is noteworthy that FFAs, either when added to isolated tubules or when generated by phospholipase A2, confer marked protection against hypoxic tubular cell death (49, 50, 57). Thus it appears likely that GCM-mediated protection likely arises from the induction of cell injury/fatty acid increases and not from KATP channel antagonism, per se. That TEA induced no direct toxicity and conferred no protection against H/R injury supports this concept.

In conclusion, the results of the present study demonstrate that LS can confer substantial protection against experimental endotoxemic ARF. Given the fact that LS did not blunt LPS-mediated inflammation and that endotoxin induces a hemodynamic form of ARF, it is assumed that LS-mediated protection was hemodynamic in nature, presumably arising from its cardiac ionotropic/renal vasodilatory actions. The current studies also suggest a novel pathway by which LS might improve renal function during endotoxia: prevention of ANG II-mediated MC contraction, which theoretically should increase Kf and, hence, GFR. Finally, this study offers new insights into the influence of KATP channels on the evolution of hypoxic or ischemic tubular cell death. The data suggest that 1) KATP channel agonists do not directly exacerbate ATP depletion/reoxygenation injury (suggesting that a modicum of safety exists for LS if used in severe renal hypoperfusion states; e.g., sepsis, heart failure); and 2) KATP channel antagonists do not,
as a class of agents, protect against hypoxic tubular cell death. Although GCM may confer some protection against hypoxic tubular injury, this would seem to arise from non-K_{ATP} channel-specific effects. Given these observations and the dearth of effective agents for treating endotoxemic ARF, further exploration of LS as a possible renal protective agent in sepsis syndrome appears warranted at this time. Glomerular micropuncture and intrarenal hemodynamic studies could be particularly illuminating in this regard.

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