Rapid stimulation of the hepatic glycine-cleavage system in rats fed on a single high-protein meal

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Glycine catabolism was studied in isolated rat liver mitochondria by measuring the release of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ glycine. Mitochondria isolated from rats fed on a high-protein (60% casein) diet for 5 days showed an enhanced ability to catabolize glycine compared with mitochondria from rats fed on a normal-protein (15% casein) diet. Glycine catabolism was also stimulated in normal protein-fed rats if they ingested a single high-protein meal for 2 h before being killed, thus illustrating the rapid response of the glycine-cleavage system to protein intake. The stimulation of glycine catabolism in rats given a high-protein diet or meal was not evident if the mitochondria were incubated in the absence of P_i (omitting ADP had no effect on the rate). Mitochondria from high-protein- and normal-protein-fed rats did not differ in their ability to accumulate glycine, a process which occurred far too rapidly to impose a limit on the rate of flux through the glycine-cleavage system. The stimulation of glycine catabolism by high-protein feeding was not associated with a change in mitochondrial matrix volume. Furthermore, mitochondria from rats fed on a high-protein meal maintained an enhanced ability to catabolize glycine compared with those from rats fed on a normal-protein meal when incubated in hypo-osmotic solutions of very low osmolality. When mitochondria from high-protein- or normal-protein-fed rats were maximally activated by incubation in the presence of $0.25\ \mu\text{M}\text{-Ca}^{2+}$, the rates of glycine catabolism were high, but similar, showing that the stimulation of glycine catabolism by high-protein feeding does not involve an increase in the total capacity of the system. These findings show that hepatic glycine catabolism is stimulated rapidly by high-protein feeding, a response that we suggest is involved in the disposal of the excess glycine in the diet.

INTRODUCTION

The hepatic glycine-cleavage system is responsible for the bulk of glycine catabolism in mammals. It is an enzyme complex in the mitochondrial inner membrane (Hiraga *et al.*, 1972), consisting of a pyridoxal phosphate-dependent glycine decarboxylase, a lipoic acid-containing aminomethyltransferase, a N^5,N^{10} -methylenetetrahydrofolate-synthesizing protein, and a flavin-containing dihydrolipoyl dehydrogenase. Although the components and mechanism of the reaction have been established (Kikuchi, 1973), the metabolic regulation of the glycine-cleavage system is poorly understood. High concentrations of branched-chain oxo acids have been shown to inhibit the system (O'Brien, 1978; Kochi *et al.*, 1986). The locus of this inhibition is proposed to be the dihydrolipoyl dehydrogenase which is shared by the glycine-cleavage system and the α -oxo acid dehydrogenases. Hampson *et al.* (1983) reported that the glycine-cleavage system is sensitive to the NAD(H) and NADP(H) redox couples. Conditions which lead to the oxidation of the mitochondrial nicotinamide nucleotides stimulate glycine-cleavage-system flux, whereas reducing conditions inhibit glycine catabolism.

These observations do not explain the very rapid rate of hepatic glycine removal after a high-protein meal (Fafournoux *et al.*, 1990; Ishikawa, 1976; Remesy *et al.*, 1983; Yamamoto *et al.*, 1974). A high-protein meal lowers the concentration of glycine in liver (Fafournoux *et al.*, 1990; Yamamoto *et al.*, 1974), suggesting a stimulation of intra-hepatic metabolism rather than of membrane transport. Feeding a diet high in protein raises circulating levels of branched-chain amino acids (Schauder, 1984) and also leads to a lower mitochondrial NAD^+/NADH ratio in liver (Peret *et al.*, 1981) and hence, by the mechanisms proposed previously, would be expected to inhibit glycine catabolism. That they do not suggests that they are physiologically ineffective and

that other regulatory mechanisms must exist. Jois *et al.* (1989) reported that glycine catabolism is stimulated by glucagon in isolated hepatocytes. The stimulatory effect of glucagon on glycine catabolism is also evident in mitochondria isolated from rats that had been injected with glucagon 25 min before being killed. A rapid activation of glycine catabolism is also suggested by the findings of Couet *et al.* (1990) that, when glucagon was infused into humans, plasma glycine levels were lowered. Since ingestion of a high-protein meal is known to increase circulating glucagon concentrations (Robinson *et al.*, 1981), this hormone may provide an important signal for the hepatic removal of glycine after such a meal. We therefore hypothesized that the hepatic glycine-cleavage system would be activated in animals fed on a high-protein diet or meal, and that this would be evident in mitochondria isolated from these animals. In the present paper we report that intact liver mitochondria isolated from rats maintained on a high-protein diet or fed on a single high-protein meal exhibit an enhanced ability to catabolize glycine as compared with those from rats fed on a normal-protein diet or meal.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (200–350 g) were maintained on a standard Purina pellet diet (*ad libitum*) until the treatment period, at which time they were switched to a purified diet offered in powder form, again *ad libitum*. Lights were off from 20:00 to 08:00 h. Animals rapidly adjusted to the purified diet, as indicated by a marginal change in body weight on day 1 of the diet, followed by normal weight gain on subsequent days. In a few instances, where weight gain did not occur, the animal was removed from the study.

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In experiments designed to investigate the effect of changing the protein content of the diet on glycine catabolism, rats were fed on a high-protein or normal-protein diet for 5 days. On day 5 at 14:00 h the animals were put in clean cages and were offered the alternate diet (high-protein-fed rats received the normal-protein diet, and vice versa). The animals were killed at 09:00 h the following day. A similar protocol was used to investigate the effect of a single meal on glycine catabolism, except that in these experiments the animals were fasted for a short period (14:00–20:00 h) to prevent any food intake during this period, and thus ensuring that the animals ate promptly when they were given a meal at 20:00 h. Controls were killed at 20:00 h. The others received the various meals at 20:00 h and were subsequently killed at 22:00 h and midnight.

In experiments involving glucagon, rats maintained on the standard pellet diet were injected (intraperitoneally) with 0.1 mg of glucagon/100 g body wt, dissolved in 0.9% NaCl/0.05% BSA. Control rats were injected with vehicle. Rats were killed 20–30 min after injection.

Diets

The diets were modified AIN-76 diets (Bieri *et al.*, 1977). The high-protein diet was made up of (g/100 g of diet): casein, 59.8; L-methionine, 0.15; corn starch, 6.1; sucrose, 19.2; corn oil (Mazola), 5; vitamin mixture (AIN 76), 3.5; mineral mixture (AIN 76), 1; alphacel, 5; choline bitartrate, 0.2. For the normal-protein diet casein was decreased to 14.8 g/100 g in favour of sucrose and corn starch, which were increased to 53.3 and 17 g/100 g respectively.

Isolation of liver mitochondria

Mitochondria were isolated as described by Jois *et al.* (1989). The isolation medium consisted of: mannitol, 225 mM; sucrose, 75 mM; EGTA, 1 mM; Hepes, 5 mM; pH 7.4. The mitochondrial pellet was resuspended in 0.5 ml of the above medium to give a concentration of 50–70 mg of mitochondrial protein/ml as determined by the biuret test with BSA as standard (Gornall *et al.*, 1949). The yield of mitochondria was similar for high-protein- and normal-protein-fed rats and were acceptable if they displayed a respiratory control ratio greater than 4 with 2-oxoglutarate as substrate. Oxygen uptake was measured with a Clark-type electrode in KCl (140 mM)/KH₂PO₄ (4 mM)/MgCl₂ (2.5 mM)/EDTA (1 mM)/Hepes (5 mM)/BSA (1 mg/ml), pH 7.4. Rates of succinate oxidation were determined at ADP and succinate concentrations of 0.5 mM and 5 mM respectively.

Incubations

Flux through the glycine-cleavage system was measured by quantifying the release of ¹⁴CO₂ by mitochondria incubated in the presence of [1-¹⁴C]glycine (Jois *et al.*, 1989). The standard incubation medium (KCl-based medium) consisted of: KCl, 125 mM; NaCl, 5 mM; MgCl₂, 2.5 mM; K₂HPO₄, 2.5 mM; Mops, 10 mM; Tris/HCl, 7 mM; EGTA, 1 mM; [1-¹⁴C]glycine, 5 mM (300–500 d.p.m./nmol); ADP, 1 mM; pH 7.4. The osmolarity of this medium was 306 mosm. The flux rate was linear for up to 20 min and for up to 4 mg of mitochondrial protein/ml. Incubations were routinely carried out at 30 °C for 10–15 minutes at a final protein concentration of 1 mg/ml. Addition of 0.3 ml of 30% (w/v) HClO₄ terminated the incubation and released the CO₂, which was trapped in a centre well equipped with filter paper immersed in 0.3 ml of NCS tissue solubilizer. CO₂ was collected for 1 h after which time the centre wells were transferred to scintillation vials containing Omnifluor and counted for radioactivity. In some experiments we used a simplified incubation medium which consisted of: mannitol, 225 mM; sucrose,

75 mM; Hepes, 5 mM, pH 7.4; EGTA, 1 mM; [1-¹⁴C]glycine, 5 mM. The osmolarity of this medium was 315 mosm. To this medium K₂HPO₄ (2.5 mM) or ADP (1 mM) was added as required. This medium was utilized when we examined the requirement for phosphate and ADP in the stimulation of flux through the glycine-cleavage system.

The fate of C-2 of glycine was investigated by monitoring the release of ¹⁴CO₂ from mitochondria incubated in the presence of [2-¹⁴C]glycine and by determining serine production. In these incubations the reaction was stopped by addition of 0.3 ml of 30% (w/v) sulphosalicylic acid, and ¹⁴CO₂ was collected as above. For determination of serine, samples were first centrifuged for 4 min in an Eppendorf microcentrifuge (12000 g) to remove protein, and then a portion of the supernatant was adjusted to pH 2.2 with LiOH. The concentration of serine was measured on a Beckman model 121-M amino acid analyser as described by Lee (1974).

For incubations in which the free Ca²⁺ concentration was regulated, EGTA was maintained at 1 mM while CaCl₂ was varied from 0 to 0.95 mM to give the desired level of free calcium. Preparation of stock solutions of EGTA and EGTA + CaCl₂ was described previously (Denton *et al.*, 1978). Free Ca²⁺ was calculated from CaCl₂/EGTA ratios by using the computer program EQCAL (Biosoft, Milltown, NJ, U.S.A.) in which the equilibrium composition is determined by a free-energy-minimization method (Eriksson, 1979). The stability constants reported by Fabiato & Fabiato (1979), adjusted for temperature, were used in the calculation.

Glycine accumulation and matrix volume

Glycine transport was measured in intact mitochondria in the presence of 13 μM-rotenone to inhibit glycine oxidation. Mitochondria were preincubated, with shaking, at a concentration of 2 mg/ml for 6 min at 30 °C in the normal incubation medium. Transport was initiated by the addition of 5 mM-[1-¹⁴C]glycine (sp. radioactivity 400 d.p.m./nmol), and samples were taken at 15 and 45 s and immediately separated from the medium by centrifugation in an Eppendorf centrifuge (10000 g for 1 min). The movement of glycine into the mitochondria was quantified by determining the radioactivity associated with the pellet. In these experiments, parallel incubations were carried out with unlabelled glycine and ³H₂O (20 μCi/ml) and [U-¹⁴C]sucrose (10 μCi/ml) to determine the matrix space and also to correct for glycine distributed in the extra-matrix space.

For other experiments, matrix volume was determined by rapidly separating mitochondria from the incubation medium by centrifugal filtration through an oil mixture consisting of bromododecane and dodecane (20:1, v/v). Mitochondria were preincubated as described above in a final volume of 1.25 ml at a protein concentration of 4 mg/ml. After 6 min, 15 μl of ³H₂O (20 μCi/ml) and 15 μl of [¹⁴C]sucrose (10 μCi/ml) were added. A 0.5 ml sample was immediately transferred to an Eppendorf centrifuge tube (1.5 ml), which contained 100 μl of 15% HClO₄ acid overlaid by 0.7 ml of the oil mixture. Mitochondria were separated from the medium through the oil layer into the acid layer by centrifugation in an Eppendorf centrifuge (10000 g for 1 min.). The acid layer was counted for ³H and ¹⁴C radioactivity by using ScintiVerse E (Fisher Scientific, Dartmouth, NS, Canada) scintillation fluid in a LKB 1214 scintillation counter. Corrections were made for both ³H overspill into the ¹⁴C channel and ¹⁴C overspill into the ³H channel. The mitochondrial matrix space was calculated as the difference between ³H₂O and [¹⁴C]sucrose spaces. The protein sedimented by centrifugation of mitochondria was determined in a parallel series of experiments in which the mitochondrial suspension (0.5 ml) was taken from the same incubation flasks. In this case, Eppendorf tubes used for

separating mitochondria contained 1.25 M-KCl in place of HClO_4 . The protein in the pellet was measured by the biuret procedure.

Chemicals

Diet components were obtained from ICN (Cleveland, OH, U.S.A.), except for the corn starch and L-methionine, which were purchased from Sigma (St. Louis, MO, U.S.A.). $[1-^{14}\text{C}]$ glycine, $[2-^{14}\text{C}]$ glycine, $^3\text{H}_2\text{O}$, $[\text{U}-^{14}\text{C}]$ sucrose and Omnifluor were obtained from Dupont-New England Nuclear (Mississauga, Ont., Canada). Other reagents were of analytical grade.

Presentation of data

Experiments are reported as means $\pm$ S.E.M. Data were compared by using the Wilcoxon-Mann-Whitney test, except for values presented in Table 1, which were analysed by a three-factor analysis of variance, with comparisons made by the Tukey multiple-comparisons test. In this case, the flux through the glycine-cleavage system was compared in the various incubation media by using liver mitochondria from rats fed on the high-protein or normal-protein diet for 2 or 6 days.

RESULTS

Stimulation of glycine catabolism by a high-protein diet or meal

Glycine catabolism was stimulated in mitochondria from rats fed on a high-protein diet for 6 days, compared with rates in mitochondria from rats fed on a normal-protein diet (Fig. 1). The stimulation was especially pronounced at the lower, physiological, concentrations of glycine where it was about 6-fold, compared with 2-fold at very high concentrations. It was not necessary to feed the rats on the high-protein diet for 6 days for the stimulation to appear. Rats fed for 2 days on these diets yielded glycine-cleavage-system rates similar to those obtained in mitochondria from rats fed for 6 days (results not shown). When mitochondria from high-protein-fed rats were incubated in a medium that did not contain P_i , the stimulated rate of glycine decarboxylation was not evident (Table 1). The stimulation was, however, independent of the presence of ADP.

Table 2 illustrates the rapidity with which an altered nutritional

Fig. 1. Glycine decarboxylation in mitochondria from high-protein- and normal-protein-fed rats

Mitochondria were isolated at 09:00 h and incubated in the KCl-based medium described in the Materials and methods section. Results are means $\pm$ S.E.M. for four separate experiments. Glycine decarboxylation in mitochondria from high-protein-fed rats (●) was significantly higher than that in mitochondria from normal-protein-fed rats (○) at each of the glycine concentrations tested (Wilcoxon-Mann-Whitney test, $P < 0.05$).

Table 1. Glycine-cleavage system flux in the presence and absence of P_i

Mitochondria from rats fed on a high-protein or normal-protein diet for 2 days were isolated at 09:00 h as described in the Materials and methods section. Mitochondria were incubated in the mannitol/sucrose-based incubation medium supplemented with 1 mM-ADP and/or 2.5 mM- K_2HPO_4 . Results are means $\pm$ S.E.M. for four separate experiments: *significantly different from rates in mitochondria, isolated from normal-protein-fed rats, incubated in the same medium (Tukey multiple comparisons test, $P < 0.05$).

Diet	Additions	Rate (nmol/min per mg of mitochondrial protein)
High-protein	ADP, K_2HPO_4	$0.62 \pm 0.17^*$
	ADP	0.11 ± 0.02
	K_2HPO_4	$0.46 \pm 0.06^*$
Normal-protein	ADP, K_2HPO_4	0.11 ± 0.01
	ADP	0.09 ± 0.01
	K_2HPO_4	0.14 ± 0.02

stimulus affects glycine catabolism in subsequently isolated mitochondria. When rats fed on the normal-protein diet for 5 days were switched to the high-protein diet the day before killing, glycine decarboxylation in subsequently isolated mitochondria occurred at enhanced rates (0.41 ± 0.10 nmol/min per mg of protein). Switching rats previously fed on the high-protein diet to the normal-protein diet the day before killing resulted in rates characteristic of normal-protein-fed rats (0.09 ± 0.03 nmol/min per mg of protein). In fact, changes in glycine-cleavage-system flux were affected rapidly upon ingestion of a single meal. Stimulated rates of glycine catabolism were seen in mitochondria

Table 2. Effect of changing the protein content of the diet on the glycine-cleavage system flux in isolated rat liver mitochondria

Rats were fed on a diet (HP, high-protein; NP, normal-protein) for 2 or 5 days and subsequently given the same diet or a different one for a shorter period (the meal). For rats fed for 5 days, the diet was switched at 14:00 h on day 5, 18 h before killing. In the other experiments, the animals were deprived of food between 14:00 and 20:00 h. Controls were killed at 20:00 h. The others were given the various diets and were subsequently killed at 22:00 h or at midnight. Results are means $\pm$ S.E.M. with the numbers of experiments in parentheses. Data were compared by a Wilcoxon-Mann-Whitney test: *significantly different from the normal-protein-fed group fed 18 h on the high protein diet; †significantly different from the control; ‡significantly different from those that received a meal that matched the diet to which they had been accustomed.

Diet	Time on diet (days)	Meal	Period of feeding	Rate (nmol/min per mg of mitochondrial protein)
HP	5	NP	18 h	0.09 ± 0.03 (4)*
NP	5	HP	18 h	0.41 ± 0.10 (4)
HP	2	—	—	0.22 ± 0.05 (5)
HP	2	HP	4 h	0.43 ± 0.05 (3)
HP	2	NP	4 h	0.08 ± 0.02 (5)†‡
HP	2	HP	2 h	0.38 ± 0.06 (3)
HP	2	NP	2 h	0.10 ± 0.009 (4)†‡
NP	2	—	—	0.08 ± 0.01 (5)
NP	2	NP	4 h	0.14 ± 0.02 (3)†
NP	2	HP	4 h	1.02 ± 0.30 (5)†‡
NP	2	NP	2 h	0.13 ± 0.03 (3)
NP	2	HP	2 h	0.66 ± 0.20 (4)†‡

from normal-protein-fed rats given a single high-protein meal for 2 or 4 h (0.66 ± 0.23 and 1.02 ± 0.27 nmol/min per mg of protein respectively). A normal-protein meal given to rats previously fed on the high-protein diet led to a decreased ability in subsequently isolated mitochondria to catabolize glycine (0.10 ± 0.01 and 0.08 ± 0.02 nmol/min per mg of protein at 2 and 4 h respectively). Thus the glycine-cleavage-system flux in liver mitochondria is rapidly and dramatically modulated by the protein content of a single meal.

Stimulation of serine production from glycine

The glycine-cleavage system releases C-1 of glycine as CO_2 and transfers C-2 to tetrahydrofolate to form methylenetetrahydrofolate. The methylene carbon of methylenetetrahydrofolate may be either oxidized (Hampson *et al.*, 1983) or transferred to a second glycine molecule to form serine via serine hydroxymethyltransferase. We therefore studied the fate of C-2 of glycine by measuring the conversion of $[2-^{14}\text{C}]$ glycine into $^{14}\text{CO}_2$ as well as measuring the quantity of serine produced. In these experiments serine is, quantitatively, the more important product, even under State-3 conditions, where methylenetetrahydrofolate oxidation is greatly enhanced (Table 3). Oxidation of methylenetetrahydrofolate, as monitored by the release of $^{14}\text{CO}_2$ from $[2-^{14}\text{C}]$ glycine, is somewhat increased by high-protein feeding, while serine production is greatly increased.

Effects of Ca^{2+}

We have recently shown that, in isolated mitochondria, flux through the glycine-cleavage system is sensitive to sub-micromolar concentrations of free Ca^{2+} (Brosnan *et al.*, 1990; Jois *et al.*, 1990). We therefore investigated the effect of Ca^{2+} on the stimulation of glycine catabolism in mitochondria from rats fed on the high-protein and normal-protein diets. Ca^{2+} had no effect on glycine decarboxylation below about $0.1 \mu\text{M}$ (Fig. 2). But a remarkable stimulation occurred over the range 0.1 – $0.25 \mu\text{M}$ free Ca^{2+} . When maximal stimulation had occurred ($0.25 \mu\text{M}$ - Ca^{2+}), there was no longer a statistical difference between the glycine-cleavage-system flux in mitochondria from normal-protein- and high-protein-fed rats. When glycine concentration was varied, but Ca^{2+} was maintained at $0.25 \mu\text{M}$, there was no difference in the rate of glycine catabolism in mitochondria from high- and normal-protein-fed rats at any of the glycine concentrations tested (Fig. 3).

Movement of glycine into mitochondria

The delivery of glycine to the glycine-cleavage system is a potential site for metabolic regulation. Glycine transport was examined in intact mitochondria isolated from rats fed for 2 days on the diets (Table 4). Mitochondria from high-protein- and

Fig. 2. Sensitivity of glycine-cleavage-system flux to free Ca^{2+} in the medium

Mitochondria were isolated from rats fed on the high-protein (●) or normal-protein (○) diet for 2 days. Mitochondria were incubated in the KCl-based medium (5 mM-glycine) supplemented with $\text{CaCl}_2/\text{EGTA}$ solutions of various ratios (see the Materials and methods section). Results are means $\pm$ S.E.M. for three separate experiments. One S.E.M. bar has been omitted for clarity.

normal-protein-fed rats did not differ in their ability to accumulate glycine, which was not inhibited by rotenone, suggesting that movement was independent of respiration. Since the net accumulation of glycine was the same at 15 and 45 s in each of the mitochondrial preparations, it is clear that transport was too rapid to be measured by these methods and that a rate based even on the 15 s data is an underestimate. This means that the initial rate of glycine transport was in excess of 32 nmol/min per mg of mitochondrial protein (8 nmol/mg in 15 s, multiplied by 4). Thus glycine delivery to the mitochondria is far too rapid to impose a limit even on the maximum rate of glycine decarboxylation, which we find to be about 2.5 nmol/min per mg in the presence of $0.25 \mu\text{M}$ free Ca^{2+} (Fig. 3).

Incubations in hypo-osmotic media and measurement of mitochondrial matrix volume

A number of hormone actions on mitochondrial function can be mimicked by incubation in hypo-osmotic media (Halestrap, 1989). Among others, these include stimulation of glutaminase, citrulline synthesis and pyruvate metabolism. To this can be added the glycine-cleavage system (Brosnan *et al.*, 1990; Halestrap *et al.*, 1990). Halestrap (1989) has argued that an

Table 3. Serine production and the release of C-1 and C-2 of glycine as CO_2 by mitochondria from high-protein- and normal-protein-fed rats

Mitochondria were incubated (with or without ADP) for 15 min in the KCl-based medium in the presence of 5 mM-glycine labelled in C-1 or C-2 (300–500 d.p.m./nmol). The difference in serine levels in the incubation medium at $t = 0$ min and $t = 15$ min divided by the incubation time is serine production. Results are means $\pm$ S.E.M. for four separate experiments.

Diet (2 days)	ADP	$^{14}\text{CO}_2$ or serine (nmol/min per mg of mitochondrial protein)		
		$^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ glycine	$^{14}\text{CO}_2$ from $[2-^{14}\text{C}]$ glycine	Serine production
High protein	(–)	0.49 ± 0.04	0.028 ± 0.004	0.56 ± 0.10
	(+)	0.40 ± 0.06	0.14 ± 0.02	0.24 ± 0.08
Normal protein	(–)	0.057 ± 0.008	0.013 ± 0.003	0.071 ± 0.040
	(+)	0.081 ± 0.010	0.092 ± 0.009	0.063 ± 0.020

Fig. 3. Glycine decarboxylation in mitochondria from high-protein- and normal-protein-fed rats versus glycine concentration in the presence of $0.25 \mu\text{M}$ free Ca^{2+}

Mitochondria were isolated from rats fed on the high-protein (●) or normal-protein (○) diet for 2 days. Mitochondria were incubated in the KCl-based medium supplemented with CaCl_2 as described in the Materials and methods section. Results are means $\pm$ S.E.M. for four separate experiments. Glycine decarboxylation in mitochondria from high-protein-fed and normal-protein-fed rats was not significantly different at any of the concentrations of glycine used (Wilcoxon-Mann-Whitney test, $P > 0.05$).

important part of the action of several glucogenic hormones involves an increase in mitochondrial matrix volume. Accordingly, it was of importance to determine how hypo-osmoticity affected glycine catabolism in mitochondria from animals fed on a high-protein or a normal-protein meal and to determine mitochondrial matrix volume in such mitochondria.

Fig. 4 shows experiments in which we examined the effects of osmolarity on flux through the glycine-cleavage system. We studied mitochondria from rats given a high-protein or a normal-protein meal, as well as from rats given a glucagon injection. These experiments clearly show a marked stimulation of glycine-cleavage-system flux in hypo-osmotic media. The stimulation brought about by hypo-osmoticity does not require P_i . However, in situations where glycine-cleavage system flux was stimulated,

Fig. 4. Effect of hypo-osmotic incubation on the rate of glycine decarboxylation in mitochondria from rats fed on a high-protein meal or injected with glucagon

(a) Rats fed on the normal-protein diet for 3 days were given a high-protein (●, ○) or normal-protein (▲, △) meal for 2 h. (b) Rats fed on the standard pellet diet were injected (intraperitoneally) with 0.1 mg of glucagon/ 100 g body wt. (■, □) or 0.9% NaCl/ 0.05% BSA (▼, ▽). Solutions with different osmolarities were prepared by varying the concentration of KCl. Incubations were conducted in the presence (●, ▲, ■, ●) or absence (○, △, □, ▽) of 2.5 mM K_2HPO_4 . Results are the means $\pm$ S.E.M. of three or four separate experiments (one S.E.M. bar has been omitted for clarity): * significantly different from incubations in the same medium with mitochondria of rats fed on the normal-protein meal (a) or from rats injected with vehicle (b).

by glucagon injection or a high-protein meal, the flux even under hypo-osmotic conditions was further stimulated by P_i . Thus the stimulation brought about by hypo-osmoticity is of a different nature from the physiological stimulation brought about by feeding a high-protein diet or meal, which is absolutely dependent on the presence of P_i (Table 1). Similarly, mitochondria from glucagon-injected rats displayed a marked stimulation of flux through the glycine-cleavage system when incubated hypo-osmotically in the absence of P_i (Fig. 4b). It was clearly important to determine the mitochondrial matrix volume in mitochondria from high-protein- and normal-protein-fed rats. Table 5 shows that matrix volume was the same for mitochondria from rats

Table 4. Glycine accumulation in intact mitochondria

Mitochondria were isolated from rats fed on the high-protein or normal-protein diet for 2 days. Mitochondria were preincubated for 6 min in the normal isolation medium with or without $13 \mu\text{M}$ rotenone before addition of 5 mM -glycine ($[1\text{-}^{14}\text{C}]$ glycine, 400 d.p.m./nmol). Mitochondria were then rapidly sedimented 15 or 45 s later and the accumulation of glycine was assessed by determining the radioactivity in the pellet (see the Materials and methods section). Corrections for extra-matrix glycine were as described in the Materials and methods section. Results are means $\pm$ S.E.M. for six separate experiments, except for the 45 s determinations, which are means of two experiments.

Diet	Time	Glycine accumulation (nmol/mg of mitochondrial protein)	
		No rotenone	+ rotenone
High protein	15 s	8.55 ± 1.14	8.50 ± 0.90
	45 s	10.32	5.80
Normal protein	15 s	7.28 ± 0.98	8.04 ± 1.06
	45 s	8.83	8.13

Table 5. Matrix volume and flux through the glycine-cleavage system in mitochondria from rats fed on a high- or normal-protein meal

Rats fed on the normal-protein diet for 3 days were given a high-protein or normal-protein meal for 2 h. Matrix volume was determined by separating mitochondria from the incubation medium by centrifugal filtration through oil as described in the Materials and methods section. Results are means $\pm$ S.E.M. for four separate experiments. K_2HPO_4 was present at a concentration of 2.5 mM where indicated.

Meal	K_2HPO_4	Rate (nmol/min per mg of mitochondrial protein)	Matrix volume ($\mu\text{l}/\text{mg}$ of mitochondrial protein)
High-protein	(-)	0.11 ± 0.02	1.23 ± 0.09
	(+)	0.45 ± 0.06	1.33 ± 0.09
Normal-protein	(-)	0.09 ± 0.01	1.32 ± 0.07
	(+)	0.13 ± 0.02	1.29 ± 0.03

given a high- or normal-protein meal. This was true even when P_i was omitted from the incubations. Thus matrix volume remained unchanged despite large differences in the rate through the glycine-cleavage system.

DISCUSSION

The results of this investigation indicate that hepatic glycine catabolism is activated after a high-protein meal. The effect elicited *in vivo* on the glycine-cleavage system is both rapid and dramatic: the stimulation occurs within 2 h from the start of a meal and involves a 4–6-fold enhancement of flux through the glycine-cleavage system as measured in isolated mitochondria (Table 1). In fact, the protein content of the last meal is the single major determinant of the rate of glycine oxidation. Regardless of the rats' prior diet history, mitochondria from all rats fed on the 15%-casein meal oxidized glycine at a rate less than

0.15 nmol/min per mg, whereas the rate in mitochondria from animals fed on the 60%-casein meal was 0.38 nmol/min per mg or more.

A number of mechanisms have been described whereby hepatic catabolism of amino acids is regulated. One mechanism occurs via rapid induction of enzymes with short half-lives and whose levels subsequently decrease to near zero (Krebs, 1972). Another mechanism involves regulation by covalent modification, where activities of enzymes such as phenylalanine hydroxylase and branched-chain oxo-acid dehydrogenase are modulated by phosphorylation/dephosphorylation of enzyme subunits (Fisher & Pogson, 1984). The regulation of the glycine-cleavage system does not appear to occur through either of these mechanisms. The rapid stimulation of glycine decarboxylation in response to a single protein meal makes enzyme induction unlikely. This contention is supported by the data of Fig. 3, which show that in the presence of optimal concentrations of Ca^{2+} glycine decarboxylation rates in mitochondria from high-protein- and normal-protein-fed rats are similar. Covalent modification is also unlikely, since the mitochondria were washed three times during the isolation procedure with no particular precautions taken to preserve, for example, phosphorylation state. The effect is more long-lived than typically labile covalent modification.

Similar long-lived effects have been shown for a number of mitochondrial metabolic processes following stimulation by glucagon (see Halestrap, 1986). We have recently shown that the glycine-cleavage system is stimulated by glucagon; the stimulation is evident in isolated hepatocytes incubated in the presence of the hormone and in liver mitochondria isolated from a rat given glucagon (intraperitoneally) 25 min before death (Jois *et al.*, 1989). Here we report that the stimulation of glycine catabolism by high-protein feeding has similar characteristics to that elicited by glucagon. We consider it likely that the high circulating glucagon concentration that is evident after a high-protein meal (Robinson *et al.*, 1981) may be an important signal in the stimulation of the glycine-cleavage system at this time. However, the stimulation after a high-protein meal may be more complex, as a variety of hormonal changes will occur. Evidence that glucagon alone may not be the only signal affecting these mitochondria comes from experiments on succinate oxidation. Succinate oxidation is reported to increase after glucagon injection (Yamazaki, 1975). In our hands we found that glucagon

injection increased succinate oxidation rate by 42% (from 170 to 240 ng-atoms of O/min per mg). However, when we performed similar measurements on mitochondria from rats fed on the different protein diets there was no difference in succinate oxidation (206 versus 189 ng-atoms/min per mg for high- and normal-protein diets respectively). This emphasizes the complexity of the situation *in vivo* and the need to consider factors in addition to glucagon.

The physiological significance of these findings are readily apparent. Ingestion of a high-protein diet (or meal) necessarily involves ingestion of increased amounts of glycine. Since the body has no real capacity to store amino acids, other than the synthesis of functional proteins, excess amino acids must be catabolized. The pathway for the catabolism of glycine involves its conversion into serine in a fashion that consumes two molecules of glycine with the formation of one molecule each of serine and CO_2 (THF, tetrahydrofolate; CH_2 -THF, methylene-tetrahydrofolate):

That this pathway is active is apparent from the increased serine production which we report here (Table 3). The fate of the serine may be direct oxidation to CO_2 or, more likely, conversion into glucose, since gluconeogenesis is considered to be normal prandial process after ingestion of protein (Jungas *et al.*, 1991).

This work was supported by the Medical Research Council of Canada. S.E. was supported by a Fellowship from the School of Graduate Studies, Memorial University of Newfoundland. We thank Mr. D. E. Hall for the amino acid analyses.

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Received 9 September 1991/12 November 1991; accepted 2 December 1991