Effect of Sulfonylureas on Triglyceride Metabolism in the Rat Liver

POSSIBLE ROLE OF THE LYSOSOMES IN HEPATIC LIPOLYSIS

LUC J. DEBEER, JOSSE THOMAS, GUY MANNAERTS, and PAUL J. DESCHEPPER

From the Laboratory of Pharmacology, School of Medicine, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

ABSTRACT It has been suggested previously that chlorpropamide and other hypoglycemic sulfonylureas interfere with hepatic triglyceride breakdown. Since ketogenesis from endogenous hepatic lipid stores is a measure of hepatic triglyceride hydrolysis, ketogenesis derived from endogenous lipids as well as ketogenesis derived from exogenously added isotopic oleate was determined in isolated hepatocytes from fasted rats in an attempt to identify the nature of the direct effects of sulfonylureas on hepatic lipid metabolism. Ketogenesis from endogenous lipids was inhibited by 1 mM chlorpropamide, while ketone production from exogenous oleate did not change. The effect of chlorpropamide on hepatic triglyceride metabolism was further studied in the isolated perfused liver of normal rats in the presence of a continuous [^{3}H]oleate infusion and in isolated liver cells incubated in the presence of [^{3}H]oleate. In liver perfusion experiments, 1 mM chlorpropamide enhanced the incorporation of tritium into triglycerides (but not other lipid classes) and increased both liver triglyceride content and triglyceride secretion. Using isolated cells similar effects could be demonstrated at 0.5 mM chlorpropamide.

Chlorpropamide, tolbutamide, and carbutamide, all of which inhibited endogenous ketogenesis in isolated liver cells, also inhibited lysosomal triglyceride lipase activity in rat liver homogenates. The drugs were not inhibitory towards alkaline lipase activity. Demethylglycodiazin (2-benzolsulfonamido-5-(β-hydroxyethoxy)-pyrimidin), which did not inhibit endogenous ketogenesis in isolated liver cells, did not affect lysosomal lipase activity. The lysosomotropic drug chloroquine was markedly antiketogenic when tested in liver cells.

The reduction in endogenous ketogenesis, the enhanced accumulation of liver triglycerides, and the stimulation of hepatic triglyceride output by chlorpropamide are ascribed to an interference of the drug with hepatic triglyceride breakdown. The present results also suggest that the lysosomes play a significant role in hepatic lipolysis.

INTRODUCTION

For the last two decades hypoglycemic sulfonylureas have been widely used in the oral treatment of maturity onset diabetes. In addition to stimulating insulin secretion, a variety of extrapancreatic effects have been ascribed to these drugs, amongst which is a direct antiketogenic action on the liver (1–4). Although the antiketogenic effect of the sulfonylureas seems to be well established, the site of their inhibitory action is less clear. Hasselblatt (2) has shown that glycodiazin[1] and tolbutamide inhibit ketogenesis from endogenous hepatic lipids but not from an exogenously added tracer amount of [1-^{14}C]palmitate in liver slices. Similarly, experiments carried out in our laboratory (3, 4) have demonstrated that the antiketogenic effect of chlorpropamide and carbutamide in the isolated rat liver perfused with a fatty acid-free medium largely disappears upon addition of octanoic or oleic acid to the perfusion medium.

The main difference between ketogenesis from endogenous lipids vs. ketogenesis from exogenous fatty acids is that in the former case, hepatic

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[1] Glycodiazin belongs to the sulfonamidepyrimidine group which is structurally related to the sulfonylurea group.

TABLE I

Effect of Chlorpropamide on Ketogenesis in Isolated Hepatocytes from Fasted Rats

Time	Additions	Ketogenesis		
		Total	Exogenous	Endogenous
		μmol ketones/10⁸ cells per 30 min		
0–30 min				
	Control (6)	21.33±0.62	12.96±1.12	8.37±0.98
	1 mM Chlorpropamide (6)	18.59±0.56 $P < 0.001$	12.02±0.94 NS	6.57±0.94 $P < 0.05$
	5 mM Chlorpropamide (6)	11.73±0.45 $P < 0.001$	9.93±0.66 NS	1.85±0.65 $P < 0.005$
30–60 min				
	Control (5)	17.81±1.06	5.48±1.03	12.33±0.72
	1 mM Chlorpropamide (5)	15.05±0.86 $P < 0.02$	5.90±0.62 NS	9.15±1.28 $P < 0.025$
	5 mM Chlorpropamide (5)	7.82±0.42 $P < 0.001$	4.89±0.54 NS	2.93±0.39 $P < 0.001$

Isolated hepatocytes from fasted rats were incubated for 30 or 60 min in the presence of 0.3 mM [1-^{14}C]oleic acid (initial concentration). Calculation of exogenous and endogenous ketogenesis is described under Methods. Results are expressed as means±SEM of the number of separate cell preparations given in parentheses. Statistical significance of experimental vs. control results was established by the *t* test for paired data.

triglycerides must be hydrolyzed by a triglyceride lipase before the liberated fatty acids can enter the activation and oxidation pathways which are common to ketogenesis from either endogenous or exogenous fatty acids. Since activation and oxidation of exogenous fatty acids were apparently not affected by the drugs in the above mentioned experiments, their inhibitory effect on endogenous ketogenesis has been postulated to involve an inhibition of hepatic triglyceride lipase activity (glycerol ester hydrolase E.C. 3.1.1.3).

In the present experiments we have examined in detail the direct effects of sulfonylureas on hepatic lipid metabolism. The results to be presented confirm the antiketogenic effects of these drugs from endogenous but not exogenous substrate and demonstrate that they cause an increase in hepatic triglyceride storage and release. In addition it is shown that the antiketogenic sulfonylureas inhibit lysosomal lipase activity.

METHODS

Animals. Livers were taken from male Wistar rats weighing 100–120 g and maintained on stock laboratory diet. For studies on ketogenesis, rats were starved for 24 h. Fed rats were used in the other experiments.

Liver perfusions. The operative technique was that of Hems et al. (5) modified as follows: the animals received no heparin before cannulation of the portal vein. Instead, after insertion of the portal cannula, the inferior caval vein was cut at a point below the kidney and the liver was immediately flushed with 20 ml of perfusion medium. The usual operative procedure was then continued. The closed circuit perfusion apparatus has been described in detail previously (3). Livers were perfused at 36°C for 135 min with a cell-free medium (6) containing 200 mg/dl of glucose. Tritiated oleate (67 μCi/μmol) was converted to its sodium salt as described previously (6). 100 or 150 μmol of the labeled fatty acid, bound to albumin (6), was added as a priming dose at min 11. From min 13 on, 136 or 180 μmol/100 g rat per 60 min of the tritiated fatty acid were infused over the next 122 min resulting in average steady concentrations of 1.16 mM oleate (range: 1.07–1.24 mM) and 1.40 mM oleate (range: 1.30–1.60 mM), respectively. Chlorpropamide was dissolved in a minimal volume of 1 N sodium hydroxide, diluted with fresh perfusion medium, adjusted to pH 7.4, and added as a single dose at min 16.

Analysis of the perfusion medium and the liver. Samples of perfusate were removed at various times during the experiment. Lipids were extracted from 1 ml of medium with 20 ml of chloroform:methanol (2:1, vol/vol) (7). The extracts were washed with 0.2 vol of 0.1 N sodium hydroxide to remove excess FFA and evaporated to dryness under nitrogen. The residue was dissolved in 0.5 ml of chloroform and placed on 2-g silicic acid columns to remove phospholipids (8). The columns were eluted with 10 ml of chloroform and the eluates analyzed for triglycerides by the method of Van Handel and Zilversmit (9).

At the end of the experiment, livers were rapidly flushed with 25 ml of ice cold perfusion medium, and frozen between metal clamps previously cooled in liquid nitrogen. The frozen tissue was then pulverized in a mortar precooled with liquid nitrogen. Lipids were extracted from approximately 1 g of liver powder with 20 vol of chloroform:methanol (2:1, vol/vol) (7). The extracts were washed with 0.2 vol of 0.02% Mg Cl_2 (wt/vol) and evaporated to dryness under nitrogen. The residue was taken up in 2.5 ml of chloroform per gram tissue and 0.5 ml of this volume was passed through silicic acid columns for the chemical determination of triglycerides as described above. 50- or 100-μl aliquots were spotted on thin-layer silicic acid chromatography plates (Merck A.G., Darmstadt, W. Germany) and the lipids separated according to Skipski et al. (10).

Lipid bands were located with a 2′, 7′-dichlorofluorescein spray and scraped off into liquid scintillation counting vials. The silicic acid powder was shaken for 60 min with 10 ml of Instagel (Packard Instrument, Co., Inc., Downers Grove, Ill.) and counted in a liquid scintillation spectrometer. Appropriate corrections were made for quenching and counting efficiency by the external standardization method.

Isolated liver cells. Hepatocytes were prepared by the method of Zahlten and Stratman (11). 5×10^6 cells were incubated in duplicate in siliconized glass scintillation counting vials in a final volume of 2 ml of Krebs-Henseleit bicarbonate buffer containing 2.3 g of defatted (12) albumin per 100 ml. The vials were gassed with O_2-CO_2 (95:5, vol/vol) and incubated with shaking at 37°C for 30 or 60 min. Control experiments revealed that more than 80% of the cells excluded trypan blue at the end of a 60-min incubation period (after two washes to remove the albumin).

In ketogenesis experiments the reactions were stopped with perchloric acid, and β-hydroxybutyrate (13) and acetoacetate (14) were determined enzymatically in the neutralized supernate. When labeled oleate was present (0.3

μmol of [1-^{14}C]sodium oleate per ml of incubation mixture; approximately 1.3 × 10^6 dpm, Table I), β-hydroxybutyrate was converted to acetoacetate and the radioactivity associated with the carbonyl and carboxyl group of acetoacetate measured according to McGarry et al. (15). Appropriate corrections for blanks and recoveries were performed. The known specific activity of the added oleate allowed calculation of the amount of ketones derived from the radioactive substrate (exogenous ketogenesis). The difference between total ketogenesis (sum of enzymatically determined acetoacetate plus β-hydroxybutyrate) and the radioactive ketones gave the amount derived from hepatic lipid stores (endogenous ketogenesis).

In triglyceride experiments, 11 μmol of glucose and 1 μmol of [^{3}H]oleate (approximately 5 × 10^6 dpm) per ml of incubation mixture were present. In these experiments the cells were separated from the incubation medium by centrifugation for 1 min at 250 *g* and cell and medium lipids were extracted and analyzed by the methods used in the liver perfusion experiments.

Preparation of homogenates. 20% (wt/vol) liver homogenates was prepared in 250 mM sucrose, containing 1 mM EDTA, pH 7.4. Glass homogenizers fitted with a loose teflon pestle (Arthur H. Thomas Co., Philadelphia, Pa.) were used throughout. The homogenates were diluted 6-fold with sucrose/EDTA for the determination of alkaline lipase activity or 10-fold with distilled water to disrupt the lysosomal membranes for measurements of acid lipase activity. 0.1 ml of the final dilution was used in the assay. Protein was determined according to Lowry et al. (16) using bovine serum albumin as standard.

Assay of triglyceride lipase activity. Triglyceride lipase activity was measured by a modification of the method of Krauss et al. (17). The substrate mixture contained 5.55 × 10^5 dpm of glyceryltri[1-^{14}C]oleate (55 mCi/mmol), 0.08–5 μmol of unlabeled triolein, 16.7 mg of defatted (12) bovine serum albumin, and 0.5 mg of Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.) per ml of buffer. These constituents were emulsified by homogenizing the mixture for 2 min in ice with an Ultra-Turrax homogenizer (Janke and Kunkel, Staufen, W. Germany). Within 30 min of preparation, 0.9 ml of this emulsion was mixed with 0.1 ml of homogenate and incubated in a shaking water bath at 37°C for 20 min when acid lipase activity was measured and for 60 min when alkaline lipase activity was measured. The liberated fatty acids were subsequently extracted from the incubation mixture (18) and their radioactivity measured by liquid scintillation spectrometry. Results are expressed as μmol FFA released/mg protein per 60 min after correction for extraction and counting efficiency. Each determination was done in duplicate and appropriate zero time controls were always performed. At alkaline pH, FFA release was linear for 150 min and over a protein concentration range of 0.1–1 mg per assay. At pH 4.4, linearity was observed over the same protein concentration range, but lipase activity declined with time after 40 min.

Chemicals. Enzymes and coenzymes used for ketone body determinations were obtained from Boehringer Gmbh, Mannheim, W. Germany; [9,10-^{3}H]oleic acid, [1-^{14}C]oleic acid, and glyceryltri[1-^{14}C]oleate from The Radiochemical Centre, Amersham, Great Britain; unlabeled oleic acid from Fluka A. G., Buchs, Switzerland; unlabeled triolein and Triton X-100 from BDH. Chemicals Ltd., Poole, Great Britain; and silicic acid (70–230 mesh), sodium metaperiodate, arsenic trioxide, chromotropic acid, and 2′,7′-dichlorofluorescein from Merck A. G. Chloroquine diphosphate and collagenase type I were purchased from Sigma Chemical Co., St. Louis, Mo. We gratefully acknowledge the gift of the following drugs: chlorpropamide from Pfizer, Ltd., Sandwich, England; tolbutamide and carbutamide from Hoechst A. G., Frankfurt, W. Germany; demethylglycodiazin (2-benzolsulfonamido-5-(β-hydroxyethoxy)-pyrimidin) from Schering A. G., Berlin, W. Germany. Stock solutions of the drugs were prepared by dissolving them in a minimum volume of 1 N NaOH, and diluting with Tris-HCl buffer, pH 8, to a final concentration of 0.2 M. Addition of up to 50 μl of these solutions did not alter the pH of the incubation mixtures. Addition of the same volume of NaOH-Tris-HCl did not affect ketogenesis or triglyceride lipase activity in control experiments.

Statistical methods. Results are expressed as mean ±SEM. Significance of differences between means was established by Student's *t* test for independent (perfusion experiments) or paired (hepatocytes) means.

TABLE II

Effect of Chlorpropamide on Incorporation of [^{3}H]Oleate into Various Hepatic Lipid Classes

Condition	Triglycerides	Diglycerides	Phospholipids	Cholesterol esters
	dpm/100 g body wt (×10^{-6})			
Control (4)	11.74±1.62	0.084±0.016	2.19±0.35	0.102±0.012
5 mM Chlorpropamide (5)	18.95±1.72*	0.100±0.032	2.26±0.26	0.095±0.012

Incorporation of tritium into lipids was determined in livers perfused for 135 min in the presence of an average steady concentration of 1.4 mM oleate. Chlorpropamide, when present, was added as a single dose at min 16. Results are expressed as means ±SEM of the number of experiments given in parentheses.
* Indicates significance at the $P < 0.02$ level.

RESULTS

Effect of chlorpropamide on ketogenesis in isolated hepatocytes. Table I shows the effects of 1 and 5 mM chlorpropamide on ketone body production by isolated hepatocytes from fasted rats incubated in the presence of 0.3 mM [1-^{14}C]oleic acid. Ketogenesis derived from endogenous lipid stores was inhibited by chlorpropamide in a dose-dependent way. In contrast, the amount of ketones originating from labeled oleate was not significantly affected by the drug. These observations corroborate and extend Hasselblatt's findings (2) and strongly suggest that sulfonylureas interfere with hepatic lipolysis.

Effect of chlorpropamide on incorporation of [^{3}H]oleate into various lipid classes in the isolated perfused rat liver. An inhibition of hepatic triglyceride breakdown by chlorpropamide should result in an increased labeling of the triglyceride fraction in the isolated liver perfused with labeled long-chain fatty acids. Table II shows the incorporation of tritium into various hepatic lipid fractions when livers were perfused in the presence of [^{3}H]oleate. 5 mM chlorpropamide did not affect the incorporation of radioactivity in phospholipids, diglycerides, and cholesterolester, but definitely increased the radioactivity

associated with the triglyceride fraction (161% of the control value).

Effect of chlorpropamide on incorporation of [^{3}H]oleate in liver triglycerides and on triglyceride content of the perfused liver. Since 5 mM chlorpropamide exceeds by far the therapeutic blood levels, the effects of 0.5 and 1 mM chlorpropamide were also studied (Table III). At 0.5 mM a suggestion of increased triglyceride synthesis was seen, but it was not statistically significant ($0.05 < P < 0.10$). Incorporation was significantly increased at 1 mM drug concentration and chemical determination of liver triglycerides revealed an increase in hepatic triglyceride content. For the newly synthesized triglycerides ([^{3}H]oleate incorporation) an increase of 8.7 μmol of triglycerides/100 g rat per 120 min was observed while the chemical determination showed an increase of 11.4 μmol/100 g per 120 min above the control value. This difference possibly reflects a chlorpropamide effect upon the unlabeled triglyceride fraction already present in the liver before infusion of the labeled fatty acids.

Effect of chlorpropamide on triglyceride secretion by the perfused liver. The liver is the site of production of the serum triglycerides present in very low density lipoproteins (19–21). Since an enhanced accumulation of hepatic triglycerides might change hepatic triglyceride secretion rates, the triglyceride content of the perfusion medium was followed after 64 and 124 min of perfusion in the presence of oleate. Table IV indicates that 1 mM chlorpropamide enhanced the secretion of triglycerides in the perfusion medium whereas 0.5 mM chlorpropamide caused a similar although not statistically significant effect.

TABLE III

Effect of Chlorpropamide on [^{3}H]Oleate Incorporation in Hepatic Triglycerides and on Hepatic Triglyceride Content

Condition	[^{3}H]Oleate incorporation into triglyceride	Total hepatic triglyceride content
	μmol/100 g body wt	
1.16 mM [^{3}H]Oleate Control	41.05±2.27 (9)	ND
0.5 mM Chlorpropamide	49.42±3.73 (9)	ND
1.40 mM [^{3}H]Oleate Control	30.25±2.24 (9)	77.25±5.41 (9)
1 mM Chlorpropamide	38.77±2.24 (7)*	88.62±2.28 (11)‡

Livers were perfused for 135 min in the presence of an average steady concentration of 1.16 mM [^{3}H]oleate or 1.40 mM [^{3}H]oleate. When present, chlorpropamide was added as a single dose at min 16. Results are expressed as mean ±SEM of the number of experiments given in parentheses. To make comparison with the increase in total triglyceride content, radioactive oleate incorporation into triglycerides has been divided by three to give an estimate of triglyceride synthesis.

* Indicates significance at the $P < 0.025$ level.

‡ Indicates significance at the $P < 0.05$ level.

TABLE IV

Effect of Chlorpropamide on Hepatic Triglyceride Secretion

Condition	Perfusion Interval 75 min	Perfusion Interval 135 min
	μmol TG/100 g body wt	
Control (10)	1.37±0.20	3.77±0.78
0.5 mM Chlorpropamide (10)	1.58±0.19	4.15±0.53
Control (9)	1.57±0.32	4.05±0.61
1 mM Chlorpropamide (10)	1.97±0.28	6.04±0.56*

Triglyceride secretion was measured in the perfusate of the perfusion experiments described in Table II. Results are means±SEM of the number of experiments given in parentheses.

* Indicates significance at the $P < 0.05$ level.

Effect of chlorpropamide on triglyceride metabolism in isolated liver cells. The results of Tables III and IV were confirmed and extended using isolated liver cells as shown in Table V. As expected, in experiments with a single dose of [^{3}H]oleate (1 mM initial concentration) incorporation of label into cell triglycerides declined with time while the opposite was true for medium triglycerides. During the first 30 min, liver cells synthesized triglycerides (TG)[2] at the rate of 30.7 μmol TG/100 g body wt per 60 min (10^8 cells = 1 g liver (11); 5.8 g liver = 100 g fed rat) compared with 15–20 μmol TG/100 g body wt per 60 min in the perfusion experiments of Table III. Triglyceride secretion by liver cells between 30 and 60 min (Table V) proceeded at the rate of 3.26 μmol TG/100 g body wt per 60 min. During the 2nd h of perfusion experiments (Table III), the secretion rate was 2.44 μmol TG/100 g body wt per 60 min. The agreement between the two experimental situations demonstrates that isolated liver cells may safely be used for this type of experiment.

In the presence of chlorpropamide, incorporation of [^{3}H]oleate into both liver cell and incubation medium triglycerides increased in a dose-dependent way. Unlike the perfusion experiments, changes with 0.5 mM chlorpropamide were significant at 60 min.

Effect of pH on lipolytic activity of rat liver homogenates. Both the inhibition of endogenous ketogenesis as well as the increased incorporation of label into triglycerides point towards an interference

[2] *Abbreviation used in this paper:* TG; triglycerides.

TABLE V

Effect of Chlorpropamide on Triglyceride Metabolism in Isolated Liver Cells

Conditions	Incubation period . . .	Triglycerides of liver cells		Triglycerides of incubation medium	
		30 min	60 min	30 min	60 min
		μmol [^3H]oleate incorporated/10^6 cells			
Control		8.85±0.46	12.88±0.53	0.17±0.02	1.02±0.10
0.5 mM Chlor-propamide		9.55±0.26	14.19±0.56	0.20±0.02	1.14±0.12
		(13) $0.05 < P < 0.10$	(13) $P < 0.001$	(13) $P < 0.05$	(13) $P < 0.025$
Control		7.80±0.57	11.30±0.68	0.18±0.03	1.01±0.09
1 mM Chlor-propamide		9.21±0.63	13.33±0.80	0.24±0.02	1.34±0.14
		(13) $P < 0.001$	(13) $P < 0.001$	(9) $P < 0.02$	(9) $P < 0.02$
Control		6.75±0.62	10.24±0.81	0.17±0.04	1.00±0.09
2.5 mM Chlor-propamide		9.46±0.98	13.93±1.04	0.62±0.07	1.82±0.05
		(8) $P < 0.001$	(8) $P < 0.001$	(4) $P < 0.005$	(4) $P < 0.005$

Incorporation of [^3H]oleate into the triglycerides of liver cells and incubation medium was measured after 30 or 60 min of incubation (initial oleate concentration: 1 mM). Results represent the mean±SEM of the number of separate cell preparations given in parentheses. Statistical significance was established using the paired *t* test.

of the drug with hepatic lipolysis. Before testing the effect of sulfonylureas on hepatic lipolytic activity a pH profile was performed. In agreement with the findings of others (22, 23), triglyceride lipase activity of rat liver homogenates, determined at 5 mM triolein concentration in the present experiments, showed two pH optima (Fig. 1): a sharp peak at pH 4.4, which represents the lysosomal enzyme (22–27), and a broad peak around pH 8–9, subdivided into a maximum at pH 8.2, and one at pH 9.2. These maxima most probably reflect the activities of the microsomal enzyme with a pH optimum at 8 (24) and of the plasma membrane enzyme which has its optimum at pH 9–9.5 (23, 24).

Effect of sulfonylureas on hepatic triglyceride lipase activity. Fig. 2 shows triglyceride lipase activity as a function of substrate concentration at pH 8.2 (Fig. 2A) and at pH 9.2 (Fig. 2B). Using the Lineweaver-Burk method, an apparent K_m of 0.28 mM and a V_{max} of 0.231 μmol FFA/mg protein per 60 min were calculated for the lipase activity at pH 8.2. At pH 9.2, these values were 0.77 mM and 0.308 μmol FFA/mg protein per 60 min. Fig. 3 shows the lysosomal lipase activity, assayed at pH 4.4, vs. substrate concentration. The acid lipase did not show straight forward hyperbolic kinetics over the range of substrate concentrations used.

Chlorpropamide,[3] tolbutamide, and carbutamide, all of which were antiketogenic in isolated hepatocytes (Table VI), significantly inhibited lysosomal lipase activity. In contrast, demethylglycodiazin, the demethylation product of glycodiazin, which retains its blood sugar lowering capacity in vivo (2), but which is no longer antiketogenic in liver slices (2) or isolated hepatocytes (Table VI) was much less inhibitory. The same drugs had little or no effect on the alkaline lipases (Fig. 2).

The fact that all the antiketogenic sulfonylureas tested inhibited acid lipase activity points towards a role of the lysosomes in hepatic triglyceride breakdown. Further evidence for this concept comes from the observation that the lysosomotropic drug chloroquine

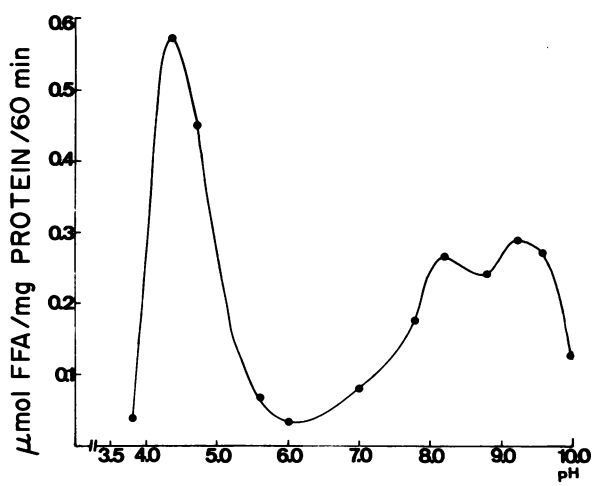

FIGURE 1 pH profile of triglyceride lipase activity in rat liver homogenates. Triglyceride lipase was measured in the presence of 5 mM triolein. The following buffers (ionic strength = 0.1) were used: sodium acetate buffer, pH 3.8, 4.4, 5.0, 5.6; sodium phosphate buffer, pH 6.0, 6.6, 7.0, 7.8; Tris-HCl buffer, pH 8.2; glycine-NaOH buffer, pH 8.8, 9.2, 9.6, and 10.0.

[3] While all drugs were routinely tested at 5 mM, chlorpropamide was used at 4 mM concentration in the acid lipase assays because of its poor solubility at acid pH.

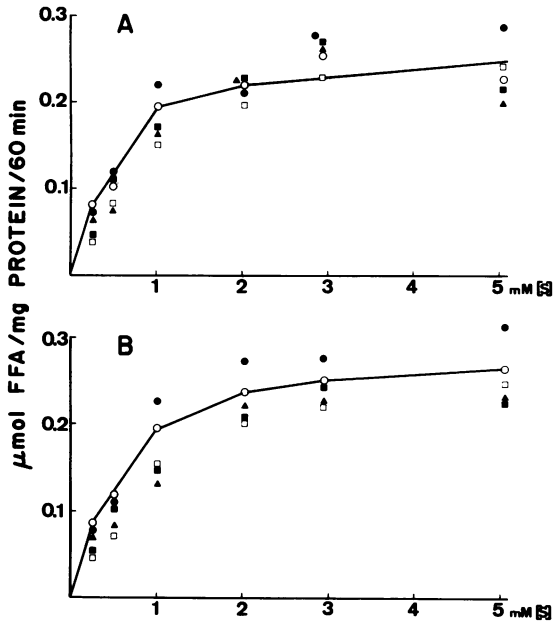

FIGURE 2 Effect of drugs on alkaline triglyceride lipase activities. Triglyceride lipase activity was measured in rat liver homogenates at pH 8.2 (A) and pH 9.2 (B). Each point represents the mean of duplicate determinations in four different homogenates. Control, ○; 5 mM chlorpropamide, ●; 5 mM tolbutamide, ■; 5 mM carbutamide, □; 5 mM demethylglycodiazin, ▲. [S], triolein substrate concentration.

(28) displayed a marked antiketogenic effect when tested in isolated hepatocytes (Table VI). The effect of chloroquine on lysosomal lipase was also directly examined. The lipolytic rate, determined at 5 mM triolein concentration, was 0.580±0.103 μmol FFA released/mg protein per 60 min in the controls (n = 4), whereas in the presence of 5 mM chloroquine this rate was only slightly reduced to 0.461±0.57 μmol FFA/mg protein per 60 min.

Finally, it is noteworthy that in the fasted rat, which has an increased rate of endogenous ketogenesis as compared to the fed rat, we observed a significant decrease in alkaline lipase activity (53 and 74% at pH 8.2 and 9.2, respectively of the lipase activity in the fed rat), while the lysosomal enzyme activity did not significantly change (115% of the activity in the fed rat).

DISCUSSION

Both in the perfused rat liver and in isolated hepatocytes, chlorpropamide increased the incorporation of labeled oleic acid into liver triglycerides. The experiments with isolated hepatocytes further indicate that the chlorpropamide effect on hepatic triglycerides is dose-dependent. Besides enhancing the incorporation of label into triglycerides, chlorpropamide also increased the total triglyceride content of the liver. Incorporation of label into other lipid fractions was unaffected.

Presumably the effects of chlorpropamide on hepatic triglyceride metabolism and the known antiketogenic effect of various sulfonylureas (1–4) share a common base. As shown in Table I, chlorpropamide inhibits ketogenesis in isolated hepatocytes by reducing the amount of ketones derived from endogenous lipid stores. Ketone body production from the labeled exogenous oleate was unaffected. The main difference between ketogenesis from endogenous triglycerides and exogenous fatty acids is that endogenous ketogenesis requires a hydrolysis step, catalyzed by a triglyceride lipase, before the fatty acids can be oxidized to ketones (and CO_2). Thus, it seems logical to conclude from the data presented in Table I that chlorpropamide interferes with the hydrolysis of hepatic triglycerides, which would result in a diminished endogenous ketogenesis as well as in an increased incorporation of label into hepatic triglycerides. This conclusion is further strengthened by the fact that chlorpropamide, tolbutamide, and carbutamide, which inhibit endogenous ketogenesis in isolated heptocytes, also inhibit triglyceride lipase activity in liver homogenates and, more specifically, the apparent lysosomal enzyme. In contrast, demethylglycodiazin, which is not antiketogenic in isolated liver cells, is essentially without effect on the lysosomal lipase.

Taken together, the present data imply that the lysosomes play a role in the breakdown of hepatic triglyceride stores. Further support for the involvement of the lysosomes in hepatic lipolysis comes from the observation that the lysosomotropic drug chloroquine

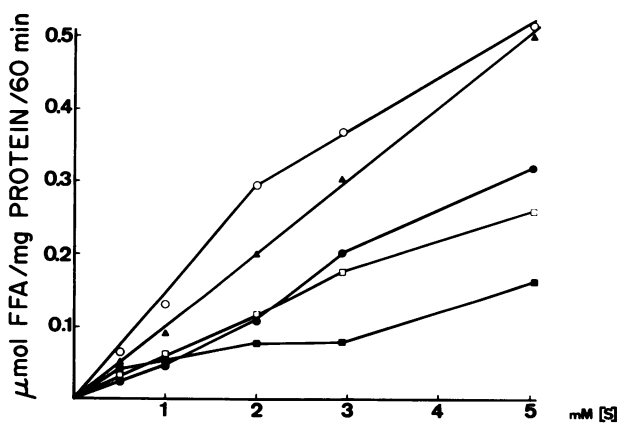

FIGURE 3 Effect of drugs on lysosomal triglyceride lipase. Triglyceride lipase activity was measured in rat liver homogenates at pH 4.4. Each point represents the mean of duplicate determinations in four different homogenates. Control, ○; 4 mM chlorpropamide, ●; 5 mM tolbutamide, ■; 5 mM carbutamide, □; 5 mM demethylglycodiazin, ▲. [S], triolein substrate concentration.

(28) also inhibits endogenous ketogenesis in liver cells. It is a known fact that chloroquine strongly affects various lysosomal functions. In cultured fibroblasts, low concentrations of chloroquine inhibit intralysosomal proteolysis (29), intralysosomal digestion of mucopolysaccharides (30), and intralysosomal catabolism of both protein and lipid of low density lipoproteins (31). In contrast, with the antiketogenic sulfonylureas the inhibitory effect of chloroquine on acid lipase activity in homogenates was rather small. However, Wibo and Poole (29) have shown that in cultured fibroblasts exposed to 100 μM chloroquine the intralysosomal concentration of the drug may reach 75–100 mM. This dramatic concentration effect, characteristic of a lysosomotropic drug, could explain the marked antiketogenic effect of 50 μM chloroquine observed in isolated hepatocytes as well as the moderate inhibitory effect of 5 mM chloroquine on the lysosomal lipase in liver homogenates. However, chloroquine might also inhibit lysosomal lipase activity in intact lysosomes by altering the intralysosomal pH (32) without having any major direct inhibitory effect on the enzyme.

As shown in Tables IV and V, chlorpropamide also stimulates hepatic triglyceride secretion. The enhanced secretion rates are most likely due to the above proposed interference of the drug with hepatic triglyceride breakdown, which results in an increased accumulation of liver triglycerides. Other metabolic situations are known where a relative increase in hepatic triglyceride content results in an increased secretion. The fed liver, which esterifies considerable amounts of FFA, secretes more triglycerides than the fasted liver, which preferentially oxidizes the incoming fatty acids (33). Moreover, sucrose feeding, which enhances esterification, also causes enhanced rates of triglyceride secretion (34, 35).

The observations reported here indicate that concentrations of 0.5 and 1 mM chlorpropamide, which lie within the range of therapeutic blood levels (36), are capable of altering hepatic triglyceride metabolism in acute experiments. It is not clear, however, whether the increased hepatic triglyceride levels might ultimately predispose to the development of fatty liver or whether the enhanced triglyceride secretion rates might cause hypertriglyceridemia. As with all in vitro and animal experiments these data cannot be directly extrapolated to human therapy. To our knowledge, no clinical reports have appeared clearly demonstrating that chronic sulfonylurea treatment might cause the above mentioned adverse effects. Such studies might be complicated by the fact that fatty liver as well as hyperlipidemia are not uncommon in diabetes. Nevertheless, it should be noted that Greenberg et al. (37) found higher lipoprotein levels in patients who had been switched from insulin to tolbutamide therapy, although blood sugar control was unchanged, and that Knick et al. (38) found higher serum triglyceride levels in sulfonylurea-treated patients than in insulin-treated diabetics, when serum triglycerides were plotted against the daytime mean sugar profile.[4] It would, therefore, seem appropriate to examine more closely plasma triglycerides in patients chronically treated with sulfonylureas. Moreover, since sulfonylureas seem to interfere with hepatic intralysosomal lipid breakdown, it is not precluded that these drugs might also influence plasma lipid levels by inhibiting the peripheral intralysosomal breakdown of low density lipoproteins (39).

TABLE VI
Effect of Drugs on Endogenous Ketogenesis by Isolated Rat Hepatocytes

Conditions	Ketone body production	Control
	μmol/10⁶ cells per 30 min	%
Control (n = 3)	8.55±1.29	100
+2.5 mM Chlorpropamide	1.43±0.44*	17
+2.5 mM Tolbutamide	2.82±0.58*	33
+2.5 mM Carbutamide	5.68±0.74*	66
+2.5 mM Demethylglycodiazin	7.69±0.51	90
Control (n = 8)	13.02±0.94	100
+50 μM Chloroquine	5.55±1.50*	43

Isolated hepatocytes from fasted rats were incubated without substrate. Results are expressed as means±SEM of the number of experiments given in parentheses (paired data).
* Indicates significance at the $P < 0.05$ level.

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[4] Calculated as the arithmetical mean of three measured blood sugar levels (at 8.00, 11.00, and 16.00 h [38]).

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