Cyclosporin A Causes a Hypermetabolic State and Hypoxia in the Liver: Prevention by Dietary Glycine

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ABSTRACT

Acute cyclosporin A (CsA) treatment inhibits mitochondrial respiration, yet effects of chronic treatment remain unclear. Accordingly, the effects of chronic CsA on oxygen metabolism in perfused rat liver and isolated mitochondria were investigated. Basal rates of oxygen uptake of around 120 μmol/g/h in isolated perfused livers from vehicle-treated controls were elevated about 1.6-fold by chronic CsA treatment. In the presence of ammonium chloride, a substrate for urea synthesis, oxygen uptake was about 150 μmol/g/h and was increased about 1.7-fold by CsA, indicating that chronic CsA treatment causes a robust hypermetabolic state in the liver. In isolated mitochondria, state 3 rates of oxygen uptake were increased about 1.6-fold by chronic CsA treatment. Since significant increases in oxygen consumption could cause hypoxia, the hypoxia marker pimonidazole was given. Pimonidazole binding in the liver was increased about 3-fold by chronic CsA. Moreover, intracellular calcium in Kupffer cells isolated from vehicle-treated rats was not altered by CsA addition; however, in cells isolated from chronic CsA-treated rats, CsA increased intracellular calcium about 15-fold and prostaglandin E2 (PGE2) production 3.5-fold. Importantly, dietary glycine (5%) largely blocked chronic CsA-induced activation of Kupffer cells, blunted production of PGE2, prevented the hypermetabolic state, and minimized tissue hypoxia. Taken together, it is concluded that chronic CsA treatment causes a hypermetabolic state leading to hypoxia and injury to the liver. It is hypothesized that CsA activates Kupffer cells and increases production of PGE2, which alters mitochondria leading to a hypermetabolic state. Glycine inhibits activation of Kupffer cells thus preventing liver injury.

Cyclosporin A (CsA), a fungal cyclic polypeptide, is widely used clinically as an immunosuppressive agent (Borel et al., 1976; Margreiter et al., 1983). CsA significantly improves graft survival following renal, cardiac, pancreatic, bone marrow, and hepatic transplantation; however, recipients have to maintain therapy for the rest of their lives. In addition, this drug is used in the treatment of a variety of autoimmune diseases such as idiopathic nephritic syndrome, inflammatory bowel disease, psoriasis, and rheumatoid arthritis (Berg et al., 1986).

Unfortunately, CsA has a number of side effects, including renal, hepatic, cardiovascular, alimentary, skin, and neural toxicity (Farthing and Clark, 1981; Sibley et al., 1983). Kidney damage is the most frequent toxicity observed, although, hepatic injury also limits the clinical application of CsA, especially after liver transplantation. For example, a study in Hannover showed that 4/20 cases of hepatic dysfunction following liver transplantation were caused by CsA (Muraca et al., 1993). Hepatotoxicity of CsA is characterized by cholestasis, hyperbilirubinemia, hyperproteinemia, increased alkaline phosphatase, elevated transaminases and bile salts in the blood, inhibition of protein synthesis, and disturbed lipid secretion in both man and animals (Lorber et al., 1987; Whiting and Thomson, 1989; Muraca et al., 1993; Hillebrand et al., 1999). Light microscopic and electron microscopic alterations such as dilatation of the endoplasmic reticulum, loss of ribosomes, centrilobular fatty infiltration, and focal hepatocyte necrosis have been observed in livers from CsA-treated animals (Farthing and Clark, 1981; Ryffel et al., 1983). Inhibition of ATP-dependent bile salt export carrier in the canalicul membrane and the P-glycoprotein transporter is probably involved in cholestasis caused by CsA (Bohme et al., 1994; Thalhammer et al., 1994); however, mechanisms by which CsA causes hepatic injury are still not clear.

Previous studies showed that CsA significantly inhibits respiration in mitochondria isolated from the liver and kidney (Jung and Pergande, 1985; Fournier et al., 1999), and giant mitochondria have been observed after chronic CsA treatment (Mihatsch et al., 1981). Inhibition of oxidative phosphorylation could theoretically reduce energy supply

ABBREVIATIONS: CsA, cyclosporin A; HBSS, Hank's balanced salt solution; fura-2, 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxyl]-2-(2′-amino-5′-methylphenoxy)-ethane-N,N,N′,N′′-tetaacetic acid; PGE2, prostaglandin E2; [Ca2+]i, intracellular Ca2+; ANOVA, analysis of variance.
thus causing cell damage, but the effects of CsA on oxygen metabolism in intact cells are not clear. Therefore, the purpose of this study was to investigate the effects of CsA on oxygen metabolism using a rat liver perfusion model in which all cell types are present and lobular architecture is maintained mimicking the physiological situation, yet blood-borne hormones are eliminated. Since glycine is known to protect hepatocytes from hypoxic injury (Zhong et al., 1996), its effects on CsA hepatotoxicity were also explored.

Materials and Methods

Animals. Male Sprague-Dawley rats weighing 199 ± 4 g (mean ± S.E.M.) were fed semisynthetic powdered diets (Harlan Teklad Co., Madison, WI) starting 3 days prior to CsA treatment. The control diet was AIN-76A + 5% casein, and the glycine diet was AIN-76A + 5% glycine. Rats were given either CsA (25 mg/kg) dissolved in a vehicle (olive oil containing 1.25% dehydrated alcohol) resulting in a final concentration of 10 mg of CsA/ml or an equivalent volume of vehicle alone daily by oral gavage for 4 weeks. Higher doses of CsA are needed in rats than humans due to lower sensitivity (Farthing et al., 1966). Isolated mitochondria were incubated in a buffer containing 100 mM KCl, 50 mM sucrose, 20 mM Tris-HCl, and 5 mM MgCl2. Protein levels in tissue homogenates were measured with the bicinchoninic acid assay using a commercially available kit.

Liver Perfusion and Urea Synthesis. After 4 weeks of CsA treatment, animals were anesthetized with pentobarbital sodium (50 mg/kg), and livers were removed surgically and perfused via a cannula inserted into the portal vein with Krebs-Henseleit bicarbonate buffer (pH 7.4, 37°C) saturated with an oxygen/carbon dioxide (95:5) mixture in a nonrecirculating system (Thurman and Scholz, 1969). Perfusion flow rate was 4 ml/g/min, and this rate was kept constant during the whole procedure using a precision perfusion pump. Oxygen consumption in the effluent perfusate was monitored continuously with a Teflon-shielded, Clark-type oxygen electrode. Oxygen uptake was calculated from the difference between influent and effluent oxygen concentrations, the flow rate, and the liver wet weight.

To evaluate urea synthesis, NH4Cl (3 mM), lactate (2.0 mM), ornithine (5.0 mM), and methionine sulfoximine (0.15 mM) were infused into the liver for 20 min. Effluent perfusate was collected at 2- to 3-min intervals, and urea in the perfusate was measured using a Sigma kit (Sigma, St. Louis, MO). Urea synthesis was calculated from the concentration of urea in the effluent perfusate, the flow rate, and the liver wet weight.

Clinical Chemistry. On the day of sacrifice, blood samples were taken from the vena cava, and serum was kept at −20°C until analysis. Alkaline phosphatase and aspartate transaminase were measured using analytical kits from Sigma. Total bilirubin was determined in sera by direct spectrophotometry at 454 nm.

Mitochondrial Respiration. Mitochondria were isolated from livers by standard procedures of differential centrifugation (Remmer et al., 1966). Isolated mitochondria were incubated in a buffer containing 100 mM KCl, 50 mM sucrose, 20 mM Tris-HCl, and 5 mM Tris-phosphate. Oxygen uptake was measured in a closed vessel (2.0 ml) with a Clark-type oxygen electrode after addition of succinate (2.5 mM) and rotenone (10 μM) or glutamate (2.5 mM) and malate (2.5 mM). To determine state 3 rates of respiration, ADP (0.25 mM) and rotenone (10 μM) or glutamate (2.5 mM) and malate (2.5 mM) were added. Mitochondrial protein was determined using the method of Lowry et al. (1951).

Assessment for Hypoxia in the Liver. Pimonidazole, a 2-nitroimidazolyl compound (hypoxpyrobe-1; NPI, Inc., Belmont, MA), is reductively activated at low oxygen concentrations and binds to cell molecules that possess free thiol groups. Pimonidazole adducts accumulate in vivo in intact awake animals and represent tissue hypoxia directly at the cellular level (Durand and Raleigh, 1998). Pimonidazole hydrochloride was dissolved in normal saline at a concentration of 120 mg/ml and injected into the tail vein (120 mg/kg) 24 h after the last dose of CsA. Livers were perfused with Krebs-Henseleit bicarbonate buffer (pH 7.4, 37°C) 2 h later to remove blood, and pimonidazole binding was determined in liver homogenates using a competitive enzyme-linked immunosorbent assay (ELISA) procedure described elsewhere (Durand and Raleigh, 1998). Protein levels in tissue homogenates were measured with the bicinchoninic acid assay using a commercially available kit.

Protein-bound pimonidazole in liver sections was also determined immunohistochemically. Paraffin blocks of formalin-fixed liver tissue were sectioned at 6 μm, and pimonidazole adducts were detected with a biotin-streptavidin-peroxidase indirect immunostaining method (Durand and Raleigh, 1998). Sections were hydrated and treated briefly with 0.01% protease (pronase E) and exposed to mouse monoclonal antipimonidazole IgG antibody (batch number 4.3.11.3) in phosphate-buffered saline-Tween for 30 min at room temperature. Rat adsorbed horse anti-mouse antibody was then applied to the sections for 10 min. Once the antibody-biotin-peroxidase complex was formed, 3,3′-diaminobenzidine chromogen was added as the peroxidase substrate. After the immunostaining procedure was completed, a counterstain of hematoxylin was applied followed by mounting with crystal mount solution. Some slides were stained with hematoxylin and eosin.

Kupffer Cell Preparation and Culture. Kupffer cells were isolated by collagenase digestion and differential centrifugation using Percoll (Amersham Pharmacia Biotech AB, Uppsala, Sweden) 24 h after the last dose of CsA. The liver was perfused through the portal vein with Ca2+- and Mg2+-free Hanks' balanced salt solution (HBSS) at 37°C for 5 min at a flow rate of 26 ml/min. Subsequently, perfusion was with HBSS containing 0.025% collagenase IV (Sigma) at 37°C for 5 min. After the liver was digested, it was excised and cut into small pieces in collagenase buffer. The suspension was filtered through nylon gauze mesh, and the filtrate was centrifuged at 450g for 10 min at 4°C. Cell pellets were resuspended in buffer, parenchymal cells were removed by centrifugation at 50g for 3 min, and the nonparenchymal cell fraction was washed twice with buffer. Cells were centrifuged on a density cushion of Percoll at 1000g for 15 min, and the Kupffer cell fraction was collected and washed with buffer again. Viability of cells determined by trypan blue exclusion was >90%. Cells were seeded onto 25-mm glass coverslips in 2 ml of RPMI 1640 (Invitrogen, Grand Island, NY) with 10% fetal bovine serum, 10 mM HEPES, and antibiotics (100 U/ml penicillin G and 100 μg/ml streptomycin sulfate) at 37°C with 5% CO2. Nonadherent cells were removed after 1 h by replacing buffer, and Kupffer cells were cultured for 24 h prior to measurement of intracellular calcium or PGE2.

Measurement of Intracellular Ca2+ ([Ca2+]i). Intracellular calcium was measured fluorometrically using the fluorescent calcium indicator dye fura-2 and a microspectrofluorometer (InCyt Im2 imaging system; Intracellular Imaging, Inc., Cincinnati, OH). Kupffer cells were incubated in modified HBSS (115 mM NaCl, 5 mM KCl, 0.3 mM Na2HPO4, 0.4 mM KH2PO4, 5.6 mM glucose, 0.8 mM MgSO4, 1.26 mM CaCl2, 15 mM HEPES, pH 7.4) containing 5 μM fura-2-acetoxymethyl ester (Molecular Probes Inc., Eugene, OR) at room temperature for 60 min. Coverslips plated with Kupffer cells were rinsed and placed in chambers with buffer at room temperature and stimulated with 1 μg/ml CsA. Changes in fluorescence intensity of fura-2 at excitation wavelengths of 340 nm and 380 nm and emission at 510 nm were monitored in individual Kupffer cells for 24 h prior to measurement of intracellular calcium or PGE2.

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wavelengths of 340 nm and 380 nm, and \( R_{\text{max}} \) and \( R_{\text{min}} \) are values of \( R \) at \([\text{Ca}^2+]_{\text{max}}\) and \([\text{Ca}^2+]_{\text{min}}\), respectively. The values of these constants were determined at the end of each experiment, and a dissociation constant of 135 nM was used (Grynkiewicz et al., 1985).

**Measurement of PGE2 in Conditioned Media from Cultured Kupffer Cells.** Kupffer cells isolated from rats fed a control or glycine-containing diet were kept in primary culture. Twenty-four hours later, cells were exposed to CsA (1 μg/ml) or an equal volume of vehicle (1 μl of dimethyl sulfoxide) for 4 h, and conditioned media were collected. PGE2 was analyzed by competitive radioimmunoassay using 125I-labeled PGE2 from Advanced Magnetics (Cambridge, MA) (Qu et al., 1996).

**Statistical Analysis.** ANOVA plus Student-Newman-Keuls test was used. Differences were considered significant at the \( p < 0.05 \) level.

## Results

**Body Weight Gain and Food Consumption.** Average food consumption in all groups studied was around 6 to 7 g/100 g body weight/day (data not shown). In rats fed the control diet, body weight increased from about 200 to 300 g over the 4 weeks of study. However, body weight gain was ~40% less in the glycine diet + vehicle, control diet + CsA, and glycine diet + CsA groups (data not shown). Reasons for these differences in weight gain are not currently understood.

**Oxygen Uptake and Urea Synthesis.** Typical liver perfusion experiments are shown in Fig. 1. The ratio of liver weight/body weight was not statistically or significantly different among the control groups, CsA-treated group, and CsA + glycine diet group. Basal oxygen uptake in rats fed the control diet and the vehicle was around 120 μmol/g/h; upon infusion of NH4Cl, a substrate for urea synthesis, values increased rapidly to about 150 μmol/g/h in about 2 min (Fig. 1A). CsA treatment for 4 weeks increased oxygen uptake by about 1.6-fold in the presence and absence of NH4Cl (Fig. 2A and B). Thus, chronic treatment with CsA causes a hypermetabolic state in the liver. Moreover, increases in oxygen uptake caused by CsA both in the presence and absence of NH4Cl were largely blocked by glycine (Fig. 2A and B).

Urea synthesis was barely detectable under basal conditions (Fig. 1B) but increased gradually to new steady state values about 10 min after NH4Cl infusion was begun (Fig. 1B). Maximal urea synthesis during NH4Cl infusion was about 125 μmol/g/h in livers from rats fed either control or glycine-containing diets (Fig. 2C). Chronic CsA treatment increased urea synthesis 1.3-fold in rats fed a control diet, an effect blocked totally by dietary glycine (Fig. 2C).

**Oxygen Uptake in Liver Mitochondria.** Hepatic oxygen uptake in the perfused liver is due almost entirely to mitochondria in the perfused liver (Thurman and Scholz, 1969). Since chronic CsA caused a hypermetabolic state, the effect of CsA on respiration of isolated mitochondrial was examined. When succinate was used as the substrate, state 3 rates of oxygen uptake by mitochondria from rats fed a control diet and the vehicle were around 80 nmol/min/mg of protein, and the respiratory control ratio (state 3/state 4) was around 5. Previous studies showed that acute treatment with CsA inhibits mitochondrial respiration (Jung and Pergande, 1985). Consistent with these reports, incubation of mitochondria isolated from untreated rats with CsA in vitro significantly decreased state 3 rates of oxygen uptake by about 70% (data not shown). However, after 4 weeks of treatment with CsA in vivo, basal state 3 respiration was increased to around 125 nmol/min/mg of protein (Fig. 3). In contrast, respiration was not increased in mitochondria from rats fed dietary glycine and exposed to chronic CsA treatment. Interestingly, when glutamate/malate was used as the substrate, rates of mitochondrial respiration were not significantly different in rats receiving CsA or vehicle (data not shown), indicating that complex I of the respiratory chain is not involved.

**Hypoxia in the Liver.** Figure 4 depicts representative images of pimonidazole adducts detected immunohistochemically. In livers from vehicle-treated controls and from rats fed glycine and the vehicle (Fig. 4, upper and lower left panels), pimonidazole adducts accumulated slightly in pericentral regions of the liver lobule due to the natural low oxygen tension.
of these areas physiologically. Chronic treatment with CsA extended pimonidazole binding from pericentral regions to periportal areas (Fig. 4, upper right panel). Importantly, dietary glycine prevented the increase caused by CsA almost completely (Fig. 4, lower right panel).

While immunohistochemistry detects predominantly protein-bound adducts, quantitation of pimonidazole binding with ELISA detects both protein and nonprotein adducts.
Levels of pimonidazole adduct formation in control livers were 173 ± 20 pmol/mg of protein (data not shown); glycine diet alone did not alter this background value. In contrast, CsA increased pimonidazole binding about 2.6-fold, effects which were prevented largely with dietary glycine (data not shown).

Liver Damage. It is known that CsA causes hyperbilirubinemia (Muraca et al., 1993). Indeed, serum bilirubin values ranged from 0.79 to 0.85 mg/dl in rats fed either control or glycine diets (data not shown) but were increased ~2.5-fold by CsA treatment. Dietary glycine largely blocked hyperbilirubinemia caused by CsA (data not shown). Alkaline phosphatase tended to be elevated in the CsA group, an effect also blocked by glycine, but CsA treatment did not significantly alter blood transaminase levels (data not shown). Chronic CsA treatment caused widespread cell swelling in the liver (Fig. 5), and dietary glycine largely blocked this effect (Fig. 5).

Effects of Cyclosporin A and Glycine on Intracellular Calcium Levels and Prostaglandin E2 Production by Isolated Kupffer Cells. Previous studies showed that activation of Kupffer cells, the resident macrophages in the liver, leads to prostaglandin release, which increases mitochondrial respiration (Qu et al., 1996); therefore, [Ca^{2+}]_i was measured to investigate if chronic CsA activates Kupffer cells. CsA (1 μg/ml) did not increase [Ca^{2+}]_i in cells from rats treated with olive oil vehicle (Fig. 6A); however, it increased [Ca^{2+}]_i to values over 100 nM in cells from rats treated chronically with CsA. These data indicate that chronic CsA treatment sensitizes Kupffer cells to CsA. Dietary glycine (Fig. 6B) or addition of glycine (1 mM) to buffer 3 min prior to exposure to CsA (1 μg/ml) also blocked the increases in [Ca^{2+}]_i caused by CsA (Fig. 7A). In calcium-free buffer, CsA (1 μg/ml) did not increase [Ca^{2+}]_i in cells from rats treated chronically with CsA (Fig. 7B), indicating that this increase in [Ca^{2+}]_i depends on the presence of extracellular calcium. Basal concentrations of PGE2 in conditioned media were not different in all groups studied (data not show). Addition of CsA (1 μg/ml) did not significantly alter PGE2 in cultured Kupffer cells from rats that were fed control diet and given olive oil vehicle (Fig. 8) but dramatically increased it to about 3320 ng/ml in cells from rats given chronic CsA treatment (Fig. 8). Consistent with data presented above, increases in production of PGE2 were largely blocked by glycine diet (Fig. 8).

Discussion

Chronic Cyclosporin A Causes a Hypermetabolic State and Hypoxia in the Liver. While kidney injury is the most frequent toxicity of CsA, hepatic injury also limits its clinical application. A previous study showed that ~25% of hepatic dysfunction following liver transplantation was caused by CsA toxicity (Muraca et al., 1993). Hepatotoxicity of CsA includes cholestasis, increased enzyme release, hypoproteinemia, disturbed lipid secretion, and pathological changes such as dilatation of the endoplasmic reticulum, loss of ribosomes, centrilobular fatty infiltration, and focal hepa-
tocyte necrosis (Farthing and Clark, 1981; Ryffel et al., 1983; Lorber et al., 1987; Hillebrand et al., 1999). Mechanisms by which CsA causes hepatic injury are not clearly understood; however, it has been shown to inhibit ATP-dependent transporters and P-glycoprotein resulting in cholestasis (Bohme et al., 1994; Thalhammer et al., 1994). Long-term CsA treatment decreased glutathione (Galan et al., 1999), which could also impair bile acid-independent bile flow. In addition, CsA inhibits biliary excretion of bilirubin via the multiple organic anion transport system (Roman et al., 1990), making cholestasis and hyperbilirubinemia frequent manifestations of CsA hepatotoxicity. Acute CsA also inhibits respiration of isolated mitochondria (Fournier et al., 1999), which could be involved in its hepatotoxicity. In the present study, chronic CsA paradoxically increased oxygen consumption in isolated perfused liver (Figs. 1 and 2). Synthesis of urea, a process highly dependent on ATP supply, was also elevated after chronic CsA (Figs. 1 and 2), indicating that increases in oxygen consumption are not due to uncoupling of oxidative phosphorylation. High rates of oxygen consumption could theoretically cause hypoxia leading to liver injury. Indeed, binding of pimonidazole, a 2-nitroimidazole hypoxia marker, was increased about 3-fold by chronic treatment with CsA (Fig. 4). In livers from vehicle-treated controls, pimonidazole adducts accumulated minimally in oxygen-poor pericentral regions of the liver lobule; however, chronic treatment with CsA increased pimonidazole binding significantly (Fig. 4). Taken together, these data are consistent with the hypothesis that chronic CsA causes a hypermetabolic state leading to hypoxia in the liver, which may be responsible, at least in part, for its hepatotoxicity.

Fig. 5. Effects of CsA and glycine on hepatic histology. Conditions as in Fig. 1. Livers were perfused briefly with Krebs-Henseleit bicarbonate buffer to remove blood, fixed with 1% paraformaldehyde, and sections were stained with hematoxylin and eosin. Original magnification, 100×. Representative images in each treatment group (n = 4 in each group): upper left panel, control diet + vehicle; lower left panel, glycine diet + vehicle; upper right panel, control diet + CsA; and lower right panel, glycine diet + CsA.

Role of Kupffer Cells. It is well known that acute CsA inhibits mitochondrial respiration (Jung and Pergande, 1985; Fournier et al., 1999). Paradoxically, chronic CsA causes a hypermetabolic state in the liver (Figs. 1 and 2) due to increases in mitochondrial respiration (Fig. 3). Consistent with this observation, a previous study also showed that chronic CsA increased oxygen consumption in mitochondria...
from renal cells (Lemmi et al., 1989). How chronic CsA increases mitochondrial respiration is unclear. One possibility is that CsA increases components of the respiration chain, although, this effect is not due to changes in complex I since mitochondrial respiration was not altered when glutamate/malate was used as the substrate. In contrast, when succinate was used as the substrate, higher rates of respiration occurred, indicating that complex II is likely increased (Fig. 3).

Alternatively, CsA might increase mitochondria respiration indirectly. It is well known that activation of Kupffer cells is responsible for increased oxygen consumption due to ethanol, endotoxin, peroxisome proliferators, and hypoxia/reoxygenation (Casteleijn et al., 1988; Bradford et al., 1993; Bojes and Thurman, 1996; Rivera et al., 1998), and destruction of Kupffer cells blunts the hypermetabolic effects of these agents (Marsman et al., 1992; Rivera et al., 1998). Moreover, Kupffer cells are the major hepatic source of eicosanoids, which increase cell respiration (Dieter et al., 1987; Altin and Bygrave, 1988; Casteleijn et al., 1988). For example, it was shown that eicosanoids play an important role in stimulating hepatic metabolism by endotoxin (Rivera et al., 1998), and prostaglandin E2 is involved in the hepatic hypermetabolic state caused by ethanol treatment (Qu et al., 1996). Prostaglandin E2 stimulates oxygen consumption by increasing cAMP and activating a cAMP-dependent protein kinase (Qu et al., 1999). Here, CsA only increases intracellular calcium and PGE2 production in Kupffer cells from rats treated with CsA chronically but not in cells from control rats (Figs. 6 and 8). This is consistent with the hypothesis that CsA sensitizes Kupffer cells, which then become activated upon reexposure to CsA (Fig. 6), resulting in overproduction of
prostaglandins (Fig. 8) that cause a hypermetabolic state. It is also possible that chronic overproduction of PGE₂ increases components of the mitochondrial respiration chain.

In support of this hypothesis, a previous study from this laboratory showed that PGE₂ treatment caused mitochondria enlargement (W. Qu and R. G. Thurman, unpublished data), and it is known that swelling of mitochondria stimulates respiration (Armenton et al., 1982). How CsA sensitizes data), and it is known that swelling of mitochondria stimulates respiration (Armenton et al., 1982). How CsA sensitizes Kupffer cells remains unknown.

**Glycine Prevents Hepatotoxicity Caused by Cyclosporin A.** Chronic CsA causes hypoxia, which may lead to injury and dysfunction of the liver. Dietary glycine prevented the hypermetabolic state by blocking activation of Kupffer cells and production of PGE₂. Consistent with this hypothesis, intracellular calcium and PGE₂ did not increase in Kupffer cells from rats fed CsA and a glycine-containing diet after acute in vitro addition of CsA (Figs. 6 and 8). In the central nervous system, glycine activates an anion channel that increases chloride influx (Ito and Cherubini, 1991) and causes hyperpolarization of the plasma membrane, making calcium channels more difficult to open (Wheeler et al., 1999). Glycine increases intracellular calcium stimulated by lipopolysaccharide in a variety of cells including neutrophils, lymphocytes, and Kupffer cells, most likely by this mechanism (Ikejima et al., 1996; Stachlewitz et al., 1997). In this study, in vitro addition of CsA increases intracellular calcium in Kupffer cells from CsA-pretreated rats, an effect that depends on extracellular calcium (Fig. 7B). Either acute addition or dietary supplementation with glycine blocked this activation of Kupffer cells caused by CsA (Figs. 6B and 7A), supporting the hypothesis that glycine works by activating glycine-gated chloride channels.

Previous studies from this laboratory have shown that glycine, a simple nontoxic amino acid, protects against renal toxicity of CsA (Thurman et al., 1997). In this study, it also prevents hepatotoxicity. Thus, dietary supplementation of patients taking CsA with glycine should be useful to prevent more than one side effect of this widely used immunosuppressive drug.

**References**


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