Original articles

TRANSPLANT International

Transplant Int (1990) 3: 2–7

Effect of cyclosporin A, azathioprine, and prednisolone on carbohydrate metabolism of rat hepatocytes*

W. Riegel[1], E. Stephan[2], C. Ballé[1], A. Heidland[2], and W. H. Hörl[1]

[1] Department of Medicine, Division of Nephrology, University of Freiburg, Hugstetterstrasse 55, D-7800 Freiburg, Federal Republic of Germany
[2] Department of Medicine, University of Würzburg, D-8700 Würzburg, Federal Republic of Germany

Received May 12, 1989/Received after revision October 23, 1989/Accepted November 10, 1989

Abstract. The effect of different immunosuppressive drugs (prednisolone, azathioprine, cyclosporin A) on liver carbohydrate metabolism in the rat was investigated. Daily administration of prednisolone (3 mg/kg body weight) and azathioprine (2 mg/kg body weight) intraperitoneally for 2 weeks caused significantly lower liver glycogen content than that in NaCl-treated controls. Liver glucose and lactate content, as well as plasma glucose, glucagon, and serum insulin concentration of these animals, remained unchanged. There were no differences in any of these parameters between cyclosporin A (15 mg/kg body weight)-treated and vehicle (olive oil/ethanol)-treated animals. Prednisolone caused significantly lower glucose production in isolated rat hepatocytes using Na-pyruvate as the substrate, whereas glucose production was unchanged in hepatocytes of azathioprine-treated rats using pyruvate or L-serine as substrates. Glucose production from pyruvate or serine was significantly inhibited by cyclosporin A compared to the vehicle, but did not differ from the effects of azathioprine and prednisolone. Lactate production was significantly lower in cyclosporin-treated animals than in those given either the vehicle or azathioprine. Cyclosporin A completely reversed the inhibition of hepatocyte glycogen consumption caused by the vehicle. However, glycogen production in the presence of cyclosporin A was comparable to the effects of prednisolone and azathioprine. Finally, hepatocyte ketone body production using pyruvate as the substrate was higher in the presence of all immunosuppressive drugs. In the presence of serine, acetoacetate production increased in rats treated with 50 mg/kg body weight cyclosporin A, and β-hydroxybutyrate production in animals receiving 15 and 50 mg/kg body weight cyclosporin A.

Key words: Cyclosporin A, carbohydrate metabolism, rat hepatocytes – Immunosuppression, carbohydrate metabolism, rat hepatocytes – Carbohydrate metabolism, immunosuppression, rat hepatocytes – Hepatocytes, carbohydrate metabolism, immunosuppression

Immunosuppressive therapy for patients after solid organ transplantation is complicated by the deterioration of glucose tolerance. For example, corticosteroids are known to alter carbohydrate metabolism by acting on hepatic gluconeogenesis, glycogen synthesis, and peripheral glucose metabolism [3]. Corticosteroids are the most reliable causative factor for post-transplant hyperglycemia [2, 10, 22]. Cyclosporin A exerts toxic effects on the liver [27, 33, 36]. Exocrine and endocrine pancreas metabolism are also known to be influenced by therapeutic doses of cyclosporin A [23, 38]. The presence of glucose intolerance following cyclosporin A administration was first suggested by Gunnarsson et al. [14] and then corroborated by numerous groups (for review, see Hörl et al. [18]). Recently, Yoshimura et al. [39] reported that altered carbohydrate metabolism in transplant recipients ameliorates after changing the immunosuppressive regimen from cyclosporin A to azathioprine, despite higher prednisolone doses.

Studies from our laboratory have demonstrated that cyclosporin A, in a dose of 50 mg/kg body weight, inhibits glycogen synthesis in rat liver homogenates [25]. The present study compares liver carbohydrate metabolism in rats treated with prednisolone, azathioprine, or cyclosporin A. The gluconeogenetic ability, lactate, and ketone body production of rat hepatocytes were also investigated under these different immunosuppressive drugs.

Materials and methods

Experimental protocol

Wistar rats with an initial body weight of approximately 200 g were used for the experiment. The animals were housed in metabolic cages with free access to tap water. Rats were randomly allocated to different groups. All treated rats were weight-matched with control animals throughout the study. Body weight of rats treated with immunosuppressive drugs and of controls was determined daily and did not differ significantly throughout the study.

* This article is dedicated to Professor K. Kochsiek, Chief of the Medical Department, University of Würzburg, FRG, on the occasion of his 60th birthday

Offprint requests to: W. Riegel

Animals studied

Group 1. Twenty control rats were injected twice daily with 0.9% sodium chloride (0.1 ml) intraperitoneally. Groups of three animals each were housed in single cages at constant humidity and temperature with a 12-hour light/dark cycle. The rats had free access to tap water and standard rat chow (Altromin, Lippe', Westphalia, FRG). Two weeks after starting the experiments, the animals were sacrificed. Blood was withdrawn from the abdominal aorta of eight rats in order to determine blood and serum parameters as outlined below. The liver was removed from five other rats and quick-frozen in liquid nitrogen. All tissue samples were then crushed in order to prevent postmortem alterations of metabolite concentrations. Hepatocytes were isolated from the remaining seven animals.

Rats in group 1 acted as controls for those in groups 2, 3, and 4. All animals were anesthetized with thiobutabarbital (Inactin, 100 mg/kg; Byk-Gulden, Konstanz, FRG).

Group 2. Twenty-two rats were injected intraperitoneally with prednisolone, 3 mg/kg body weight, daily (Solu-Decortin, Merck, Darmstadt, FRG). Blood was withdrawn from nine of the animals 2 weeks after starting the experiments. The liver was removed from five animals and hepatocytes were isolated from eight others.

Group 3. Twenty rats were injected intraperitoneally with azathioprine, 2 mg/kg body weight, daily (Imurek, Wellcome, Burgwedel, FRG). After 2 weeks the rats were anesthetized. Blood was taken from seven animals, the liver removed from five, and hepatocytes isolated from eight others.

Group 4. Sixteen rats were orally fed 250 µl of the vehicle for cyclosporin A (olive oil containing 12.25 vol% ethanol) twice daily for 2 weeks. Thereafter, the rats were anesthetized. Blood was taken from six rats, the liver removed from five, and hepatocytes isolated from five, as described above. This group acted as a control for group 5.

Group 5. Twenty rats were fed cyclosporin A, 15 mg/kg body weight, in two daily doses (250 µl each) for 2 weeks. The cyclosporin A (Sandoz, Basel, Switzerland) was dissolved in the vehicle. Anesthesia, removal of blood from seven rats, removal of the liver from five, and isolation of hepatocytes from eight were the same as for group 4. Cyclosporin A trough levels in the blood were determined at the time of sacrifice by a commercially available radioimmunoassay (Sandoz, Nürnberg, FRG). The last cyclosporin A dose was administered 12 h before the animals were sacrificed. It has previously been shown that such a dose causes blood levels of 1284 ± 82 ng/ml [25].

In separate experiments, eight rats were fed cyclosporin A, 50 mg/kg body weight, in two daily doses for 2 weeks, causing cyclosporin A blood levels of 4664 ± 276 ng/ml [25]. Hepatocytes were isolated and incubated with serine or alanine as substrates. The results were compared with those obtained from rats treated with 15 mg/kg body weight cyclosporin A or the vehicle.

Hepatocyte isolation and incubation procedure

For isolation of hepatocytes, the liver was perfused with Krebs-Ringer bicarbonate buffer without calcium. Cells were isolated according to the Berry and Friend method [6], using 0.05% collagenase (type IV, Sigma, Munich, FRG) as the digestive agent. Hepatocytes were washed several times after the isolation procedure. Hepatocyte viability was tested using trypan blue and was found to be in excess of 90%.

Hepatocytes were incubated in Krebs-Ringer bicarbonate buffer (pH 7.4) for 30 min at 37° C under a gas mixture of 5% carbon dioxide and 95% oxygen. Sodium pyruvate was used as the main gluconeogenetic substrate and L-serine as the glucoplastic amino acid, in concentrations of 10 m*M* (both from Sigma). The incubation was stopped by adding 30% perchloric acid and the samples were quickly frozen. Measurements were taken in neutralized supernatant.

Analytical methods and measurements

Blood lactate, pyruvate, acetoacetate, and β-hydroxybutyrate levels were measured enzymatically using modified microassays in 500 µl samples of deproteinized (5% perchloric acid) whole blood [4]. The coefficient of variation for these determinations was between 5% and 7%. Serum glucose, urea, and creatinine were determined by routine methods (Hitachi 737, Behring, FRG). Serum insulin levels were measured using a highly sensitive enzyme-immunoassay (Boehringer, Mannheim, FRG). Glucagon was assayed in an aprotinin-EDTA plasma by a radioimmunoassay (Serono, Freiburg, FRG).

For determination of phosphoenolpyruvate-carboxykinase activity, tissue samples were homogenized in a buffer containing 0.25 m*M* saccharose, 25 m*M* Tris/HCl, and 7.4 m*M* $MgCl_2$ (pH 7.4). Enzyme activity was determined in a 100,000 *g* supernatant, as described by Seubert and Huth [30].

Glycogen was measured in liver homogenates [25] and isolated hepatocytes, according to the Bergmeyer method [5], with the following modifications. Acid supernatant was precipitated by 67% (v/v) ethanol. The glycogen pellet was dissolved in 0.5 ml of 50 m*M* acetate (sodium) buffer (pH 4.8), which contained 1 mg/ml amyloglucosidase (50 IU/mg; Merck, Darmstadt, FRG). After 30 min of incubation at 56° C, glucose was assayed enzymatically using glucose dehydrogenase (Merckotest Gluc-DH, Merck, Darmstadt, FRG). Tissue glucose concentration was assayed enzymatically

Table 1. Serum insulin, plasma glucagon, serum glucose, and whole blood metabolic intermediates of rats treated with intraperitoneally injected saline (group 1), 3 mg/kg body weight prednisolone (group 2), or 2 mg/kg body weight azathioprine (group 3), and with orally applied vehicle (olive oil/ethanol; group 4) or 15 mg/kg body weight cyclosporin A (group 5) for 2 weeks. Data are given as mean values ± SEM. *N*, Number of observations

	Control	Prednisolone 3 mg/kg body weight	Azathioprine 2 mg/kg body weight	Vehicle	Cyclosporin A 15 mg/kg body weight
Group	1	2	3	4	5
N	(8)	(9)	(7)	(6)	(7)
Insulin (IU/ml)	10.9 ± 0.7	9.1 ± 0.9	11.0 ± 0.3	8.2 ± 0.3	8.1 ± 0.9
Glucagon (pg/ml)	99 ± 13	92 ± 16	102 ± 11	126 ± 12	95 ± 8
Glucose (mg/dl)	161 ± 10	143 ± 9	157 ± 13	152 ± 4.5	153 ± 4.5
Lactate (mmol/l)	1.9 ± 0.3	2.2 ± 0.5	1.7 ± 0.3	1.7 ± 0.3	1.1 ± 0.2
Pyruvate (µmol/l)	124 ± 28	69 ± 9	76 ± 12	91 ± 8	59 ± 4
β-OH-Butyrate (µmol/l)	131 ± 14	140 ± 13	155 ± 14	197 ± 26	181 ± 17
Acetoacetate (µmol/l)	33 ± 3	45 ± 7	40 ± 3	44 ± 8	46 ± 8

Table 2. Metabolic intermediates and PEP-CK activity in the liver of rats treated with intraperitoneally injected saline (group 1), 3 mg/kg body weight prednisolone (group 2), or 2 mg/kg body weight azathioprine (group 3) and with orally applied vehicle (olive oil/ethanol; group 4) or 15 mg/kg body weight cyclosporin A (group 5) for 2 weeks. Data are given as mean values ± SEM. *N*, Number of observations. * $P < 0.05$, compared with control group

	Control	Prednisolone 3 mg/kg body weight	Azathioprine 2 mg/kg body weight	Vehicle	Cyclosporin A 15 mg/kg body weight
Group	1	2	3	4	5
N	(5)	(5)	(5)	(5)	(5)
Lactate (μg/mg protein)	2.42 ± 0.66	1.99 ± 0.2	2.13 ± 0.44	3.0 ± 0.2	2.8 ± 0.1
Glucose (μg/mg protein)	21.0 ± 7.3	20.1 ± 2.0	24.4 ± 5.9	20.5 ± 1.8	17.9 ± 0.7
Glycogen (μg/mg protein)	114 ± 12	56 ± 8*	55 ± 7*	99 ± 10	95 ± 12
Phosphoenolpyruvate-carboxykinase	8.45 ± 0.67	8.19 ± 3.90	7.66 ± 2.98	6.36 ± 1.80	9.71 ± 1.64
(μmol × min^{-1} × g wt. weight^{-1})					
N	(4)	(3)	(3)	(4)	(3)

using the glucose-oxidase method (Merckotest GOD-PAP, Merck, Darmstadt, FRG). Lactate was measured enzymatically by the method given by Bergmeyer [4].

Statistics

The results are expressed as the mean ± standard error of the mean. An analysis of variance (ANOVA) was performed using a commercially available statistical package from Hewlett Packard. Differences were accepted as statistically significant with $P < 0.05$. In the tables and figures below, these differences are indicated only between groups 2 (prednisolone), 3 (azathioprine), 4 (vehicle) and 1 (control), and between group 5 (cyclosporin A) and 4 (vehicle).

Results

Five different groups of rats treated with saline IP (group 1), prednisolone IP (group 2), azathioprine IP (group 3), vehicle p.o. (group 4), or cyclosporin A p.o. (group 5) were investigated. Serum insulin, plasma glucagon, serum glucose, and whole blood metabolic intermediates were unchanged when prednisolone and azathioprine-treated rats were compared with control animals and cyclosporin-treated rats were compared with rats receiving the vehicle (Table 1). Liver glycogen content of controls (114 ± 12 μg/mg protein) was significantly lower after treatment with prednisolone (56 ± 8) or azathioprine (55 ± 7). No statistically significant differences were observed between vehicle (99 ± 10)-treated and cyclosporin (95 ± 12 μg/mg protein)-treated rats. Hepatic glucose and lactate content, as well as PEP-CK activity, were without significant differences between the groups (Table 2).

Glucose production of isolated rat hepatocytes with pyruvate as the substrate was reduced after treatment with prednisolone ($P < 0.05$; Fig. 1). Azathioprine treatment caused only a nonsignificant decrease in glucose production. In group 4 (olive oil/ethanol), hepatocyte glucose production was enhanced. Treatment with cyclosporin A reduced glucose production markedly using serine ($P < 0.05$) as well as pyruvate ($P < 0.001$) as substrates (Fig. 1). Treatment with the vehicle increased lactate production and cyclosporin A, again, inhibited hepatocyte lactate production (213 ± 27 vs 349 ± 36 nmol × mg protein^{-1} × 30 min^{-1}). Treatment with azathioprine enhanced lactate production in the presence of pyruvate as the substrate (Fig. 2).

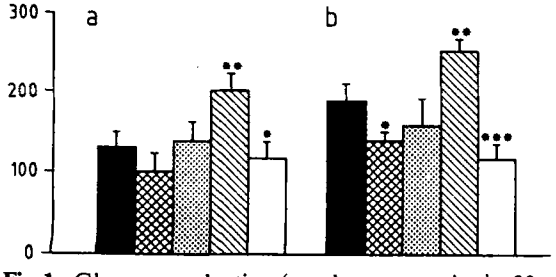

Fig. 1. Glucose production (nmol × mg protein^{-1} × 30 min^{-1}) from L-serine (**a**) or Na-pyruvate (**b**) (each 10 m*M*) of hepatocytes isolated from rats injected IP with saline (group 1) (■) ($n = 7$), 3 mg/kg body weight prednisolone (group 2) (▩) ($n = 8$), or 2 mg/kg body weight azathioprine (group 3) (▩) ($n = 8$) and orally treated with the vehicle (olive oil/ethanol; group 4) (▨) ($n = 5$) or 15 mg/kg body weight cyclosporin A (group 5) (□) ($n = 8$) for 2 weeks. Data are given as mean values ± SEM. * $P < 0.05$, *** $P < 0.001$ (groups 2, 3, and 4 versus group 1; group 5 versus group 4)

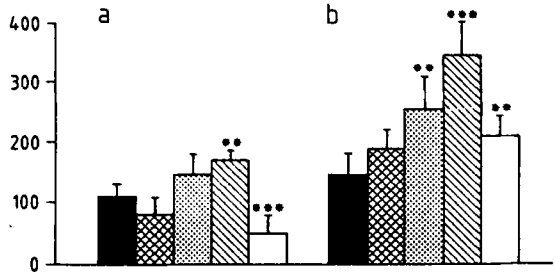

Fig. 2. Lactate production (nmol × mg protein^{-1} × 30 min^{-1}) from L-serine (**a**) or Na-pyruvate (**b**) (each 10 m*M*) of hepatocytes isolated from rats injected IP with saline (group 1; $n = 7$), 3 mg/kg body weight prednisolone (group 2; $n = 8$), or 2 mg/kg body weight azathioprine (group 3; $n = 8$) and orally treated with the vehicle (olive oil/ethanol; group 4; $n = 5$) or 15 mg/kg body weight cyclosporin A (group 5; $n = 8$) for 2 weeks. Data are given as mean values ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (groups 2, 3, and 4 versus group 1; group 5 versus group 4). Symbols as for Fig. 1

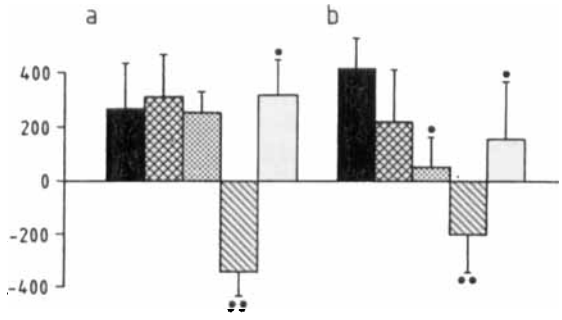

Fig. 3. Hepatocyte glycogen (nmol glucose equivalents × mg protein^{-1} × 30 min^{-1}) in the presence of L-serine (**a**) or Na-pyruvate (**b**) (each 10 m*M*) of rats injected IP with saline (group 1; $n = 7$), 3 mg/kg body weight prednisolone (group 2; $n = 8$), or 2 mg/kg body weight azathioprine (group 3; $n = 8$) and with orally applied vehicle (olive oil/ethanol; group 4; $n = 5$) or 15 mg/kg body weight cyclosporin A (group 5; $n = 8$) for 2 weeks. Data are given as mean values ± SEM. * $P < 0.05$ (groups 2, 3, and 4 versus group 1; group 5 versus group 4). Symbols as for Fig. 1

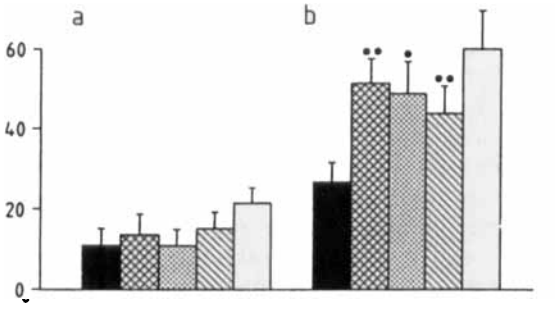

Fig. 4. Hepatocyte acetoacetate production (nmol × mg protein^{-1} × 30 min^{-1}) from L-serine (**a**) or Na-pyruvate (**b**) (each 10 m*M*) of rats injected IP with saline (group 1; $n = 7$), 3 mg/kg body weight prednisolone (group 2; $n = 8$) or 2 mg/kg body weight azathioprine (group 3; $n = 8$) and orally treated with the vehicle (olive oil/ethanol; group 4; $n = 5$) or 15 mg/kg body weight cyclosporin A (group 5; $n = 8$) for 2 weeks. Data are given as mean values ± SEM. * $P < 0.05$, ** $P < 0.01$ (groups 2, 3, and 4 versus group 1; group 5 versus group 4). Symbols as for Fig. 1

Hepatocyte glycogen was not influenced by treatment with azathioprine or prednisolone when using serine as the substrate (Fig. 3). In the presence of pyruvate, however, glycogen was diminished in hepatocytes of prednisolone-treated, and more pronounced in those of azathioprine ($P < 0.05$)-treated, animals. In group 4 (vehicle), a clear consumption of glycogen occurred. After medication with cyclosporin A (group 5), hepatocytes again produced glycogen from both serine and pyruvate (Fig. 3).

With pyruvate as the substrate, the isolated hepatocytes of untreated controls synthetized higher amounts of acetoacetate than when serine was used as the substrate. Treatment with azathioprine, prednisolone, the vehicle, or cyclosporin A increased ketone body production from pyruvate to a similar extent (Fig. 4).

Figure 5 shows the effect of 15 mg/kg and 50 mg/kg body weight cyclosporin A on hepatocyte glucose, pyruvate, and lactate production using the gluconeogenic amino acids serine and alanine as substrates. Cyclosporin A caused significant inhibition of these three parameters in the presence of both substrates. β-Hydroxybutyrate formation was significantly stimulated with serine as the substrate, after administration of both 15 mg/kg and 50 mg/kg body weight cyclosporin A (Fig. 6). Acetoacetate production from both substrates was enhanced after 50 mg/kg body weight cyclosporin A (Fig. 6).

Discussion

Prednisolone action on carbohydrate metabolism and ketogenesis

It is well established that corticosteroids affect hepatic carbohydrate metabolism. In the presence of insulin, glucocorticoids may stimulate hepatic glycogen synthesis [3, 12]. Furthermore, the gluconeogenic capacity of the liver is enhanced. This latter effect is most likely due to the permissive role of steroids in the induction of phosphoenolpyruvate-carboxykinase (PEP-CK) by glucagon or catecholamines [9, 20, 21, 29]. Insulin depresses the activity of the enzyme [35] and the gluconeogenic capacity [1]. The slight increase in PEP-CK activity in prednisolone-treated nondiabetic animals (Table 2) is in agreement with experiments conducted by Gunn et al. [13].

Glucocorticoids also increase the glycogen content of the tissue, even in the fasting condition, and allow glycogen synthesis to occur, even at low levels of glycemia [17]. In the present study, liver glycogen was reduced in corticoid-treated rats. Their hepatocytes produced lower glucose and glycogen in vitro, predominantly with pyruvate as the substrate (Figs. 1, 3). The ketogenic effect of glucocorticoids has been described by Agius et al. [1]. In hepatocyte monolayer cultures, insulin prevents the steroid-dependent increase in ketogenesis. The present experiments may confirm these observations, as in vivo prednisolone treatment was followed by a slight increase in blood ketone body concentration; in vitro (insulin-absent), however, prednisolone significantly enhanced ketogenesis (Fig. 4).

Acetoacetate production increased in hepatocytes of corticosteroid and azathioprine-treated rats only when pyruvate was the substrate (Fig. 4). There exists for serine, but not for pyruvate, an alternative pathway to 2-phosphoglycerate. The enzymes involved are serine aminotransferase, glycerate dehydrogenase, and glycerate kinase [31]. Because of the high substrate concentration of 10 m*M* serine in our experiments, this alternative pathway plays an important role in gluconeogenesis, whereas with

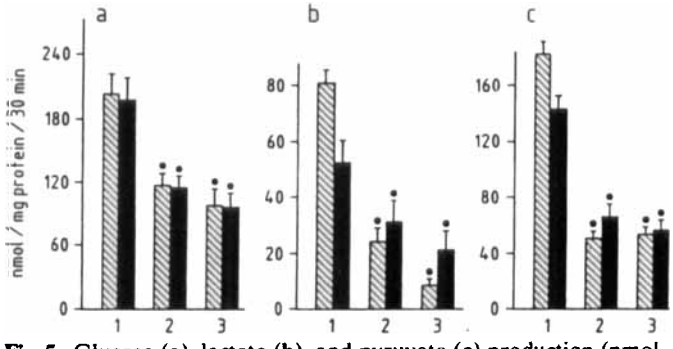

Fig. 5. Glucose (**a**), lactate (**b**), and pyruvate (**c**) production (nmol × mg protein^{-1} × 30 min^{-1}) from L-serine (▨) and alanine (■) (each 10 m*M*) of hepatocytes isolated from rats orally treated with **1**: vehicle (olive oil/ethanol; $n = 5$); **2**: 15 mg/kg body weight ($n = 8$); **3**: 50 mg/kg body weight ($n = 8$) cyclosporin A for 2 weeks. Data are given as mean values ± SEM. * $P < 0.05$ (versus vehicle)

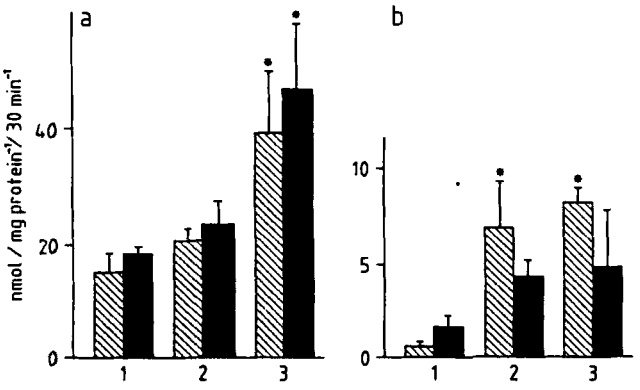

Fig. 6. Acetoacetate (**a**) and 3-OH-butyrate (**b**) (nmol × mg protein^{-1} × 30 min^{-1}) from L-serine (▨) and alanine (■) (each 10 m*M*) of hepatocytes isolated from rats orally treated with **1**: vehicle (olive oil/ethanol; $n = 5$); **2**: 15 mg/kg body weight ($n = 8$); **3**: 50 mg/kg body weight ($n = 8$) cyclosporin A for 2 weeks. Data are given as mean values ± SEM. * $P < 0.05$ (versus vehicle)

lower (physiological) concentrations, this pathway might be quantitatively negligible. The lack of elevated ketogenesis from serine favors the hypothesis of an enhanced gluconeogenic use of this substrate.

Effect of fatty acids and ethanol

Animals in group 4 were treated with approximately 45 mg/day ethanol and 400 mg/day olive oil (vehicle). This treatment was chosen because the commercially available cyclosporin A (Sandimmun) for oral treatment is always dissolved in ethanol plus olive oil. After treatment with the vehicle, plasma concentrations of glucose, lactate, and pyruvate were not changed, whereas insulin was slightly decreased and both glucagon and β-hydroxybutyrate concentration were increased (Table 1).

A stimulatory effect of palmitic acid [8] and oleic acid [37] in vitro on hepatocyte gluconeogenesis from pyruvate has been shown. The effect of ethanol on gluconeogenesis is very dependent on the gluconeogenic substrate. Ethanol inhibits gluconeogenesis from lactate by increasing the cytosolic NADH/NAD$^+$ ratio, but stimulates gluconeogenesis from pyruvate. The increased formation of lactate from pyruvate or serine may be indicative of a change in the cytosolic redox state rather than of any change in flux through gluconeogenesis.

After isolation of hepatocytes, net consumption of glycogen was observed. From the present experiments it cannot be said whether this glycogenolytic effect is due to the fatty acid or the ethanol compound of the vehicle. Since marked glycogenolysis did not occur until hepatocytes were isolated, it can be concluded that in vivo hormonal or other factors counterbalanced the glycogenolytic effect of the vehicle and that these factors in vitro obviously were no more effective. Thus, glycogen breakdown was observed in vitro (Fig. 3).

Effect of cyclosporin A

Compared to the rats in group 4 (vehicle only), rats treated with cyclosporin A showed no differences in the blood concentrations of insulin, glucose, ketone bodies, or hepatic glycogen content. Circulating glucagon was slightly decreased, thus increasing the insulin:glucagon ratio (0.067 ± 0.006 in group 4 as compared to 0.096 ± 0.024 in group 5). In vivo, however, cyclosporin A (in a high dose of 50 mg/kg body weight) caused a significant reduction in hepatic glycogen content and glycogen synthetase [25]. This latter effect is explained by reduced circulating insulin levels. Yale et al. [38] demonstrated that therapeutic doses of cyclosporin (10 mg/kg body weight per day) induce reversible glucose intolerance, and that an increase in the cyclosporin dose augments the glucose intolerance, due to inhibition of insulin secretion and/or synthesis. Similar data were obtained by Garvin et al. [11] in the canine model. In the present study, we used cyclosporin A in the therapeutic dosage of 15 mg/kg body weight per day. With this dosage pancreatic function was well preserved. Therefore, the marked metabolic alterations observed in group 5 as compared to those in group 4 (i.e., inhibition of stimulated glycogenolysis with a consequent decrease in glucose and lactate production) cannot be explained by insulin deficiency. It has to be supposed that cyclosporin A exerts its glycogenolytic inhibiting effect either by direct action on glycogen synthesis or by an insulin-independent hormonal mechanism. We were able to demonstrate in isolated hepatocytes of rats treated with low-dose cyclosporin A [26] that the activities of glycogen synthetase and phosphorylase were essentially unchanged compared to vehicle treatment. However, one should keep in mind that these experiments were done in the absence of substrates and that they are not fully comparable with the present study. Interestingly, in the present experiments, cyclosporin A did not enhance hepatic glycogen content in vivo but rather antagonized the vehicle-induced glycogenolysis in isolated hepatocytes.

Nicchitta et al. [24] showed that cyclosporin A increases receptor-mediated Ca^{2+} fluxes and the cellular Ca^{2+} content. Thus, cyclosporin A should be able to facilitate the glycolytic and glycogenolytic effects of all hormones acting by enhancement of cytosolic-free calcium (e.g., alpha adrenergic agonists or vasopressin). Another experimental finding was that in isolated hepatocytes from cyclosporin A-treated rats, glycogen synthesis did not respond to stimulation by insulin. Moreover, although the number of glucagon receptors was increased, the latter hormone did not activate glycogenolysis [7]. These findings of decreased sensitivity to insulin and glucagon or increased sensitivity to vasopressin, angiotensin II, and alpha adrenergic agonists obviously cannot explain the stimulation of glycogen synthesis by cyclosporin A under the in vitro conditions used. It is surprising that the rate of glycogen synthesis is greater than that of glucose synthesis in these cells, since glycogen synthesis of isolated hepatocytes of starved rats is normally rather low.

Effect of azathioprine

The hepatic toxicity of azathioprine has been reported both in experimental animals and in transplant patients [15, 16, 32]. Azathioprine and its metabolite 6-mercaptopurine have been implicated in hepatocellular disease [19, 28, 32] and pancreatitis [34]. In contrast, no data are avail-

able about its influence on hepatic carbohydrate metabolism. In our studies, azathioprine treatment evoked a metabolic pattern similar to that after prednisolone medication, namely, unchanged blood parameters (Table 1), decreased hepatic glycogen content (Table 2), and diminished glucose and glycogen production of isolated hepatocytes (Figs. 1, 3).

In conclusion, prednisolone and azathioprine, administered over a period of 2 weeks in a therapeutical dose to female Wistar rats, caused a decrease in liver glycogen. Moreover, it can be said that in vitro, cyclosporin A reverses the vehicle-induced alterations of hepatocyte carbohydrate metabolism.

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