Biochemical Pharmacology, 1960, Vol. 3, pp. 305–315. Pergamon Press Ltd., Printed in Great Britain.

EFFECTS OF HYPOGLYCIN ON CERTAIN ASPECTS OF GLUCOSE AND FATTY ACID METABOLISM IN THE RAT

K. W. McKERNS, H. H. BIRD, E. KALEITA, B. S. COULOMB and E. C. DE RENZO

Biochemistry Department, Biochemical Research Section,
Lederle Laboratories, American Cyanamid Company, Pearl River, N.Y.

(*Received* 12 *September* 1959; *revised* 18 *March* 1960)

Abstract—Hypoglycin (2-amino-methylene*cyclo*propanepropionic acid), isolated from the seeds of *Blighia sapida*, induced a marked hypoglycemia when administered to rats. Little direct effect of this compound was observed on glucose metabolism, either on glucose oxidation to respiratory CO_2 or on conversion of glucose to liver lipid or muscle glycogen. Synthesis of glycogen from glucose in diaphragm muscle was inhibited by hypoglycin without altering glucose uptake.

The major effects of hypoglycin were those concerned with fatty acid metabolism. The administration of hypoglycin to rats caused an increase in the level of non-esterified fatty acids in the serum, an increase in the total lipids of the liver and a significant inhibition in the conversion of butyrate or stearate to respiratory CO_2. Liver mitochondria isolated from hypoglycin-treated rats showed an impaired ability to form high energy phosphate bonds associated with the oxidation of pyruvate and malate. Hypoglycin had no effect on adrenal cortical output.

Since hypoglycin lowered blood glucose level only after 3 or 4 hr, the active compound may be a metabolite or the effects on blood sugar may be secondary to some primary metabolic block. Methylene*cyclo*propanepyruvic acid and methylene*cyclo*propaneacetic acid, both likely metabolites, do produce hypoglycemia. The possibility that such intermediates interfere with the oxidation of fatty acid substrates is briefly discussed.

INTRODUCTION

HYPOGLYCIN, a compound isolated from the seeds of *Blighia sapida*,[1,2] is capable of inducing hypoglycemia in several animal species. This compound has aroused considerable interest recently as a result of the elucidation of its structure as α-amino-methylene*cyclo*propanepropionic acid.[3–6] Although studies have been reported on certain metabolic effects of hypoglycin, its mode of action in inducing hypoglycemia remains obscure.

A primary metabolic effect of hypoglycin has been postulated to be an inhibition of liver glycogen formation.[1,7] On the other hand, recent experiments in our laboratory demonstrated the ability of hypoglycin to induce lipid infiltration in the liver and to produce a several-fold increase in the serum non-esterified fatty acid (NEFA) level in intact rats.[8] Also, on the basis of histological evidence, Chen *et al.*[9] reported fat infiltration in the livers of hypoglycin-treated rats. Further investigation into the effect of hypoglycin on fatty acid metabolism therefore appeared to be of interest.

This paper describes the effects of this interesting hypoglycemic agent on certain aspects of glucose and fatty acid metabolism. A comparison of some of these effects with those produced by insulin or tolbutamide (N-*p*-tosyl-N′-*n*-butyl urea) is also presented.

EXPERIMENTAL

Materials

Crystalline zinc-insulin was provided by Dr. O. K. Behrens of Eli Lilly and Company. Tolbutamide was purchased from the Upjohn Company. Hypoglycin was isolated from the seed of *Blighia sapida* by Dr. R. S. de Ropp and shown to be analytically pure. Mr. J. C. Van Meter prepared the methylene*cyclo*propanepyruvic acid by enzymatic deamination of hypoglycin and methylene*cyclo*propaneacetic by decarboxylation of this pyruvic acid derivative. Dr. S. Safir provided the 4-pentenoic acid. Radioactive glucose and fatty acids were purchased from the New England Nuclear Corporation.

Assays

Blood glucose,[10] liver and muscle glycogen[11] and liver total lipid[12] were determined by published procedures. Glucose uptake and glycogen synthesis were determined in rat diaphragm as described previously.[13] Adrenal cortical secretion was measured by the method of McKerns and Nordstrand.[14,15] R.Q. was determined on paired liver slices by the direct method of Dixon[16] using the Krebs–Ringer phosphate medium described by Umbreit *et al.*[17] Oxidative phosphorylation was studied in liver mitochondria using pyruvate and malate as substrates by methods similar to those described in Brody and Bain.[18]

Metabolism experiments with ^{14}C-labeled glucose or fatty acids

After the intramuscular administration of the hypoglycemic agent under study, 3–4 μc of glucose-U-^{14}C was injected intramuscularly, along with unlabeled glucose (1 g/kg body weight), either to fed or 20-hr fasted albino rats weighing about 100–150 g. Immediately thereafter, the rats were placed in a respiratory apparatus and the expired CO_2 was swept by filtered CO_2-free air into carbonate-free 20% sodium hydroxide. The sodium hydroxide was changed at $\frac{1}{2}$ hr intervals during a 2- or 4-hr experimental period and the CO_2 collected as $BaCO_3$. The radioactivity of the $BaCO_3$ precipitates was determined and the $BaCO_3$ weighed to determine total CO_2 excretion. At the end of the experimental period the rats were decapitated and selected tissues removed, weighed and subjected to the desired analysis.

The effect of hypoglycin on the metabolism of 1-^{14}C labeled fatty acids in the intact rat was studied in the following manner: 1-^{14}C-labeled acetate, butyrate or stearate was administered at a desired time after the intramuscular injection of hypoglycin. The fatty acids were injected either as saline, rat plasma or albumin suspensions. The route of administration was varied. In certain experiments non-labeled glucose was also administered intramuscularly along with the fatty acids. Respiratory CO_2 was collected and tissue analyses were performed as described below.

The metabolsim of radioactive fatty acids or glucose in liver slices was studied using the K-110 medium of Ashmore *et al.*[19] One hundred mg of tissue were incubated in 3 ml of medium for 1 hr in a modified Warburg flask containing a side arm closed with a rubber policeman; 0·4ml of 30% NaOH was then injected into the center well, followed by injection of 0·2 ml of 8 N H_2SO_4 into the main compartment. After allowing 2 hr for absorption of CO_2, the NaOH was removed and the center well washed out with 5 ml of CO_2-free water. The CO_2 was recovered as $BaCO_3$ by the addition of 0·5 ml of a solution of $BaCl_2$ (2 N) and NH_4Cl (1·6 N). The $BaCO_3$ was washed with CO_2-free water and finally with ethanol–ether (3:1).

Radioactivity measurements

All radioactivity measurements were made on samples sufficiently thin that no corrections for self-absorption had to be made. A gas-flow counter with automatic sample changer was used. Samples were counted sufficiently long that a counting error of not more than 5 per cent was ensured.

RESULTS

Effect of hypoglycin, tolbutamide or insulin on blood glucose and liver glycogen levels in rats

Fig. 1 shows typical changes in the level of blood glucose after the administration of hypoglycemic agents to fasted rats. All rats received an intramuscular dose of 1 g

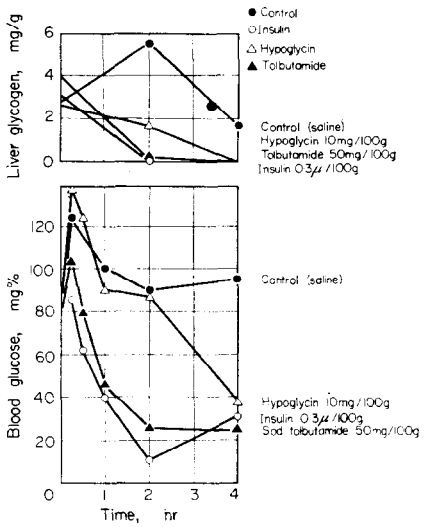

FIG. 1. Effect of hypoglycemic agents on the blood glucose and liver glycogen levels in the rat. Fasted rats were given the drug intramuscularly, followed by glucose (100 mg/100 g).

of glucose per kg of body weight along with the hypoglycemic agent. Both insulin and tolbutamide caused a rapid lowering in the level of blood glucose. The levels of blood glucose after hypoglycin-treatment followed the same pattern as the control for the first 2 hr, but dropped to low levels during the next 2 hr. Similar changes in the blood glucose levels were seen in other experiments after the intraperitoneal injection of hypoglycin. The associated changes in the levels of liver glycogen are also shown in Fig. 1. The liver glycogen level rose only in the case of the controls. The maximum rise was observed within 2 hr.

Effect of in vivo *administration of hypoglycin, tolbutamide or insulin on oxidative phosphorylation in liver mitochondria*

Since hypoglycin treatment caused a rapid infiltration of lipid into the liver,[8] it appeared of interest to determine oxidative phosphorylation capacity of liver mitochondria isolated from hypoglycin-treated rats. In these experiments the hypoglycemic agents were administered to starved rats which were killed by decapitation 4 hr later. The livers were excised and mitochondria isolated. Malate and pyruvate were used as

substrates. The results are shown in Table 1. A significant decrease in P:O ratio was observed only in the case of hypoglycin-treatment. When added *in vitro* to mitochondria prepared from untreated rats, hypoglycin had no effect (data not shown).

Table 1. Oxidative phosphorylation* in liver mitochondria prepared from fasted rats four hours after treatment with hypoglycin (15 mg/100 g), tolbutamine (100 mg/100 g), or insulin (0·2 U/100 g)

Expt. no.	Mitochondria from	N per flask (mg)	Pi† uptake (μ moles)	O_2 uptake (μ atoms)	P : O‡ control	P : O‡ tested
			Mean of 3 Flasks			
1	Control	4·4	19·7	7·6	2·61	2·04
	Hypoglycin	4·3	12·2	7·2		
2	Control	4·4	15·7	7·8	$t = 3·62$	
	Hypoglycin	4·4	12·4	7·7		
3	Control	3·8	33·2	10·5	$P<0·02$	
	Hypoglycin	3·7	26·5	9·6		
4	Control	4·6	16·2	8·5	2·20	2·27
	Tolbutamide	4·6	14·3	7·4		
5	Control	4·2	21·9	8·6		
	Tolbutamide	3·8	23·1	9·0		
6	Control	4·8	25·9	12·0	$t =$ NS	
	Tolbutamide	4·5	29·6	13·2		
7	Control	5·4	22·5	10·2	2·23	2·40
	Insulin	4·1	21·8	9·0		
8	Control	4·0	13·0	6·1	$t =$ NS	
	Insulin	4·0	10·1	6·4		
9	Control	4·0	19·3	8·3		
	Insulin	3·8	18·6	7·8		

* Determined as described by Brody and Bain.[18] Substrate concentrations: sodium pyruvate, $1·5 \times 10^{-2}$M; sodium malate, 2×10^{-3}M.
† Pi = Inorganic phosphate.
‡ Calculated from the average of the three experiments shown.

Effect of hypoglycin on the conversion of ^{14}C*-labeled glucose or fatty acids to* CO_2 *in intact rats*

The effect of hypoglycin on the conversion of glucose-U-^{14}C and acetate-1-^{14}C to CO_2 was determined at $\frac{1}{2}$ hr intervals following the administration of the ^{14}C-labeled compound. As shown in Table 2, no significant differences in $^{14}CO_2$-output from glucose-U-^{14}C were observed between treated and normal animals.

The conversion of acetate-1-^{14}C to respiratory CO_2 in starved rats was slightly lower after hypoglycin-treatment. The simultaneous administration of glucose reduced this slight difference. Acetate conversion to CO_2 was not affected in fed rats (data not shown). The slight effect on acetate metabolism in fasted rats was probably a reflection of the very low carbohydrate reserves. The total respiratory CO was reduced in all

cases. The total $^{14}CO_2$ (expressed in counts/min per 100 g of body weight) and total respiratory CO_2 at the end of the experiments are shown in Table 2.

In contrast to the results with acetate, the conversion of butyrate-1-^{14}C to $^{14}CO_2$ in

TABLE 2. THE EFFECT OF HYPOGLYCIN ON THE CONVERSION OF ^{14}C-LABELED GLUCOSE OR ACETATE TO CO_2 IN INTACT RATS

Exp. no.	Glucose-U-^{14}C				Acetate-1-^{14}C			
	1		2		3		4	
	Control	Hypoglycin (10 mg/100 g)	Control	Hypoglycin (15 mg/100 g)	Control	Hypoglycin (15 mg/100 g)	Control	Hypoglycin (15 mg/100 g)
Counts/min per 100 g	328,850	345,968	364,947	419,160	1,248,418	1,072,849	2,213,957	2,095,026
ml CO_2 per 100 g body weight	1021	636	358	268	333	195	386	264

Glucose-U-^{14}C, 4 μc/100 g, was administered intramuscularly with non-labeled glucose (100 mg/100 g) to fasted rats in exp. 1; 3 μc to fed rats in exp. 2.

Acetate-1-^{14}C, 7 μc/100 g, was administered intramuscularly to fasted rats in exp. 3; acetate-1-^{14}C (7 μc) with non-labeled glucose (100 mg/100 g) to fasted rats in exp. 4.

The radioactive compounds were given 4 hr after hypoglycin in exp. 1 and 5 min after hypoglycin in exp. 2, 3 and 4. CO_2 was collected for 4 hr in exp. 1 and for 2 hr in exp. 2, 3 and 4. Values shown are total accumulative ^{14}C-output at these times.

Data shown are for individual rats and are representative of at least two rats on each treatment.

fed rats was found to be greatly reduced when it was administered intravenously 4 hr after the administration of hypoglycin. In these experiments, butyrate-1-^{14}C was administered as a neutralized suspension with bovine serum albumin. The results of two separate experiments are given in Fig. 2. The most striking difference between the control and the hypoglycin treatment was seen in the first $\frac{1}{2}$ hr after the administration of the butyrate.

Similarly the conversion of stearate-1-^{14}C to $^{14}CO_2$ in fed animals which received hypoglycin was also reduced. Stearate was administered intramuscularly as a rat plasma suspension. Results are shown in Fig. 3.

Effect of hypoglycin on incorporation of ^{14}C-labeled fatty acids into liver lipid in vivo

Previous experiments demonstrated a marked rise in serum NEFA level and moderate increase in liver total lipid following hypoglycin-treatment.[8] It was of further interest to study the effect of hypoglycin on incorporation of ^{14}C labeled substrates into liver lipids *in vivo*. These data are shown in Table 3 along with data on the conversion of ^{14}C labeled glucose into liver lipid. They show that hypoglycin treatment had no effect on the conversion of these substrates to liver total lipid. However, specific activities were somewhat lower since the total lipids were higher. This suggests a mobilization of lipid from body stores.

Effect of hypoglycin and related compounds on conversion of glucose U ^{14}C *or acetate*-1-^{14}C *to* CO_2 *by liver slices*

In these experiments labelled glucose or acetate was incubated alone or with the

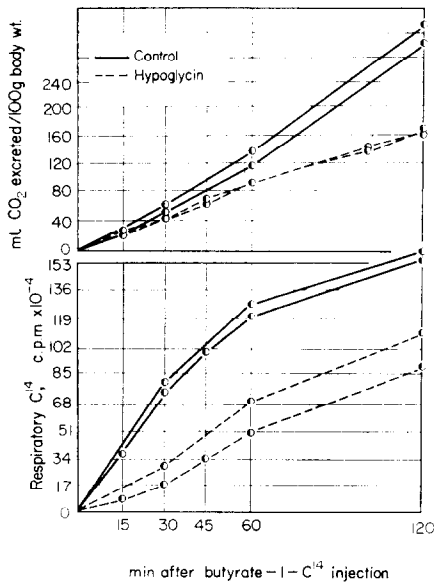

FIG. 2. Effect of the administration of hypoglycin on the metabolism of butyrate in the intact rat. Butyrate-1-^{14}C was administered intramuscularly to fed rats 4 hr after the intramuscular injection of hypoglycin. Each value is from a single rat.

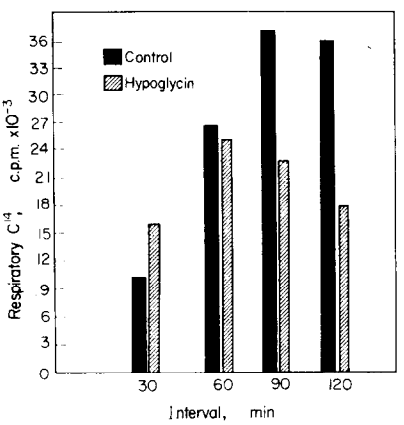

FIG. 3. Effect of hypoglycin on the excretion of $^{14}CO_2$ derived from stearate-1-^{14}C. The fatty acid was injected intramuscularly as a plasma emulsion 5 min after the intramuscular administration of hypoglycin (15 mg/100 g). Each value is from a single rat.

compound under study with liver slices obtained from fasted rats. Incorporation of ^{14}C into CO_2 was measured. The results are shown in Table 4. As expected, non-labeled acetate suppressed the conversion of acetate-1-^{14}C to CO_2. At the concentration employed, no marked effect on the conversion of either glucose or acetate to CO_2 was observed with hypoglycin, methylene*cyclo*propanepyruvic acid or methylene-*cyclo*propaneacetic acid. A slight stimulation of glucose conversion to $^{14}CO_2$ was observed in the presence of the latter compound, but further experiments must be done

TABLE 3. THE EFFECT OF THE ADMINISTRATION OF HYPOGLYCIN (15 mg/100 g) FOLLOWED BY ^{14}C-LABELED SUBSTRATES ON THE INCORPORATION OF ^{14}C INTO THE LIVER LIPIDS OF THE RAT

Exp. no.	Total counts/ min per liver	mg of lipid/ g liver	Liver wt. (g)	Total counts/ min per liver	mg of lipid/ g liver	Liver wt. (g)
Glucose-U-^{14}C						
1	9831	62·7	3·61	10,672	70·0	3·84
2	8740	56·5	5·27	7561	63·5	3·71
3	5865	59·9	2·04	6171	50·5	2·05
4	13,966	56·6	4·70	15,727	64·9	5·06
	9406	54·6	3·87	10,559	62·4	4·8
5	2460	56·3	2·79	3153	73·7	2·24
	7765	61·0	2·11	3316	78·7	2·03
Acetate-1-^{14}C						
6	4936	54·4	3·50	5512	65·2	3·95
7	5772	53·7	4·01	5610	72·2	4·17
Butyrate-1-^{14}C						
8	6176	59·3		7935	69·3	4·67
9	7867	55·1		7866	68·0	4·84

Acetate-1-^{14}C or Glucose-U-^{14}C (4 μc/100 g) administered intramuscularly with non-labelled glucose (50 and 100 mg/100 g, respectively) to fasted rats.

Butyrate-1-^{14}C (4 μc/100 g) bound to albumin administered intravenously to fed rats.

The radioactive compounds were administered 2 hr (exp. 1–4) or 4 hr (exp. 6–9) after hypoglycin. Liver lipids were measured 2 hr after the administration of ^{14}C-labeled compound.

In expt. 5 the glucose-U-^{14}C was administered 5 min after hypoglycin and the liver lipids were measured 4 hr later.

TABLE 4. THE EFFECT OF HYPOGLYCIN AND ANALOGS ON THE CO_2-PRODUCTION OF LIVER SLICES FROM GLUCOSE-U-^{14}C OR ACETATE-1-^{14}C *in vitro*

Substrate	Control	Hypo-glycin	2-Methylene *cyclo*propane pyruvic acid $CH_2 = \Delta\ CH_2COCOOH$	2-Methylene *cyclo*propane acetic acid $CH_2 = \Delta\ CH_2COOH$	Acetic acid CH_3COOH	4-Pentenoic acid $CH_2 = CHCH_2CH_2COOH$
Glucose-U-^{14}C 0·1 μc/ml	355 ±56	263 ±15	377 ±61	539		
Acetate-1-^{14}C 0·1 μc/ml	3299 ±19	2866 ±21	3470 ±31	3040 ±23		
	8994 ±18				5253 ±36	3174 ±48

Incubations were for 1 hr in K-110 buffer[19] containing 0·2% glucose, the substrate shown and 10^{-3} M of hypoglycin or analog. Values are counts/min per 100 mg tissue (±5·0) and the mean of three determinations. They represent metabolic CO_2 derived from the substrate.

in order to determine the significance of this finding. 4-Pentenoic acid significantly inhibited acetate-1-^{14}C conversion to CO_2. This compound has been reported to cause hypoglycemia in rats.[20]

Effect of hypoglycin on glucose uptake and glycogen deposition in rat diaphragm

Diaphragm from untreated or hypoglycin-treated rats was compared in these experiments. Hypoglycin was also added *in vitro* to diaphragm removed from untreated

rats. Results are shown in Table 5. No significant effect on glucose uptake was observed either when hypoglycin was added *in vitro* or when administered *in vivo*. It is interesting, however, to note the significant inhibition of net glycogen formation induced by hypoglycin both *in vivo* and *in vitro*.

Table 5. Effect of *in vivo* pretreatment with hypoglycin on the subsequent glucose uptake and glycogen formation of rat hemidiaphragm

(Starved rats were decapitated 4 hours after injection. Insulin at 1 milliunit/ml. Results expressed as mg/g of diaphragm).

	Control		Hypoglycin	
	U	S	U	S
Glucose uptake	3·14	5·00	3·43	4·95
		s.e. m. = ±0·29		
Glycogen formation	2·56	3·50	1·91	2·52
		s.e. m. = ±0·17		

	Glucose uptake	Glycogen formation
t between U and S of control =	4·54†	3·92†
t between U and S of hypoglycin =	3·71*	2·54*
t between U and U =	NS	2·71*
t between S and S =	NS	4·08*

Each value is the mean of four determinations.
U = unstimulated.
S = stimulated by insulin.
* = $P < 0.01$.
† = $P < 0.001$.
NS = not significant.

Effect of hypoglycin on R.Q. of rat liver slices

The effect of hypoglycin on R.Q. of liver slices prepared from hypoglycin-treated rats is shown in Table 6. It can be seen that the R.Q. was slightly lowered due to a higher QO_2 when hypoglycin was administered 4 hr prior to the removal of the liver.

Table 6. The effect of hypoglycin (10 mg intramuscularly/100 g) on the subsequent R.Q. of rat liver slices *in vitro*

	Control			Hypoglycin		
	QCO_2	QO_2	R.Q.	QCO_2	QO_2	R.Q.
Exp. A (2 hr) Means (12)	2·6	4·3	0·61	2·8	4·4	0·64
			No significant differences			
Exp. B (4 hr) Means (12)	3·1	4·8	0·65	3·4	5·6	0·60

t between control and hypoglycin for QCO_2 = NS
t between control and hypoglycin for QO_2 = 3·7*
t between control and hypoglycin for R.Q. = 2·32*
* $P < 0.05$

Slices were incubated in Krebs–Ringer phosphate medium. In expt. A the livers were removed 2 hr after hypoglycin-treatment. In expt. B the livers were removed 4 hr after hypoglycin-treatment.

No significant effect was apparent 2 hr after administration of hypoglycin (experiment A).

Effect of hypoglycin on adrenal corticoid secretion

It was conceivable that the pituitary–adrenal axis was involved in some of the metabolic changes brought about by hypoglycin. In order to investigate this possibility, the output of adrenal corticoids was studied. The parenteral administration of hypoglycin at 10 or 15 mg/100 g did not significantly change the adrenal corticoid secretion pattern. Also, when hypoglycin was incubated, at 200 μg to 2000 μg/ml of medium, with adrenal slices from untreated rats there was no statistically significant effect on corticoid output.

DISCUSSION

Hypoglycin apparently induces hypoglycemia in a unique manner. Although blood glucose levels are markedly decreased by 3 hr after hypoglycin treatment, little other effect on overall glucose metabolism is evident. Glucose conversion to respiratory CO_2 is unaffected and the lack of action of hypoglycin on the conversion of glucose to liver lipid or muscle glycogen has been previously reported.[8] The only direct effect on carbohydrate metabolism revealed from the present study is an inhibition of net glycogen synthesis from glucose in diaphragm. Although Patrick has reported an inhibition of glycogen formation from glucose in the liver,[7] interpretation of his results is difficult.

The dominant effects of hypoglycin are apparently those concerned with fatty acid metabolism. Following its administration *in vivo*, hypoglycin induces a pronounced increase in serum NEFA level, a moderate increase in the total lipids of the liver, and a significant inhibition of the rate of the conversion of butyrate or stearate to respiratory CO_2. Moreover, liver mitochondria isolated from hypoglycin-treated rats show an impaired ability to form high-energy phosphate bonds, an effect which has been reported to be correlated with the ability of a compound to induce fat infiltration in the liver.[21]

Hypoglycin does not immediately affect blood sugar levels as does insulin, but causes a profound lowering within 3 or 4 hr. This delayed effect may be because hypoglycin is metabolized to an active metabolite or possibly because the effects on blood sugar are secondary to some primary metabolic block. Both possibilities could occur. The fact that analogues without the double bond or the *cyclo*propane ring are not active as hypoglycemic agents suggests the uniqueness of this combination for hypoglycemic activity.[3]

The inhibition of oxidative phosphorylation observed in liver mitochondria of hypoglycin-treated rats and the lack of inhibition when hypoglycin is added *in vitro* to normal mitochondria further supports the idea that a metabolite of hypoglycin is the true hypoglycemic agent. Finally, methylene*cyclo*propanepyruvic acid and methylene*cyclo*propaneacetic acid, both likely metabolites, do produce hypoglycemia.[8] It is possible that such metabolites interfere with fatty acid oxidation either by binding coenzyme A or by interfering with the oxidation of normal fatty acid substrates in some other manner.

When administered *in vivo*, hypoglycin had no effect on the conversion of U-^{14}C-glucose or 1-^{14}C-acetate to respiratory CO_2, but it significantly inhibited the conversion of 1-^{14}C-labeled butyrate ,palmitate and stearate to CO_2. The total amount of respired

CO_2 was always reduced. These data support the idea of a metabolic block in the oxidation of fatty acids.* However, it must be pointed out that during the time interval of these studies a significant rise of serum NEFA was occurring.[8] This rise in NEFA may dilute the fatty acid pool sufficiently to account for the observed decrease in oxidation of the labeled acids.

Hypoglycin, 2-methylene*cyclo*propanepyruvic acid and 2-methylene*cyclo*propaneacetic acid had no effect on acetate metabolism in liver slices; 4-pentenoic acid, which inhibited the metabolism of acetate in the liver slice system, was only a weakly-active hypoglycemic agent in the intact animals.[23] The oxidation of butyrate by liver slices in the presence of hypoglycin or of the possible active metabolites of hypoglycin, such as 2-methylene*cyclo*propaneacetic acid, has not yet been studied.

SUMMARY

(1) Some effects of hypoglycin, α-aminomethylene*cyclo*propanepropionic acid, on certain aspects of glucose and fatty acid metabolism in the rat have been presented.

(2) Hypoglycin does not influence the conversion of glucose-U-^{14}C or acetate-1-^{14}C to respiratory CO_2 either in the intact rat or when added *in vitro* to normal liver slices.

(3) Hypoglycin markedly decreases the rate of conversion of butyrate-1-^{14}C or stearate-1-^{14}C to respiratory CO_2 in the intact rat.

(4) Liver mitochondria from hypoglycin-treated rats show an impaired ability to synthesize high-energy phosphate bonds associated with the oxidation of pyruvate and malate.

(5) The synthesis of glycogen from glucose in diaphragm is decreased by hypoglycin both when it is administered *in vivo* or added *in vitro*. Glucose uptake is unaltered in both cases.

(6) A small, but statistically significant decrease in R.Q. is observed in liver slices prepared from hypoglycin-treated rats.

(7) Hypoglycin is shown to have no effect on adrenal corticoid output when administered *in vivo* or added *in vitro* to normal adrenal slices.

(8) The evidence is discussed in connection with a possible inhibitory effect of hypoglycin on fatty acid metabolism.

Acknowledgement—The writers are indebted to Dr. P. H. Bell and to Dr. B. L. Hutchings for their interest in these studies.

* von Holt[22] has also recently suggested that hypoglycin may affect fatty acid oxidation.

REFERENCES

1. C. H. HASSALL, K. REYLE and P. FENG, *Nature, Lond.* **173**, 356 (1954).
2. C. H. HASSALL and K. REYLE, *Biochem. J.* **60**, 334 (1955).
3. R. S. DE ROPP, J. C. VAN METER, E. C. DE RENZO, K. W. MCKERNS, C. PIDACKS, P. H. BELL, E. F. ULLMAN, S. R. SAFIR, W. J. FANSHAWE and S. B. DAVIS, *J. Amer. Chem. Soc.* **80**, 1004 (1958).
4. C. VON HOLT and W. LEPPLA, *Angew. Chem.* **70**, 25 (1958).
5. S. WILKINSON, *Chem. & Ind.* **17** (1958).
6. J. A. CARBON, W. B. MARTIN and L. R. SWETT, *J. Amer. Chem. Soc.* **80**, 1002 (1958).
7. S. J. PATRICK, *J. Appl. Physiol.* **7**, 140 (1954).
8. E. C. DE RENZO, K. W. MCKERNS, H. BIRD, W. CEKLENIAK, B. COULOMB and E. KALEITA, *Biochem. Pharmacol.* **1**, 236 (1958).

9. K. K. CHEN, R. C. ANDERSON, N. C. MCGOWEN and P. N. HARRIS, *J. Pharmacol. Exp. Therap.* **121**, 272 (1957).
10. N. J. NELSON, *J. Biol. Chem.* **153**, 376 (1944).
11. W. C. STADIE, N. HAUGAARD and J. B. MARSH, *J. Biol. Chem.* **188**, 167 (1950).
12. R. A. SHIPLEY, E. B. CHUDZIK and P. GYORGY, *Arch. Biochem.* **16**, 301 (1948).
13. K. W. MCKERNS, B. COULOMB, E. KALEITA and E. C. DE RENZO, *Endocrinology* **63**, 709 (1958).
14. K. W. MCKERNS and E. NORDSTRAND, *Canad. J. Biochem.* **33**, 681 (1955).
15. K. W. MCKERNS and E. NORDSTRAND, *Canad. J. Biochem.* **33**, 209 (1955).
16. M. DIXON, *Manometric Methods* (2nd Ed.). MacMillan, New York (1951).
17. W. W. UMBREIT, R. H. BURRIS and J. F. STAUFFER, *Manometric Techniques and Tissue Metabolism.* Burgess Publishing Company (1949).
18. T. M. BRODY and J. A. BAIN, *J. Pharm. Exp. Therap.* **110**, 148 (1954).
19. J. ASHMORE, J. H. KINOSHITA, F. B. NESBITT and A. B. HASTINGS, *J. Biol. Chem.* **220**, 619 (1956).
20. H. V. ANDERSON, J. L. JOHNSON, J. W. NELSON, E. C. OLSON, M. E. SPEETER and J. J. VAVRA, *Chem. & Ind.* **11**, 330 (1958).
21. M. U. DIANZANI and S. SCURO, *Biochem. J.* **62**, 205 (1956).
22. C. VON HOLT and L. VON HOLT, *Naturwissenschaften* **45**, 546 (1958).
23. Unpublished information.