Biochemical Pharmacology, Vol. 24, pp. 1035–1041. Pergamon Press, 1975. Printed in Great Britain.

# METABOLIC EFFECTS OF HYPOGLYCEMIC SULFONYLUREAS—VI.

## EFFECTS OF CHLORPROPAMIDE AND CARBUTAMIDE ON KETOGENESIS AND ON MITOCHONDRIAL REDOX STATE IN THE ISOLATED PERFUSED RAT LIVER*

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(*Received* 5 *April* 1974; *accepted* 30 *October* 1974).

**Abstract**—The effect of 5 mM chlorpropamide and 5 mM carbutamide on fatty acid oxidation in the perfused rat liver was studied. Chlorpropamide as well as carbutamide inhibited endogenous lipid oxidation to ketone bodies. Chlorpropamide had no effect on ketogenesis from exogenously added octanoate, but inhibited ketone body production from a single dose of oleate. Ketogenesis from a continuous oleate infusion was reduced by chlorpropamide during the first 15 min of infusion but was unaffected during the next 45 min studied. Carbutamide did not decrease ketogenesis during oleate infusion. The uptake of octanoate and oleate remained unchanged in the presence of both drugs. Chlorpropamide caused a stimulation of oxygen consumption in the absence of fatty acid substrates but was unable to alter oleate-stimulated respiration. Chlorpropamide, but not carbutamide, stimulated oligomycin-inhibited respiration although to a lesser degree than 2,4-dinitrophenol. Krebs cycle flux during oleate infusion was slightly stimulated by chlorpropamide. Mitochondrial redox state, as measured by the β-hydroxybutyrate/acetoacetate ratio in the perfusate, was markedly lowered by chlorpropamide in the octanoate and oleate experiments. The lactate/pyruvate ratio was unaffected by chlorpropamide. Carbutamide did not produce any change compared with control experiments. From these experiments it is concluded that chlorpropamide and carbutamide inhibit endogenous lipid oxidation by interfering with hepatic triglyceride lipase activity. Changes in oxygen uptake and in mitochondrial redox state caused by chlorpropamide are attributed to the uncoupling activity of this drug.

Numerous studies *in vivo* and *in vitro* have established that the hypoglycemic activity of sulfonylureas can be attributed mainly to a stimulation of insulin release. However, direct extrapancreatic effects cannot be excluded. In this respect much attention has been paid to effects on liver metabolism (for literature see ref. 1). Although an antiketogenic effect in the liver has been described by several authors, the primary site of action of sulfonylureas on ketogenesis remains obscure.

Hasselblatt [2] found an inhibition of endogenous ketogenesis by tolbutamide and glycodiazine in rat liver slices, but was unable to demonstrate an inhibition of ketogenesis from exogenously added fatty acids. He suggested that the antiketogenic action was due to an inhibition of triglyceride lipase activity. Söling *et al.* [3] showed a reduction of ketogenesis from endogenous lipids in the perfused rat liver by several sulfonylureas, and this was confirmed by a previous report from this laboratory for livers perfused with chlorpropamide [4].

In contrast with Hasselblatt's findings, Boshell *et al.* [5] described an inhibition of ketogenesis from acetate by tolbutamide, and Söling and Seck [6] reported an inhibition of ketogenesis from hexanoate by glibornuride, both groups using rat liver slices. Walter *et al.* [7] using the isolated perfused rat liver observed an inhibition of ketogenesis from a single dose of oleate by tolbutamide and glibornuride, which was interpreted as an inhibition of fatty acid oxidation. The above contradictory observations prompted us to reinvestigate the antiketogenic effect of the sulfonylureas.

Earlier work from this laboratory revealed an uncoupling effect of several sulfonylureas in isolated rat liver mitochondria, and an interference of these compounds with mitochondrial substrate uptake [8, 9]. Therefore, the experiments on ketogenesis were completed with measurements of mitochondrial redox state and of hepatic oxygen consumption.

High drug concentrations (5 mM) were used in the present experiments since the aim of this study was to identify the possible site of action of these compounds, rather than to evaluate the therapeutic relevance of their effects *in vitro*.

### MATERIALS AND METHODS

*Animals.* Male Wistar rats maintained on stock laboratory diet and weighing 100–120 g were used. Starved animals had free access to water but no access to food for 24–28 hr. Diabetic animals were injected intravenously with 100 mg per kg body wt of alloxan monohydrate, 48 hr prior to use, and had free access to food. Only livers from animals

* This work was supported by grants from the "Fonds voor Wetenschappelijk Geneeskundig Onderzoek".

† Aspirant, Nationaal Fonds voor Wetenschappelijk Onderzoek.

with maximal ketonuria (Ketostix, Ames, Epernon, France) were used in the perfusion experiments.

*Liver perfusions.* The operative technique has been described by Hems *et al.* [10]. The perfusion medium (initially 100 ml) contained 2·6 g of bovine serum albumin (Armour fraction V) per 100 ml of Krebs–Henseleit bicarbonate buffer pH 7·4. The albumin was defatted according to Chen [11] and dialysed for 48 hr at 4° against 100 vol of Krebs–Henseleit buffer prior to use. The perfusion apparatus has been described in detail elsewhere [4]. Hepatic oxygen consumption was continuously monitored by Clark-type Pt–Ag electrodes placed before and after the liver [4].

*Preparation of additions.* Appropriate amounts of chlorpropamide or carbutamide were dissolved in a minimal volume of 1 N sodium hydroxide. The solution was diluted with fresh medium and adjusted to pH 7·4 before addition to the perfusion medium. Octanoic and oleic acid were converted to their sodium salts by titration of an ethanolic solution with 1 N sodium hydroxide to a phenolphthalein endpoint. The ethanol was evaporated under nitrogen and the residue dissolved in 0·5 ml saline. The solution was adjusted to 5 ml with $Ca^{2+}$-free perfusion medium and added to the perfusion system as a single dose. In infusion experiments 100 μmoles of sodium oleate, prepared as described above, were added as a priming dose. The infusion was prepared by dissolving the sodium oleate residue in distilled water. This solution was infused in the medium entering the rotating disc oxygenator at a rate of 136 μmoles of sodium oleate per 100 g rat per hr resulting in an average steady concentration of 1·1 mM oleate (range 0·96–1·36 mM).

*Analyses of perfusion medium and liver.* One ml medium samples were extracted with $HClO_4$ [4] and assayed for β-hydroxybutyrate [12] and acetoacetate [13]. 0·2 ml medium samples, taken for oleic acid determination, were extracted with 6 ml of chloroform and the extracts assayed according to Duncombe [14]. Octanoic acid in the perfusion medium was determined by gas–liquid chromatography. Two ml medium samples were extracted twice with 6 ml of a 1:1 acetone–ethanol mixture with a recovery of 95 per cent. The combined extracts were evaporated to dryness under nitrogen at room temperature. A known amount of decanoic acid in methanol was added as internal standard and the residue was made up with methanol to a final volume of 2 ml. The fatty acids were converted to their methylesters and extracted with hexane as described by Rogozinski [15]. 5 μl of the hexane extract was injected into a Hewlett-Packard 5750 G gaschromatograph equipped with a flame ionisation detector. Six foot glass columns (internal diameter 4 mm) packed with acid washed chromosorb w (80 to 100 mesh) coated with 8% butane-1,4-diol succinate (Applied Science Laboratories Inc., State College, Pa, U.S.A.) were used. Column temperature was programmed, starting at 110° and rising 20° per min after a two min post injection interval. Cyclic AMP was purified from a trichloracetic acid extract of a portion of quick-frozen liver as described by Van den Berghe *et al.* [16] and determined by the method of Breckenridge [17]. Lactate and pyruvate content of livers was determined as described previously [4].

*Chemicals.* Enzymes were obtained from Boehringer Gmbh, Mannheim, Germany; $NAD^+$, NADH and alloxan monohydrate from Sigma Chemical Company, St. Louis, Missouri, U.S.A.; oleic, decanoic and octanoic acids from Fluka A.G., Buchs, Switzerland. Oligomycin was purchased from Sigma Chemical Company and 2,4-dinitrophenol from E. Merck A.G., Darmstadt, Germany. A stock solution of both chemicals was prepared in ethanol. Chlorpropamide and carbutamide were kind gifts from Pfizer Ltd, Sandwich, Kent U.K. and from Hoechst A.G., Frankfurt a/Main, Germany, respectively. All other reagents were analytical grade.

*Statistical methods.* All values are given as arithmetical means ± standard error of the mean. The significance of differences between means was established by the Student's *t*-test. Results in Table 5 were treated as paired data, after logarithmic transformation.

## RESULTS

*Effects on ketogenesis.* A previous report from this laboratory demonstrated that 1 mM and 5 mM chlorpropamide inhibit ketone body output from endogenous lipids by the perfused rat liver [4]. Table 1 shows that 5 mM carbutamide also markedly reduces endogenous ketogenesis. These results sharply contrast with the negative findings of Creutzfeldt *et al.* [18]. Although these authors used a lower concentration of carbutamide (1·2 mM), this discrepancy is probably the result of the presence in their perfusion medium of 11·1 mM glucose, which is strongly antiketogenic. As demonstrated in exper-

Table 1. Effect of chlorpropamide and carbutamide on endogenous ketone body production by the perfused liver of normal and alloxan-diabetic rats

| Experimental conditions | Ketone body production [μmoles/30 min per g liver (wet wt)] min 0–30 | min 30–60 | min 60–90 |
|---|---|---|---|
| (1) Fasted rat | 11·89 ± 0·71 | 16·15 ± 2·61 | 14·34 ± 1·36 |
| + 5 mM carbutamide at 28 min | 13·27 ± 0·89 | 6·57 ± 0·78* | 4·84 ± 1·91* |
| (2) Fed alloxan diabetic rat | 51·97 ± 4·93 | 52·94 ± 2·79 | 39·49 ± 7·30 |
| + 5 mM chlorpropamide at 28 min | 51·31 ± 5·87 | 30·91 ± 5·34* | 19·87 ± 3·72* |

Ketone body production was measured in the perfusate of livers perfused without substrate for 90 min. Ketones were taken as the sum of β-hydroxybutyrate and acetoacetate production. Carbutamide or chlorpropamide was added at 28 min to a final concentration of 5 mM. Results are given as means ± S.E.M. with n = 4 for fasted rats and with n = 5 for diabetic rats.* $P < 0·05$.

Table 2. Effect of chlorpropamide and carbutamide on ketone body production from octanoate or oleate by the perfused rat liver

| Experimental conditions | Ketone body production [μmoles/30 min per g liver (wet wt)] min 0–30 | min 30–60 | min 60–90 |
|---|---|---|---|
| (1) 5 mM octanoate (single dose) at 28 min | 15·92 ± 1·94 | 60·40 ± 3·57 | 50·80 ± 4·14 |
| 5 mM octanoate (single dose) at 28 min + 5 mM chlorpropamide at 23 min | 13·97 ± 0·92 | 58·24 ± 4·69 | 48·26 ± 4·52 |
| (2) 1 mM oleate (single dose) at 28 min | 16·05 ± 3·53 | 54·47 ± 3·84 | 30·57 ± 2·70 |
| 1 mM oleate (single dose) at 28 min + 5 mM chlorpropamide at 23 min | 11·39 ± 2·33 | 28·53 ± 1·05* | 10·63 ± 3·08* |

| | [μmoles/15 min per g liver (wet wt)] min 30–45 | min 45–60 | min 60–75 | min 75–90 |
|---|---|---|---|---|
| (3) Oleate infusion from 28 min on | 28·19 ± 2·30 | 23·04 ± 2·67 | 22·54 ± 3·55 | 17·81 ± 2·70 |
| Oleate infusion from 28 min on + 5 mM chlorpropamide at 23 min | 18·95 ± 2·89* | 19·90 ± 2·64 | 21·71 ± 3·65 | 15·55 ± 1·85 |
| Oleate infusion from 28 min on + 5 mM carbutamide at 23 min | 29·57 ± 1·07 | 24·16 ± 1·10 | 26·00 ± 0·53 | 19·66 ± 0·30 |

Ketone body production was measured in the perfusate of livers from fasted rats, perfused for 90 min. Octanoate was added as a single dose at 28 min to a final concentration of 5 mM (expt. 1). Oleate was added as a single dose at 28 min to a final concentration of 1 mM (expt. 2). In Experiment 3, 100 μmoles of oleate (final concentration 1 mM) were added as a priming dose at 26 min, and a continuous oleate infusion of 136 μmoles/100 gram rat per hr was started at 28 min, for the next hour resulting in an average steady concentration of 1·1 mM oleate (range 0·96–1·36 mM). Chlorpropamide or carbutamide was added at 23 min, to a final concentration of 5 mM. Results are given as means ± S.E.M., n = 5. *P < 0·05.

ment 2 (Table 1) chlorpropamide retains its inhibitory activity on ketogenesis in the diabetic liver.

Table 2 shows the results of experiments on ketogenesis from exogenously added fatty acids. Chlorpropamide, added at 23 min, does not affect ketogenesis from 5 mM octanoate, added at 28 min (expt. 1). From these results it can be concluded that chlorpropamide inhibits endogenous lipid oxidation, but does not affect the β-oxidation of octanoate, nor the pathway leading from acetyl-CoA to ketone bodies.

Medium-chain fatty acids are thought to be activated inside the mitochondria [19–21], while long-chain fatty acids are activated outside. Until now it has not been established whether medium-chain and long-chain fatty acids are oxidized by the same β-oxidation system. Besides differences in activation and transport into mitochondria, medium-chain fatty acids, in contrast to long-chain fatty acids, cannot be esterified to triglycerides by the liver [22, 23]. These metabolic differences prompted us to study the effect of chlorpropamide and carbutamide also on long-chain fatty acid oxidation.

Experiment 2 of Table 2 shows that the presence of 5 mM chlorpropamide causes an immediate and significant decrease in ketogenesis from a single dose of oleate. This inhibition of ketogenesis from oleate cannot be ascribed to a blocking of endogenous lipid utilisation alone. When the rate of endogenous ketogenesis (0–30 min) is substracted from the rate observed after oleate addition in control experiments, this resulting value is still higher than the ketogenic rate in chlorpropamide experiments. In another series of experiments (expt. 3) a priming dose of 100 μmoles of oleate was given at 26 min. At 28 min oleate was infused at a constant rate of 34 μmoles per 15 min per 100 g rat, so that a steady concentration of about 1·1 mM oleate was achieved during the next hour of perfusion. Chlorpropamide decreased significantly the ketogenic rate during the first 15 min, but had no effect on further ketogenesis. Carbitamide was without effect.

Since the results of experiment 3 indicate that chlorpropamide and carbutamide have little or no effect on the oxidation of infused long chain fatty acids, inhibition of ketogenesis from endogenous lipids by these drugs seems to be due to an effect on hepatic triglyceride lipase activity, as will be discussed further.

*Effect of chlorpropamide and carbutamide on hepatic free fatty acid uptake.* In order to exclude any effect of sulfonylureas on fatty acid uptake by the liver, this uptake was measured during the experiments reported in Table 2. The data in Table 3 show that there was no significant effect on fatty acid uptake.

*Effect of chlorpropamide on hepatic cyclic AMP content.* Since cyclic adenosine-3′,5′-monophosphate (cAMP) could be involved in hormonal stimulation of hepatic triglyceride lipase [24] measurements of hepatic cAMP concentration were performed. In control experiments, the cAMP content of livers was 512 ± 42, 652 ± 78 and 622 ± 87 nmoles/g after 5, 15 and 60 min perfusion respectively (n = 4). In the presence of 5 mM chlorpropamide, the corresponding values were 519 ± 49, 682 ± 85 and 593 ± 119 nmoles/g (n = 4). These results suggest that under our experimental conditions cAMP is not involved in the possible action of chlorpropamide on hepatic lipase activity.

*Effect of chlorpropamide on hepatic oxygen uptake.* A previous report from this laboratory suggested that chlorpropamide could act as an uncoupler of oxidative phosphorylation in the perfused rat liver [4]. This uncoupling effect was confirmed by studies on isolated rat liver mitochondria [8, 9].

During the present work, the oxygen consumption by the perfused liver oxidizing 1 mM oleate (infusion) was measured in the presence or absence of

Table 3. Effect of chlorpropamide and carbutamide on octanoate or oleate uptake by the perfused rat liver

| Experimental conditions | Free fatty acid uptake [μmoles/30 min per g liver (wet wt)] min 30–60 | min 60–90 |
|---|---|---|
| (1) 5 mM octanoate (single dose) at 28 min | 42·28 ± 7·00 | 33·64 ± 2·69 |
| 5 mM octanoate (single dose) at 28 min + 5 mM chlorpropamide at 23 min | 34·22 ± 4·84 | 38·62 ± 4·25 |
| (2) 1 mM oleate (single dose) at 28 min | 11·09 ± 2·27 | 5·53 ± 0·95 |
| 1 mM oleate (single dose) at 28 min + 5 mM chlorpropamide at 23 min | 14·13 ± 0·29 | 6·67 ± 1·30 |
| (3) Oleate infusion from 28 min on | 17·48 ± 0·29 | 19·22 ± 1·06 |
| Oleate infusion from 28 min on + 5 mM chlorpropamide at 23 min | 15·81 ± 1·38 | 17·57 ± 0·72 |
| Oleate infusion from 28 min on + 5 mM carbutamide at 23 min | 19·05 ± 0·70 | 19·11 ± 0·98 |

Same livers and perfusion conditions as in Table 2. Results are given as means ± S.E.M.

chlorpropamide (expt. 3, Table 2). Upon addition of chlorpropamide to the perfusion medium at 23 min, oxygen consumption immediately increased by 13 per cent from 2·27 ± 0·17 to 2·56 ± 0·16 μmoles $O_2$ per min per g liver ($n = 5$, $P < 0·001$; not shown in Table). As shown in Table 4, hepatic oxygen consumption further increased after addition of the fatty acid substrate to the perfusion medium, but the results obtained in the presence of chlorpropamide are not significantly different from the control. In another series of experiments where the perfusion medium contained 5 mM chlorpropamide 15 min after the start of the oleate infusion (data not shown) no effect of chlorpropamide on oxygen consumption could be detected.

Livers were also perfused in the presence of oligomycin and chlorpropamide or 2,4-dinitrophenol as shown in Table 5. Oligomycin (1 mg/100 ml) markedly inhibits hepatic respiration. Chlorpropamide 5 mM causes a 13 per cent stimulation above the oligomycin-inhibited respiration while 1 mM dinitrophenol gives a 73 per cent stimulation. A stepwise elevation of the chlorpropamide concentration in 1-mM steps from 1 to 5 mM after addition of oligomycin induced a progressive stimulation of oxygen uptake, although no clear jumps in oxygen uptake were recorded (data not shown). This was probably due to the limited sensitivity of oxygen uptake measurements. Carbutamide, which does not uncouple oxidative phosphorylation in isolated mitochondria [8, 9] had no effect in similar experiments (data not shown).

*Influence of chlorpropamide on Krebs cycle flux.* Previous studies from this laboratory revealed that chlorpropamide, like other uncouplers of oxidative phosphorylation, interferes with mitochondrial substrate uptake [8]. Since inhibition of mitochondrial substrate uptake could affect Krebs cycle activity, approximate fluxes through the cycle were calculated during perfusions with oleate infusion. The calculations were made by the method of Williamson *et al.* [25] and the results are shown in Table 6. As can be seen, Krebs cycle flux was not inhibited.

*Effect of chlorpropamide and carbutamide on mitochondrial redox state.* Figure 1 shows the mitochondiral redox state as measured by the β-hydroxybutyr-

Table 5. Stimulation of oligomycin-inhibited respiration by chlorpropamide and dinitrophenol

| Experimental conditions | Experiment 1 [μmoles $O_2$/min per g liver (wet wt)] | Experiment 2 |
|---|---|---|
| No addition | 2·67 ± 0·47 | 2·12 |
| Oligomycin 1 mg/100 ml | 1·84 ± 0·47* | 1·34 |
| Chlorpropamide 5 mM | 2·08 ± 0·53* | — |
| 2,4-dinitrophenol, 1 mM | — | 2·33 |

Livers from fasted rats were perfused without substrate. At 30 min, oligomycin was added to a final concentration of 1 mg/100 ml. 5 min after oligomycin addition, when oxygen uptake was stabilized, chlorpropamide or 2,4-dinitrophenol was added to a final concentration of 5 mM and 1 mM, respectively. *$P < 0·02$; $n = 3$. 2,4-Dinitrophenol data are means of two experiments.

Table 4. Oxygen uptake by the perfused liver during oleate infusion

| Experimental conditions | Equilibration period | Perfusion period [μmoles $O_2$/15 min per g liver (wet wt)] min 30–45 | min 45–60 | min 60–75 | min 75–90 |
|---|---|---|---|---|---|
| Oleate (infusion at 28 min) | 33·19 ± 2·29 | 45·40 ± 2·58 | 45·86 ± 2·92 | 46·86 ± 2·71 | 48·53 ± 2·36 |
| Oleate (infusion at 28 min) + chlorpropamide 5 mM at 23 min | 34·04 ± 2·75 | 45·44 ± 4·15 | 45·52 ± 4·68 | 46·12 ± 4·58 | 46·97 ± 4·59 |

Same livers and perfusion conditions as in Table 2, expt. 3. Results are expressed as means ± S.E.M. Equilibration period: oxygen uptake in μmoles $O_2$/min, per g liver (wet wt) at 22 min times 15. For further explanations see text.

Table 6. Sources of oxygen consumption by the perfused liver during oleate infusion

| Source of $O_2$ consumption | Perfusion period [$\mu$moles $O_2$/min per g liver (wet wt)] min 30–45 Control | min 30–45 Chlorprop | min 45–60 Control | min 45–60 Chlorprop | min 60–75 Control | min 60–75 Chlorprop | min 75–90 Control | min 75–90 Chlorprop |
|---|---|---|---|---|---|---|---|---|
| Total $O_2$ consumption | 3·03 | 3·03 | 3·06 | 3·03 | 3·12 | 3·07 | 3·24 | 3·13 |
| Cyanide-sensitive respiration | 2·76 | 2·76 | 2·79 | 2·76 | 2·86 | 2·80 | 2·97 | 2·86 |
| $\beta$-OH butyrate production | 2·24 | 0·69 | 1·50 | 0·58 | 1·43 | 0·74 | 1·10 | 0·61 |
| Acetoacetate production | −0·07 | 0·98 | 0·40 | 0·93 | 0·46 | 1·27 | 0·41 | 0·67 |
| Ketone respiration | 2·17 | 1·67 | 1·91 | 1·51 | 1·89 | 2·01 | 1·51 | 1·28 |
| Fatty acid oxidation to $CO_2$ | 0·59 | 1·09 | 0·88 | 1·24 | 0·97 | 0·79 | 1·47 | 1·58 |
| Fatty acid oxidation to acetyl CoA | 0·18 | 0·32 | 0·26 | 0·37 | 0·29 | 0·23 | 0·43 | 0·46 |
| Fatty acid oxidation in cycle | 0·42 | 0·77 | 0·63 | 0·88 | 0·69 | 0·55 | 1·03 | 1·12 |
| Cycle flux ($\mu$moles/g per min) | 0·21 | 0·38 | 0·32 | 0·44 | 0·35 | 0·28 | 0·52 | 0·56 |

Same livers and perfusion conditions as in Table 2, expt. 3. The calculations of oxygen equivalents were based on data of Table 2 (Expt. 3), and Table 4, and were made according to Williamson [25].

ate to acetoacetate ratio in the perfusate of experiments described in Tables 1 and 2. As can be seen from Fig. 1A, chlorpropamide markedly lowers the mitochondrial redox state of diabetic livers. Chlorpropamide has the same effect in perfusions with octanoate (Fig. 1A), and in experiments where oleate was infused (Fig. 1B). In contrast, carbutamide does not affect the $\beta$-hydroxybutyrate to acetoacetate ratio under these conditions.

During the oleate infusion experiments, the mean lactate/pyruvate ratio was 18·22 (range 13·08–25·34, n = 5) in control experiments and 20·22 (range 16·95–29·73, n = 5) in the presence of 5 mM chlorpropamide.

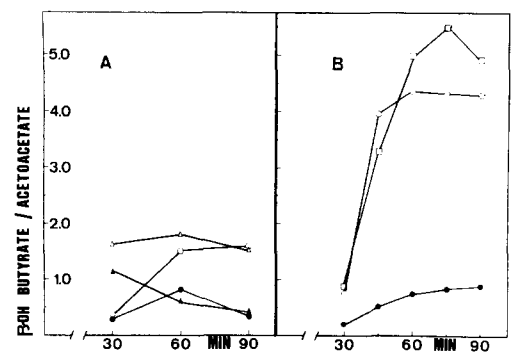


Fig. 1. $\beta$-Hydroxybutyrate to acetoacetate ratios were estimated from ketone body determinations in the perfusion fluid of the same livers as described in Tables 1 and 2. Results are expressed as means. (A). △——△ diabetic livers; ▲——▲ diabetic livers, addition of 5 mM chlorpropamide at 28 min; ○——○ fasted livers, addition of 5 mM octanoate at 28 min; ●——● fasted livers, addition of 5 mM chlorpropamide at 23 min and 5 mM octanoate at 28 min. (B). ○——○ fasted livers, oleate infusion at 28 min; □——□ fasted livers, addition of 5 mM carbutamide at 23 min and oleate infusion at 28 min; ●——● fasted livers, addition of 5 mM chlorpropamide at 23 min and oleate infusion at 28 min.

## DISCUSSION

Earlier results from this laboratory [4] and the data of Table 1 clearly show that chlorpropamide, as well as carbutamide, inhibit ketogenesis from endogenous lipids. In contrast, these drugs do not affect ketone body production from octanoate or from oleate infusion (except chlorpropamide during the first 15 min of infusion (Table 2)). These results strongly suggest that both drugs interfere with hepatic triglyceride lipase activity, as first proposed by Hasselblatt [2] for tolbutamide and glycodiazin. Indeed, the only difference in the degradation of endogenous triglycerides and exogenously added fatty acids is the prior hydrolytic cleavage of endogenous lipids. Yet another explanation could be offered for the lack of effect on ketogenesis from added fatty acids. If the sulfonylureas would act as competitive inhibitors upon the $\beta$-oxidation system or upon the enzymes of the Lynen cycle, this inhibition could be overcome by the elevated concentrations of intermediates during enhanced fatty acid oxidation. This possibility can be ruled out by the fact that chlorpropamide remains inhibitory in the diabetic liver (Table 1), which produces ketone bodies at comparable rates with livers oxidizing octanoate or oleate. The inhibition of ketone body output by chlorpropamide during the first 15 min of oleate infusion could also be attributed to an inhibition of hepatic triglyceride lipase activity. Indeed, in control experiments a portion of the free fatty acid substrate could be esterified and then converted to ketone bodies after hydrolysis of the triglycerides, while another portion of the substrate could be directly oxidized. In the presence of chlorpropamide lipolysis would be inhibited and the esterification of the free fatty acid substrate would occur only until the esterification capacity of the liver is saturated. During this period less free fatty acids would be available for ketogenesis, but later on, all the free fatty acids entering the liver would be oxidized to ketone bodies, so that no further effect of chlorpropamide could be seen. Presumably

the strong inhibition of ketogenesis from a single dose of oleate is also due to an esterification of part of the oleate. This is further supported by the observed reduction by chlorpropamide of ketogenesis from a continuous oleate infusion in perfused livers from fed rats, whose esterification capacity is probably higher than that of fasted livers. Moreover, this reduction of ketogenesis was accompanied by an increase in hepatic triglyceride content (manuscript in preparation). Nevertheless, the different effect of chlorpropamide and carbutamide during the first 15 min of oleate infusion remains unexplained.

Recently Söling and Seck [6] reported an inhibition of ketogenesis by glibornuride in liver slices incubated with hexanoate. These results could not be confirmed by us since ketogenesis from octanoate was not affected in the perfused rat liver. The discrepancy between these results might be due to a specific effect of glibornuride. However, it is more likely that this inhibition of ketogenesis could be explained by the fact that these authors used liver slices in their experiments. It has been pointed out that liver slices have a low ATP content and that they have reduced rates of ketogenesis compared with the perfused liver [26]. In addition sulfonylureas have been shown to interfere with mitochondrial energy metabolism [4, 8, 9]. A further lowering of ATP content caused by sulfonylureas could inhibit fatty acid activation and oxidation and cause a fall in oxygen consumption as has been demonstrated in isolated mitochondria incubated with fatty acids and classical uncouplers [27].

An inhibition of lipolysis by sulfonylureas has also been described in white [28–30] and brown adipose tissue [31]. This antilipolysis caused by sulfonylureas in brown tissue is not the result of a decreased cyclic AMP concentration [31]. In our experiments also the cAMP content of perfused livers was not affected by chlorpropamide. Fain suggested that the uncoupling action of sulfonylureas in brown adipose tissue was responsible for the inhibition of lipase activity. In contrast, endogenous lipid oxidation was inhibited by both chlorpropamide and carbutamide in our perfusion experiments while in previous studies with isolated rat liver mitochondria we have shown that chlorpropamide was an uncoupling agent, whereas carbutamide was not [8, 9].

Although oleate-stimulated respiration was not altered by chlorpropamide (Table 4), the jump in hepatic oxygen uptake in the absence of fatty acids and the stimulation of oligomycin-inhibited respiration caused by chlorpropamide (Table 5) indicate that this drug had uncoupling potency under our experimental conditions. Carbutamide (which is not an uncoupler), did not produce any stimulation of oxygen consumption in similar experiments. The stimulation of oxygen uptake by chlorpropamide sharply contrasts with the findings of Hasselblatt's group [32]. These authors obtained an inhibition of hepatic oxygen consumption by the perfused liver which was already seen with 0·5 mM drug concentration and which was more pronounced with a concentration of 2·5 mM. Although oxygen uptake was inhibited, liver surface fluorescence showed a shift towards oxidation of pyridine nucleotides and flavoproteins. Since we have shown that uncoupling concentrations of sulfonylureas can inhibit mitochondrial oxygen consumption by interfering with mitochondrial substrate uptake [8], this mechanism could play a role in the above-mentioned experiments. Indeed, these perfusions were carried out with fructose as substrate, which will stimulate Krebs cycle activity, while in our experiments Krebs cycle activity was very low since the livers obtained most of their energy from $\beta$-oxidation of fatty acids.

Calculations of approximate Krebs cycle flux for our livers showed no inhibition of the cycle (Table 5). Nevertheless, from Hasselblatt's and our experiments it is reasonable to conclude that some sulfonylureas do interfere with mitochondrial oxidation.

Further evidence for the interaction of chlorpropamide with mitochondria is reached from the shift towards oxidation of the mitochondrial redox state. Mitochondrial redox state can be influenced by several mechanisms. Enhanced lipid oxidation results in a more reduced redox state (see Fig. 1 A and B; control experiments). Therefore, an antilipolytic action of sulfonylureas could cause a shift towards oxidation. Since mitochondrial redox state is lowered by chlorpropamide under conditions where lipid oxidation is not affected (Table 2, experiments 1 and 3) this is presumably not the main mechanism. Moreover, carbutamide, which depresses endogenous lipid oxidation, is without effect on the redox state. Inhibition of mitochondrial substrate uptake could restrict the production of reducing equivalents by the Krebs cycle and affect the mitochondrial redox state. As discussed above, this does not seem to occur under our experimental conditions. The unchanged lactate to pyruvate ratio also argues against a possible inhibition of transfer of reducing equivalents into the mitochondria by substrate shuttles. We feel that the more oxidized state of mitochondrial pyridine nucleotides under chlorpropamide in our experiments is the result of its uncoupling action. As mentioned above, chlorpropamide was able to stimulate oligomycin-inhibited respiration. Furthermore carbutamide, which is not an uncoupling agent in isolated mitochondria, did not affect the redox state.

From the present experiments we conclude that both chlorpropamide and carbutamide inhibit hepatic ketogenesis from endogenous lipids by affecting the hepatic triglyceride lipase activity. The question therefore arises whether an inhibition of hepatic triglyceride lipase activity could affect hepatic triglyceride accumulation and secretion, which plays a central role in blood lipid homeostasis. Studies on the effect of sulfonylureas on hepatic triglyceride lipase activity are in progress. Furthermore it appears that some of the sulfonylureas are capable of affecting mitochondrial energy metabolism. The therapeutic relevance of these mitochondrial interactions remains to be established.

*Acknowledgements*—The authors wish to thank Mrs. Th. Stes-Souren and Mr. L. Govaert for expert technical assistance.

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