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THE ANTAGONISM OF THE TOXICITY OF HYPOGLYCIN BY GLYCINE

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Abstract—(1) Administration of large amounts of glycine to rats injected with the toxic amino acid hypoglycin prevents death, hypoglycaemia and hypothermia, and greatly decreases the rise in the plasma concentrations of isovalerate plus 2-methylbutyrate, butyrate and of MCPA, an end-product of hypoglycin metabolism. (2) Isovalerate given intravenously to normal rats is rapidly metabolised with a 50% decrease in plasma concentrations after 11 min, and this time is shortened to 5 min by the simultaneous administration of glycine. The hypoglycin metabolite MCPA-CoA inhibits butyryl-CoA dehydrogenase (EC 1.3.2.1) and isovaleryl-CoA dehydrogenase in liver *in vivo*, and this inhibition was largely prevented by administration of glycine at the same time as hypoglycin. (3)Preincubation of liver mitochondrial fractions with glycine and MCPA prevented the development of inhibition of β-oxidation caused by MCPA alone. (4) Glycine also decreased the inhibition of pyruvate carboxylase in intact mitochondria by MCPA or by isovalerate. (5) The inhibition of glucose synthesis from pyruvate in isolated hepatocytes by hypoglycin was decreased by inclusion of glycine in the incubation medium. (6) It was concluded that tissue concentrations of glycine are rate-limiting for the formation of the glycine conjugates of isovalerate and of MCPA, catalysed by glycine *N*-acyltransferase (EC 2.3.1.13) which has a low K_m for most acyl-CoA esters and a high K_m for glycine. Glycine therefore decreases inactivation of some acyl-CoA dehydrogenases by MCPA-CoA, and the competitive inhibition by various acyl-CoA esters of the activation by acetyl-CoA of pyruvate carboxylase, and hence decreases the inhibition of gluconeogenesis from pyruvate. (7) It is suggested that administration of large amounts of glycine may be of use in human ackee poisoning.

Administration of hypoglycin (methylenecyclopropylalanine), the toxic hypoglycaemic principle of the Jamaican ackee fruit, *Blighia sapida*, causes profound disturbances of metabolism in many animal species [1–7]. These may include hypoglycaemia, isovalericadaemia, excretion of unsaturated dicarboxylic acids [8] and hypothermia. In rats [3, 4] and mice [9] the isovalericacidaemia was greater than in two fatal human cases [8]. Hypoglycin is transaminated to MCPP† in the cytosol and is then oxidatively deaminated to MCPA-CoA in the mitochondrial matrix [7, 10]. MCPA-CoA inhibits, apparently irreversibly, several acyl-CoA dehydrogenases [11, 12]. Isovaleryl-CoA and 2-methylbutyryl-CoA, which are metabolites of leucine and isoleucine respectively, accumulate in the mitochondrial matrix when their further metabolism is blocked by inhibition of isovaleryl-CoA and 2-methylbutyryl-CoA dehydrogenases. Hypoglycaemia is caused by impairment of gluconeogenesis following inhibition of β-oxidation at the stage of butyryl-CoA dehydrogenase (EC 1.3.2.1) (13–16), and indirectly of pyruvate carboxylase (EC 6.4.1.1) by acyl-CoA esters accumulating in the matrix as a consequence of the primary inhibitions by MCPA-CoA (11,16,17). Excess concentrations of acyl-CoA esters may either be hydrolysed in all tissues to the corresponding free acids causing an acidaemia or be conjugated in the liver and kidneys to their acyl-glycine conjugates, catalysed by glycine *N*-acyltransferase (EC 2.3.1.13). This enzyme has a low K_m for most of its acyl-CoA substrates and a high K_m for glycine [18]. Therefore the rate of formation of harmless glycine conjugates of MCPA and of branched-chain fatty acids from their acyl-CoA esters depends on local tissue concentrations of glycine rather than on the total amount of glycine in the animal.

The prediction that administration of large amounts of glycine would decrease the toxicity of hypoglycin was confirmed. A preliminary account of some of this work has already appeared [19].

MATERIALS AND METHODS

Chemicals. Hypoglycin (85%) pure was isolated from ackee seeds (11,20,21). The main impurities were leucine and isoleucine and there were no qualitative differences in its biological effects compared with pure hypoglycin obtained by the hydrolysis of hypoglycin B (11,20,21). Acyl-CoA and acyl-carnitine esters were prepared as previously described [22, 23]. Isovaleryl-glycine was a gift from Dr S. J.

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† Abbreviations: MCPP, methylenecyclopropylpyruvate; MCPA, methylenecyclopropylacetate; MCPA-CoA, methylenecyclopropylacetyl-CoA; CoA, coenzyme A (sum of all forms); CoA, coenzyme A (esterified form); CoASH (free form); HEPES, *N*-2-hydroxyethylpiperazine-*N'*-ethanesulphonic acid; MOPS, 3-(*N*-morpholino)-propanesulphonic acid; EDTA, ethylenediamine-tetra-acetic acid; EGTA, ethanedioxybis-(ethylamine)-tetra-acetic acid.

Gatley. [^{14}C]Bicarbonate (specific radioactivity, 59.7 mCi/mmole) was purchased from the Radiochemical Centre, Amersham, U.K. The sources of other materials used have been given [11, 12].

Animals. Albino, male Wistar rats (usually 250–300 g) from a local inbred strain were used.

Toxicity studies with hypoglycin. Rats were deprived of food overnight and then injected intraperitoneally with hypoglycin (150 mg/kg body wt as a 1% solution in 0.14 M NaCl). Glycine (75 mg/kg body wt as an 0.33 M solution in 0.14 M NaCl) was also given intraperitoneally where appropriate with hypoglycin and then subsequently every hour for 6 hr to maintain high tissue concentrations. This amount would give an extra concentration of glycine of about 5 mM *in vivo* following each injection, assuming a uniform distribution throughout the body water without any metabolism or excretion. Controls were given 0.14 M NaCl, or 0.14 M NaCl followed by glycine. Blood samples (up to 0.1 ml) were taken from the tail vein and glucose concentrations were measured by the glucose oxidase (EC 1.1.3.4) method [12] and rectal temperatures were monitored with a digital thermometer and a thermistor probe [12]. Animals were killed by cervical dislocation and liver mitochondrial fractions prepared [11]. All animals were kept at an ambient temperature of 22°.

Determination of plasma volatile fatty acid concentrations. Blood was obtained from the tail vein of conscious rats, or through a polythene cannula (internal diameter No. 20 gauge) implanted in the right jugular vein of rats (150–270 g) anaesthetised with α-chloralose [24], and collected in heparinised tubes. The jugular veins were cannulated immediately before collection of blood [24]. Plasma volatile fatty acid concentrations were determined by gas–liquid chromatography as previously described [12]; isovaleric and 2-methylbutyric acids could not be separated by the method used.

Chromatography of acylglycine esters in urine. The 24 hr urine output from 3 groups of 3 rats in individual metabolism cages was collected in vessels containing 2 ml H_2SO_4. This (1 ml) was extracted with 2 ml of $CHCl_3$/*n*-butanol (5:1, v/v) and the extract chromatographed on sheets of silica gel with *n*-butanol/acetic acid/H_2O (5:1:1, v/v/v) against standards of authentic isovalerylglycine. The spots were detected by spraying with alkaline bromocresol purple (0.1 g/100 ml of ethanol containing 0.1 ml of conc. NH_3), and an estimate (± 10%) made of the concentrations of isovalerylglycine [25].

Enzyme assays. Palmitoyl-CoA (long-chain) dehydrogenase (EC 1.3.2.2), butyryl-CoA dehydrogenase, isovaleryl-CoA dehydrogenase and malate dehydrogenase (EC 1.1.1.37) were assayed at 20° as previously described [11,12,23].

Preparation of mitochondrial fractions. Mitochondrial fractions were prepared from livers in 0.3 M mannitol, 5 mM HEPES, 0.1 mM EGTA, pH 7.2 [11].

Measurement of β-oxidation by mitochondria. Oxygen uptake by mitochondrial fractions with 20 μM palmitoyl-carnitine as substrate was measured polarographically at 30° and pH 7.2 [11, 22] in the presence of malonate so that acyl-groups were quantitatively converted to acetoacetate and the rate of oxygen uptake was a direct measure of the flux through β-oxidation [6].

Determination of protein. Protein was determined by the method of Lowry *et al.* [26].

Measurement of pyruvate carboxylation by mitochondria. The pyruvate-dependent fixation of $^{14}CO_2$ was taken as a measure of pyruvate carboxylase activity in intact mitochondria [27]. The reaction was started by adding the mitochondrial fraction (2.6–15 mg of protein) to 2.0 ml of medium at 30°, pH 7.6, containing 120 mM KCl, 5 mM phosphate, 10 mM $KHCO_3$, 5 mM pyruvate and [^{14}C]bicarbonate to give about 1500 cpm, in sealed plastic vials with an atmosphere of 95% O_2/5% CO_2 with shaking (80 strokes/min). The reaction was stopped by the addition of cold 1.65 M $HClO_4$ to give a final concentration of 0.33 M $HClO_4$, and a small piece of solid CO_2 was added to displace unfixed $^{14}CO_2$. The mixture was then centrifuged in an Eppendorf microcentrifuge at 9300 *g* for 2 min and the radioactivity of the supernatant was determined using an Intertechnique SL 50 liquid scintillation counter [28].

Preparation of isolated hepatocytes. Isolated hepatocytes were prepared after perfusion of rat livers with collagenase [29]; these preparations usually contained about 90% of viable cells as indicated by trypan blue exclusion. The cells were suspended in Krebs–Ringer bicarbonate medium and incubated with shaking at an atmosphere of 95% O_2/5% CO_2, and glucose synthesis from 10 mM pyruvate determined as previously described [30].

RESULTS

Effects of glycine on the toxicity, blood glucose concentrations and rectal temperatures in rats given hypoglycin

The administration of large amounts of glycine to rats treated with hypoglycin dramatically decreased its toxicity and the development of hypoglycaemia and hypothermia (Fig. 1, Table 1). The maximum difference between blood glucose concentrations in groups of 6 rats given hypoglycin, or hypoglycin plus glycine, was after 6 hr (2 mM, $P < 0.01$), and after 24 hr for rectal temperatures (4.1°, $P < 0.05$). After 36 hr, two animals given hypoglycin had apparently recovered while four died with severe hypothermia (Table 1). Animals given 0.14 M NaCl had the same blood glucose concentrations and rectal temperatures as animals given glycine alone.

Effects of glycine on the concentrations of plasma volatile fatty acids in rats given hypoglycin

Acetate (0.16–0.29 mM) was the only volatile fatty acid detected in the plasma of control animals. After administration of hypoglycin, MCPA appeared and increased to a maximum concentration of 0.62 ± 0.26 mM (S.E.M.) within 6 hr (Fig. 2), and was still detectable after 24 hr. By contrast, when glycine was given after hypoglycin the maximum concentration of MCPA was 0.06 mM after 6 hr, and it was not detected after 24 hr. The combined concentrations of isovalerate and 2-methylbutyrate 12 hr after administration of hypoglycin was 9 times greater [3.14 ± 1.1 mM (S.D.)] than in rats given both

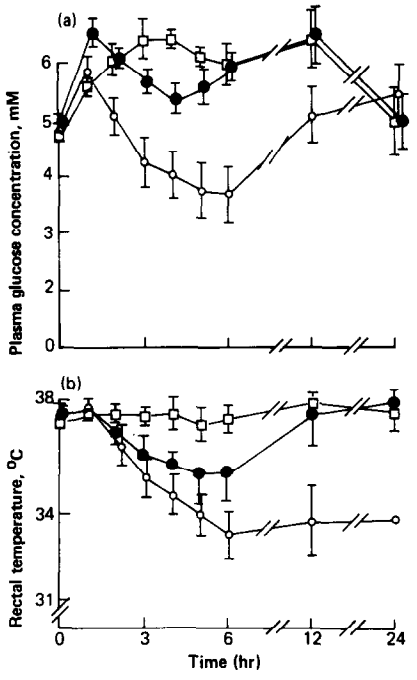

Fig. 1. The effects of glycine on blood glucose concentrations and rectal temperatures of rats given hypoglycin. Four starved rats were injected with 0.14 M NaCl (□), 6 with hypoglycin (150 mg/kg body wt) (○), and 6 with hypoglycin plus glycine (●), as described in the text. (a) Blood glucose concentrations, means ± S.E.M. (b) Rectal temperatures ± S.D.

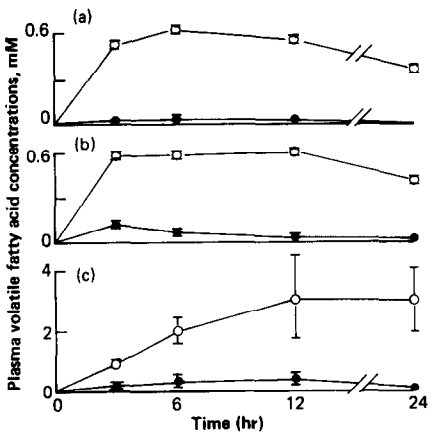

Fig. 2. The effects of glycine on the plasma concentrations of MCPA, butyrate and of isovalerate plus 2-methylbutyrate in rats given hypoglycin. Rats were starved overnight and then injected with hypoglycin (150 mg/kg body wt) and plasma volatile fatty acid concentrations were determined as described in the text. (a) MCPA after hypoglycin (○) or after hypoglycin plus glycine (●). (b) Butyrate after hypoglycin (○) or after hypoglycin plus glycine (●). (c) Isovalerate plus 2-methylbutyrate after hypoglycin (○) or after hypoglycin plus glycine (●). Values are measn ± S.D. for 6 animals.

hypoglycin and glycine [0.36 ± 0.17 mM (S.D.), $P < 0.01$] (Fig. 2). Even when plasma concentrations of MCPA were decreasing the concentrations of isovalerate plus 2-methylbutyrate rose to a maximum 24 hr after administration of hypoglycin [3.05 ± 1.1 mM (S.D.)]. Butyrate increased to a maximum concentration of about 0.6 mM within 6–12 hr in hypoglycin-treated animals, while it only rose to about 0.05 mM in those given both hypoglycin and glycine (Fig. 2).

Since butyryl-CoA dehydrogenase is inactivated in hypoglycin poisoning [9, 13], sodium butyrate (200 mg/kg body wt) was administered intraperitoneally to 3 rats, also given hypoglycin (100 mg/kg body wt), and to 3 rats given hypoglycin plus glycine (as described above), 24 hr prior to injection of butyrate. Food was withheld during the experiment. The plasma butyrate concentration in hypoglycin-treated animals after 24 hr was 0.77 ± 0.1 mM (S.D.). This increased to a maximum of 2.97 ± 0.69 (S.D.) 30 min after administration of butyrate and was still detectable 24 hr later (Fig. 3). By contrast, animals given hypoglycin plus glycine had butyrate concentrations of only 0.04 ± 0.03 mM (S.D.) 30 min after administration of butyrate, and butyrate was not detectable after 24 hr (Fig. 3). Butyrate given to control animals was rapidly cleared and could not be detected in plasma after only 5 min (not shown). All animals treated with hypoglycin and then given butyrate died within 36 hr of butyrate administration, whilst those given hypoglycin plus glycine and then butyrate survived.

Table 1. The effects of glycine on hypoglycin toxicity in rats

Compound administered	Blood glucose concentrations after 6 hr (mM)	Plasma isovalerate plus 2-methylbutyrate concentrations after 12 hr (mM)	Rectal temperature after 24 hr (°C)
0.14 M NaCl	4.48 ± 0.35 S.E.M. (4)	0	37.3 ± 0.1 S.D. (4)
Glycine	4.99 ± 0.68 (4)	0	37.0 ± 0.6 (4)
Hypoglycin*	2.71 ± 1.10† (6)	3.14 ± 1.14† S.E.M. (6)	33.9 ± 3.2‡ (6)
Hypoglycin plus glycine	4.97 ± 0.62† (6)	0.37 ± 0.17† (6)	38.0 ± 0.5‡ (6)

Starved rats were injected with hypoglycin and glycine as described in the text. The values are means ± S.E.M. or S.D. as appropriate and the number of animals is given in parentheses, these are for times of maximum differences between control and hypoglycin-treated animals which occurred at 6 hr, 12 hr and 24 hr for glucose, organic acids and for temperature respectively.

* Four animals died after 36 hr. Significance of the differences between values, † $P < 0.01$ or ‡ $P < 0.05$.

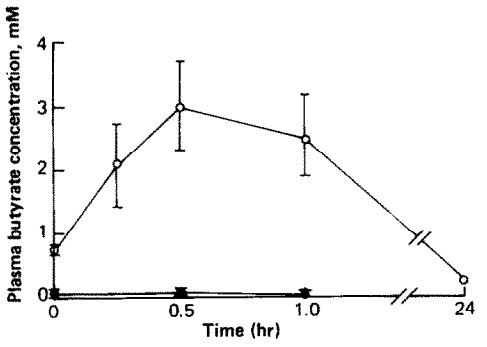

Fig. 3. The effects of glycine on plasma concentrations of butyrate in rats given hypoglycin. Rats were starved overnight and then injected intraperitoneally with hypoglycin (100 mg/kg body wt) (○) or hypoglycin plus glycine (75 mg per rat as an 0.33 M solution in 0.14 M NaCl at the same time as hypoglycin and then every hour for 6 hr) (●), 24 hr before the intraperitorieal administration of butyrate (200 mg/kg body wt). Plasma butyrate concentrations were determined as described in the text. Values are means ± S.D. for 3 animals.

Disposal of plasma isovalerate

The plasma concentrations of isovalerate 5 min after intravenous administration (200 mg/kg body wt) were 10.5 ± 1.1 mM (S.E.M.) for animals given isovalerate alone, and 6.1 ± 0.5 mM (S.E.M.) for those given isovalerate plus glycine (Fig. 4). In both cases, a semi-logarithmic plot of concentrations against time indicated biphasic elimination kinetics, with rapid lowering of isovalerate concentrations and an extrapolated initial plasma concentrations of about 15 mM. Administration of glycine decreased the time at which isovalerate fell to 50% of their initial values from about 11 min to 5 min.

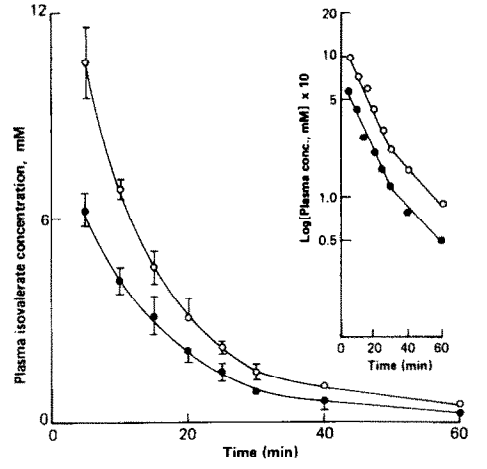

Fig. 4. The effects of glycine on the disposal of isovalerate in normal rats. Isovalerate (200 mg/kg body wt) was given intravenously through a previously implanted cannula in the right jugular vein, either alone (as 0.15 M sodium isovalerate) or together with 75 mg of glycine as 0.33 M glycine in 0.14 M NaCl. Blood samples were withdrawn at appropriate times via the cannula and replaced with an equal volume of 0.14 M NaCl or with 0.33 M glycine in 0.14 M NaCl. Plasma isovalerate concentrations were determined as described in the text; after isovalerate (○) or after isovalerate plus glycine (●). Values are means ± S.E.M. for animals. Insert: a semilogarithmic plot of this data.

Effect of glycine on the excretion of isovaleryl-glycine by rats given hypoglycin

Three groups of 3 rats were starved for 24 hr and then given hypoglycin intraperitoneally (100 mg/kg body wt). One group was then given glycine (as described above) after a further 30 min, and another after 3 hr, and the acyl-glycine excretion determined [25]. Most isovaleryl-glycine was excreted by the rats given hypoglycin alone and least by those given glycine after 3 hr (Table 2). This agrees with Kean and Rainford who confirmed the identity of isovaleryl-glycine after administration of [1-^{14}C]isovalerate [25]. Chromatograms of the urine extracts revealed a second acyl-glycine spot (R_f = 0.46). It was suggested that this was MCPA-glycine [25]. However, as it was most dense on chromatograms of urine from animals given hypoglycin alone its identity is uncertain.

Acyl-CoA dehydrogenase activities in liver mitochondrial fractions from rats given hypoglycin or hypoglycin plus glycine

Butyryl-CoA and palmitoyl-CoA dehydrogenase activities were measured in extracts of mitochondrial fractions prepared from livers taken from rats 24 hr after administration of hypoglycin. Butyryl-CoA dehydrogenase activity was inhibited by 60% compared with the controls. Palmitoyl-CoA dehydrogenase was not inhibited. Isovaleryl-CoA dehydrogenase was inhibited by about 40% and 2-methylbutyryl-CoA dehydrogenase by about 27%.

Butyryl-CoA dehydrogenase activity in extracts of mitochondrial fractions prepared from livers taken from rats 24 hr after administration of hypoglycin plus glycine was only inhibited by 20% and no inhibition of isovaleryl-CoA and 2-methylbutyryl-CoA activities was detected (Table 3).

Effect of glycine on the inhibition of palmitoyl-carnitine oxidation by isolated liver mitochondrial fractions

Preincubation of mitochondrial fractions with 0.5 mM MCPA decreased the rate and extent of oxidation of 10 μM palmitoyl-carnitine added subsequently, the palmitoyl-group only being oxidised as far as butyrate [6, 13]. Addition of high concen-

Table 2. Excretion of isovaleryl-glycine by rats given hypoglycin or hypoglycin plus glycine

Treatment	Urinary excretion of isovaleryl-glycine (mg/day/rat)	
Hypoglycin	60.0 ± 1.0	
Hypoglycin + glycine after 30 min	16.0 ± 4.0	*P < 0.02
Hypoglycin + glycine after 3 hr	25.0 ± 1.0	*P < 0.05

Values are the means ± S.E.M. for 3 rats. *P is the significance of the differences from hypoglycin alone. Experimental details are given in the text.

Table 3. Acyl-CoA dehydrogenase activities in mitochondrial fractions from livers taken from rats 24 hr after administration of hypoglycin or of hypoglycin plus glycine

Treatment	Percentage inhibition after 24 hr		
	Butyryl-CoA dehydrogenase	Isovaleryl-CoA dehydrogenase	2-Methylbutyryl-CoA dehydrogenase
Glycine	0	0	0
Hypoglycin	61 ± 6	39 ± 7	27 ± 5
Hypoglycin + glycine	21 ± 4	0	0

Values are the means ± S.E.M. for 3 animals. Suspensions of mitochondria in 10 mM phosphate, pH 7.2, were solubilised by addition of 1% Triton X-100 and the dehydrogenase activities were determined in the supernatants obtained after centrifugation at 150,000 g_{av} for 60 min [12]. The control rates were: butyryl-CoA dehydrogenase, isovaleryl-CoA dehydrogenase and 2-methylbutyryl-CoA dehydrogenase were 37 ± 8, 25 ± 7 and 20 ± 8 nmole/min/mg of protein respectively.

trations of glycine (25 mM) after preincubation with MCPA had no effect on this limited oxidation. However, addition of 8 mM glycine together with 0.5 mM MCPA essentially prevented inhibition of palmitoyl-carnitine oxidation, while 8 mM glycine alone had no effect on the rate (Fig. 5). Further, glycine allowed the complete oxidation of the palmitoyl-groups to acetoacetate (not shown).

Effect of glycine on the inhibition of gluconeogenesis in isolated hepatocytes by hypoglycin

The hepatocytes synthesised glucose at linear rates for 2 hr from 10 mM pyruvate, with very little apparent synthesis from 8 mM glycine. Addition of 4 mM hypoglycin caused up to 55% inhibition which only developed after 1 hr of incubation (Fig. 6), presumably because of the time taken to generate enough of the inhibitory metabolite MCPA-CoA [6, 12]. In the presence of 8 mM glycine the inhibition of glucose synthesis was significantly less ($P < 0.01$) (Fig. 6), maximum relief of inhibition was obtained with 8 mM glycine (not shown).

Effects of glycine on the inhibition of CO_2-fixation in liver mitochondrial fractions by MCPA and by isovalerate

Pyruvate carboxylase in the mitochondrial matrix catalyses fixation of CO_2 in the presence of pyruvate with the formation of oxaloacetate, with acetyl-CoA as an obligatory allosteric activator [31]. Although oxaloacetate largely undergoes further transformations measurement of the amount of $^{14}CO_2$ fixed (as several organic acids) is a good measure of the activity of pyruvate carboxylase. Preincubation of mitochondria with 5 mM glycine alone for 5 min had little effect on the fixation of $^{14}CO_2$. This was inhibited by 50% by preincubation with 0.05 mM MCPA, and 5 mM glycine decreased the inhibition

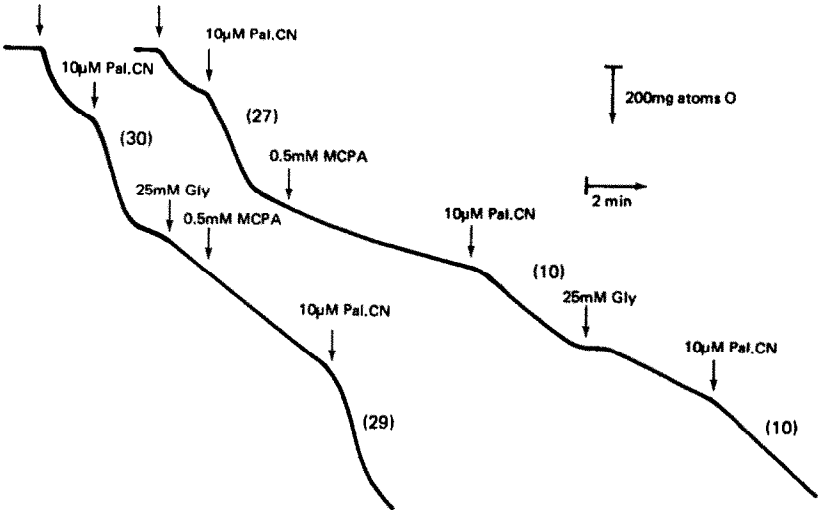

Fig. 5. The effect of glycine on the inhibition of state 3 oxidation (see Ref. [11]) of palmitoyl-carnitine by MCPA in isolated mitochondrial fractions. Rat liver mitochondria (about 9 mg of protein) were added where indicated by unlabelled arrows to 3.0 ml of medium at 30° containing 120 mM KCl, 10 mM MOPS, 5 mM $MgCl_2$, 2.5 mM phosphate, 1.0 mM EDTA, 1.0 mM ADP and 5 mM malonate, pH 7.2. Other additions were made as shown, palmitoyl-carnitine (Pal.CN), glycine (Gly). The rates of oxygen uptake (ng atoms of O per min per mg of protein) are given in parentheses.

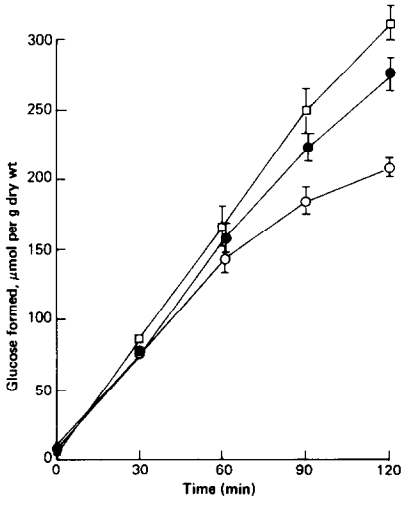

Fig. 6. The effect of glycine on the inhibition by hypoglycin of glucose synthesis from pyruvate in isolated rat hepatocytes. Hepatocytes (10 mg dry wt) were incubated in a final volume of 4.0 ml of Krebs–Ringer-phosphate medium containing 10 mM pyruvate as described in the text; glucose synthesis from pyruvate (□), pyruvate plus 4 mM hypoglycin (○) or pyruvate plus 4 mM hypoglycin and 8 mM glycine (●). Values are means ± S.E.M. for 3 incubations.

to about 20% (Fig. 7). Preincubation with 0.2 mM isovalerate also inhibited $^{14}CO_2$-fixation by 60%, while in the presence of 5 mM glycine the inhibition was 40% (Fig. 7).

DISCUSSION

The primary inhibitions in hypoglycin poisoning are of some acyl-CoA dehydrogenases, including butyryl-CoA, isovaleryl-CoA and 2-methyl-CoA dehydrogenases by MCPA-CoA, leading to accumulation of their substrates in the mitochondrial matrix (11–15). Acyl-CoA dehydrogenases concerned in the catabolism of polyunsaturated fatty acids are also inhibited [32]. Detailed arguments have been given [6, 11] against the claim that the biochemical effects of hypoglycin are simply due to sequestration of CoA (and also of carnitine) as its inert metabolites [33].

Several enzymes in liver mitochondrial fractions hydrolyse acetyl-CoA and other acyl-CoA esters. These are mainly latent and may be partly lysosomal [34–36]. Such deacylases also occur widely in extrahepatic tissues. Many have very high K_m's for their substrates (about 0.5–2 mM) and some are inhibited by CoASH [14, 35]. Their kinetic properties indicate that deacylation is a safety mechanism to oppose too much acylation of the available CoA (the total CoA concentrations in the mitochondrial matrix is about 5 mM [11]). It may be assumed that normally steady-state concentrations of CoA esters involved in metabolism are not high enough to be deacylated significantly [6, 12]. Only when there is excessive accumulation of CoA esters (either of endogenous or foreign origin) as in some inborn errors of metabolism such as isovalericacidaemia or following ingestion of some poisons including hypoglycin, is deacylation likely to become important.

Another major route for the disposal of some acyl-CoA esters with recycling of CoASH is by formation of acyl-glycine conjugates catalysed by glycine *N*-acylase, apparently limited to liver and kidney [37]. Glycine *N*-acylase occurs in the matrix of liver mitochondria [38] and has a high K_m for glycine; 3.3 mM with tiglyl-CoA as cosubstrate [18] or 15 mM according to older work [39]. Both V_{max} and K_m values for different acyl-CoA esters vary, the K_m is 0.18 mM for isovaleryl-CoA and 0.11 mM for 2-methyl-butyryl-CoA (there is no information for MCPA-CoA) [18]. The rate of conjugation of an acyl-CoA ester *in vivo* therefore depends both on

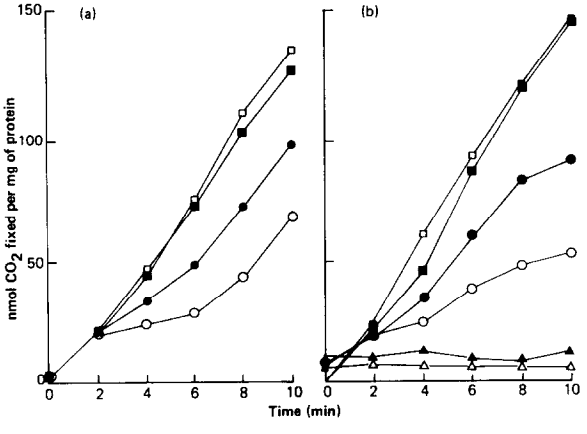

Fig. 7. The effects of glycine on the inhibition of pyruvate-dependent fixation of CO_2 by MCPA and by isovalerate in isolated rat liver mitochondrial fractions. Pyruvate-dependent fixation of CO_2 was measured as described in the text. (a) Inhibition by MCPA, additions: 5 mM pyruvate (□), 5 mM pyruvate plus 5 mM glycine (■), 5 mM pyruvate plus 50 μM MCPA (○), 5 mM pyruvate plus 50 μM MCPA, and 5 mM glycine (●). (b) Inhibition by isovalerate, additions: 5 mM pyruvate (□), 5 mM pyruvate plus 5 mM glycine (■), 5 mM pyruvate plus 0.2 mM isovalerate (○), 5 mM pyruvate plus 0.2 mM isovalerate and 5 mM glycine (●), 0.2 mM isovalerate (△), and 5 mM glycine (▲). Mitochondria were preincubated with the other additions before adding pyruvate.

its own concentration, and on that of glycine which was reported to be about 5 mM in livers of starved rats [40] and which is unlikely to be saturating. Glycine readily crosses the inner mitochondrial membrane and its concentration equilibrates between the cytosol and matrix [41]. Conjugation of acyl-CoA esters therefore competes most effectively with hydrolysis when there is a high concentration of glycine. It was predicted that artificially increasing the concentration of glycine should decrease the toxicity of hypoglycin in three ways. First, by directly decreasing the inhibition of target dehydrogenases by lowering the concentration of MCPA-CoA in liver and kidney. Second, by increasing the rate of conjugation of excess isovaleryl-CoA and of 2-methylbutyryl-CoA in these tissues should any inhibition still occur, thus decreasing the amounts of isovalerate and of 2-methylbutyrate formed by hydrolysis of these esters [19]. Third, by indirectly lowering the concentration of MCPA-CoA in peripheral mitochondria.

This prediction was fulfilled since the administration of large amounts of glycine dramatically prevented the lethal, hypoglycaemic, organicacidaemic and hypothermic effects of hypoglycin (Fig. 1, Table 1) [19]. Butyryl-CoA dehydrogenase was not inactivated sufficiently to impair β-oxidation in rats given hypoglycin and glycine, since plasma butyrate concentrations remained low (Figs. 2 and 3). Exogenous glycine, however, did not prevent small increases in plasma concentrations of isovalerate plus 2-methylbutyrate and of MCPA (Fig. 2, Table 1). Hypoglycin is also converted to MCPP, and some of this is oxidatively decarboxylated to MCPA-CoA, in extrahepatic tissues [6] so that some inhibition of branched-chain fatty acid catabolism and of β-oxidation would not be prevented in those tissues where conjugation does not occur. Because of the greater specific activities of leucine-glutamate aminotransferase (EC 2.6.1.6) in muscle, and of branched-chain 2-oxoacid dehydrogenase (EC 1.2.4.4) in liver [42], much, but not all, of the MCPP and 2-oxoisocaproate formed peripherally is transported in the blood to the liver to be converted to MCPA-CoA and isovaleryl-CoA respectively (see Ref. [6]). Further, MCPA-CoA, and excess isovaleryl-CoA and 2-methylbutyryl-CoA formed in peripheral tissues is deacylated, and the MCPA, isovalerate and 2-methylbutyrate released (normally isovalerate and 2-methylbutyrate cannot be detected in blood (Table 1)) is also transported to the liver and kidneys where some of each of these free acids is reacylated in mitochondria by an acyl-CoA synthetase (presumably butyryl-CoA synthetase (EC 6.2.1.2) [11]). The concentration of MCPA-CoA in the matrix of mitochondria depends on the balance of the rates of a 'futile cycle' between acylation and deacylation, that of acylation being dependent on the availability of circulating MCPA which in turn is partly determined by the rate of conjugation of MCPA. An increased rate of conjugation of MCPA-CoA with glycine in the liver and kidneys therefore indirectly decreases the concentration of MCPA-CoA in other tissues. Evidence for the formation of CoA esters of MCPA and of isovalerate is given by the rapid clearance of injected isovalerate in normal rats [25], which is increased further by administration of glycine (Fig. 4), and by the hypoglycaemic effects of free MCPA [43] which does not directly inhibit β-oxidation [14].

There is evidence that impaired gluconeogenesis in hypoglycin-poisoning is in part due to competitive inhibition of the activation of pyruvate carboxylase by acetyl-CoA by the excessive accumulation of acyl-CoA esters including butyryl-CoA, isovaleryl-CoA and MCPA-CoA [11, 17]. Inhibition of gluconeogenesis from pyruvate by hypoglycin or by isovalerate in isolated hepatocytes was decreased by glycine (Fig. 6). Further evidence for the proposed mechanism of the protective effect of glycine is provided by the prevention of the inhibition of β-oxidation by MCPA-CoA (Fig. 5), and of pyruvate carboxylase by MCPA or isovalerate, in mitochondrial fractions from liver (Fig. 7).

These results suggest that administration of large amounts of glycine may be of value in the treatment of ackee poisoning in man (in addition, of course, to other appropriate therapy) [19]. Glycine is both inexpensive and apparently nontoxic. It has already been used to lower plasma concentrations of isovalerate in the treatment of hereditary isovalericacidaemia where there is an absence of isovaleryl-CoA dehydrogenase [44, 45]. It is well known that administration of glycine increases the rate of elimination of benzoate and some other aromatic acids as their glycine conjugates (see Refs. [46, 47]), this effect having been reported as long ago as 1914 [48]. Conversely administration of benzoate has been used to lower plasma concentrations of glycine in hereditary hyperglycinaemia [49].

Two other compounds, L-carnitine and riboflavin, have been reported to decrease the hypoglycaemic effects and toxicity of hypoglycin [33, 50]. We have been unable to obtain any convincing evidence that L-carnitine protects against the acute hypoglycaemic or hypothermic effects of hypoglycin in mice, nor had it any effect on the organicacidaemia [9, 51]. The protection by riboflavin against the chronic toxicity of repeated administration of hypoglycin is well established [50, 51]. Riboflavin may permit the synthesis of new acyl-CoA dehydrogenases which have flavin prosthetic groups [6].

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