Catabolism of arginine and ornithine in the perfused rat liver: effect of dietary protein and of glucagon

DAN O’SULLIVAN, JOHN T. BROSNAN, AND MARGARET E. BROSNAN
Department of Biochemistry, Memorial University of Newfoundland,
St. John’s, Newfoundland, Canada A1B 3X9

Dan O’Sullivan, John T. Brosnan, and Margaret E. Brosnan. Catabolism of arginine and ornithine in the perfused rat liver: effect of dietary protein and of glucagon. Am. J. Physiol. Endocrinol. Metab. 278: E516–E521, 2000.—The rates of oxidation of arginine and ornithine that occurred through a reaction pathway involving the enzyme ornithine aminotransferase (EC 2.6.1.13) were determined using 14C-labeled amino acids in the isolated nonrecirculating perfused rat liver. At physiological concentrations of these amino acids, their catabolism is subject to acute regulation by the level of protein consumed in the diet. 14CO2 production from [U-14C]ornithine (0.1 mM) and from [U-14C]arginine (0.2 mM) was increased about fourfold in livers from rats fed 60% casein diets for 3–4 days. The catabolism of arginine in the perfused rat liver, but not that of ornithine, is subject to acute regulation by glucagon (10−7 M), which stimulated arginine catabolism by ~40%. Dibutyryl cAMP (0.1 mM) activated arginine catabolism to a similar extent. In retrograde perfusions, glucagon caused a twofold increase in the rate of arginine catabolism, suggesting an effect of glucagon on arginase in the perivenous cells.

hepatic zonation; high-protein diet; antegrade and retrograde perfusion

ARGININE OCCUPIES A CENTRAL ROLE IN INTERMEDIARY METABOLISM. In addition to its involvement in protein synthesis, it is an intermediate in the urea cycle and is required for the production of nitric oxide (13), creatine, polyamines, and agmatine (23). In most mammals, arginine arises from two principal sources, endogenous de novo production (43, 11) and the exogenous supply of this amino acid in the diet. In early studies, it has been stated that the definition of arginine as “essential” or “nonessential” depends on the species in question as well as the developmental stage under investigation (8). The term “conditionally essential” has since been coined (6) and is frequently used when describing the nutritional requirement for arginine. Thus, under certain physiological and pathophysiological conditions, endogenous production does not occur at a rate commensurate with optimum growth or recovery from disease. This has been borne out in studies that demonstrate the beneficial effects on immune function of supplying supradietary amounts of arginine (1) and recovery from trauma in rats (33). These studies have prompted debate as to whether the current criteria for assessing arginine requirements are appropriate (39).

The mechanisms that regulate whole body arginine levels have not been characterized in great detail. Arginine balance is a function of the supply of this amino acid relative to its utilization. Our own studies on rats (9) show that endogenous renal arginine synthesis is unaffected by dietary arginine. Studies in humans (4, 5) demonstrate that, under conditions in which dietary arginine is restricted, the body reduces the rate of oxidative catabolism of this amino acid. This homeostatic mechanism serves to spare arginine for its participation in the various metabolic pathways. In these experiments, there is no alteration in the rate of de novo synthesis of arginine. Both our own group (9) and Castillo and co-workers (4, 5) have made the case for a better characterization of the regulation of the catabolism of this amino acid within specific organs.

Rat liver contains all the enzymes necessary for the complete oxidation of arginine to CO2 (26). This pathway also accomplishes ornithine catabolism. The importance of ornithine aminotransferase (OAT, EC 2.6.1.13) is demonstrated in humans by the genetic disorder known as “gyrate atrophy.” OAT activity is diminished, and there is an associated increase in the circulating levels of ornithine (38) that can be decreased by restricting dietary arginine. Also, in adult mice in which the OAT gene has been inactivated, there is a marked increase in tissue ornithine concentrations (40). With respect to other possible routes of utilization, it should be emphasized that the urea cycle is not a route for the oxidation of these amino acids, because, as a cycle, it produces one molecule of ornithine for every one of arginine. Neither nitric oxide synthesis (41) nor polyamine production (34) consumes a significant amount of these substrates in the normal rat liver on a daily basis; therefore, we regard catabolism via OAT as the major route for the oxidation of these amino acids in the rat liver.

The concept of zonation of hepatic metabolism is well established (21, 30) and is of particular importance in nitrogen metabolism (18). With regard to arginine catabolism, it is clear that hepatic ornithine aminotransferase is limited to a population of hepatocytes surrounding the hepatic vein (22). We have shown by means of retrograde perfusions that these cells also contain an arginase that permits the entire catabolism of arginine in these cells (26).
Regulation by dietary and hormonal factors is a common feature of mammalian amino acid metabolism, with responses to these stimuli ranging in time from days (chronic adaptation) to minutes (acute regulation). Schimke (32) clearly demonstrated the chronic regulation of the enzymes of the urea cycle with alterations in the level of dietary protein in rats. Feeding rats a high-protein diet for four days is known to increase circulating levels of glucagon in plasma (28) more than threefold. Treatment of isolated hepatocytes with glucagon is known to stimulate the glycine cleavage system (20), glutaminase (36), and phenylalanine oxidation (12). The principal focus of this investigation is to determine whether the catabolism of arginine and ornithine in the perfused rat liver is subject to long-term regulation by the level of dietary protein and/or acute regulation by glucagon.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (240–350 g), purchased from Charles River (Montreal, Canada), were used. They were provided with free access to water and to purified diets for 3–4 days before the experiment. The diets contained either 15% (wt/wt) casein (normal protein diet) or 60% (wt/wt) casein (high-protein diet). Rarely, an animal ate the 60% diet poorly and gained little weight; such animals (~2% of total) were excluded from the study. Gabaculine, an OAT inhibitor, was administered at a level of 50 mg/kg body wt by intraperitoneal injection 2 h before the experiment. All animals were maintained under a controlled 12:12-h light-dark cycle. Before perfusion, rats were anesthetized with pentobarbital sodium (60 mg/kg ip). All protocols involving the use of animals were approved by the Institutional Animal Care Committee at Memorial University and were in accord with the Guidelines of the Canadian Council on Animal Care.

Diets. Modified AIN 76 diets were used (3). The high-protein (60% casein) diet contained the following ingredients (in g/100 g of diet): casein, 59.8; L-methionine, 0.15; cornstarch, 6.1; sucrose, 19.2; corn oil (Mazola), 5; vitamin mix (AIN 76), 1.0; mineral mix (AIN 76), 3.5; Alphacel, 5; choline bitartrate, 0.1. In case of the normal protein (15% casein) diet, casein was decreased to 14.8 g/100 g of diet, and sucrose and cornstarch were increased to 53.3 and 17 g/100 g, respectively. Thus both diets were isocaloric.

Perfusion procedures. Nonrecirculating perfusions of rat livers were carried out according to the method of Sies (35). Krebs-Henseleit medium (pH 7.4), gassed with O2-CO2 (19:1) with added lactate and pyruvate (2.1 and 0.3 mM, respectively), served as the basic perfusion medium. The flow rate was maintained at ~4.0 ml·min⁻¹·g liver⁻¹. [U-¹⁴C]arginine and [U-¹⁴C]ornithine were added at various concentrations at the times indicated in RESULTS, and the production of ¹⁴CO₂ was determined in the effluent. When the effect of glucagon was investigated, it was dissolved in 10 mM HCl and infused at a rate sufficient to give a final concentration in the influent perfusate of 10⁻⁷ M. In control experiments, the vehicle (HCl) was infused. The rate of infusion was such that no change in pH or PCO₂ was discernible. When used, dibutyryl cAMP at a concentration of 0.1 mM was added to the perfusion medium, whereas control experiments contained butyrate (0.1 mM). To ensure that livers were viable throughout the procedure, oxygen consumption, perfusate (PCO₂), and pH were monitored by means of a blood gas analyzer (model 238, Ciba Corning, Bayer, Toronto, ON, Canada). Oxygen consumption was ~2.5 μmol·min⁻¹·g liver⁻¹, and this increased by ~15% on infusion with glucagon. Retrograde perfusions were carried out according to methods developed by Häussinger (17). An initial sample of each influent medium was taken. Effluent samples were collected at 5-min intervals after that. Samples for ¹⁴CO₂ analysis were taken under mineral oil.

Measurement of ¹⁴CO₂ production. Twenty-five-millimeter Erlenmeyer flasks containing 0.4 ml of 1 N HCl were fitted with center wells containing filter paper and 0.4 ml of neutrophil chemotactic factor tissue solubilizer (Amersham Canada, Oakville, Ontario, Canada) and stoppered. Into each flask, 5 ml of perfusate were injected through the stopper. The flasks were incubated in a shaking water bath at 37°C for 1 h to ensure that all of the evolved CO₂ would be trapped in the center wells. The center wells were transferred to scintillation vials containing 10 ml of scintillation fluid and counted.

RESULTS AND DISCUSSION

Characterization of processes of arginine and ornithine catabolism. We decided to compare arginine and ornithine metabolism in livers from animals fed a high-protein diet (60% casein) with that in livers from animals fed a normal protein diet (15% casein) to examine the effects of a substantial difference in protein intake. The food consumption of rats on both these diets was similar (~5 g·day⁻¹·100 g body wt⁻¹). Because the arginine content of bovine casein is 3.7 g/100 g, we can calculate a daily dietary arginine intake of ~55 mg/100 g body wt in the rats fed the 15% casein diet, which increased fourfold in the animals fed the 60% casein diet. Ornithine is not a constituent of casein. Plasma levels of arginine and ornithine were measured in rats fed on 15, 30, or 60% casein diets for 3 days. The ornithine levels were 25.4 ± 2.5, 22.8 ± 3.4, and 29.4 ± 3.7 mmol/ml, respectively, for the animals fed the 15, 30, and 60% casein diets and were not significantly different from each other. The corresponding levels of plasma arginine were 56.2 ± 2.1, 59.3 ± 5.5, and 75.9 ± 8.1, with the concentration on the 60% casein diet being significantly increased (P < 0.05). The increased plasma arginine will be experienced by the
islets and this may contribute to increased glucagon secretion.

Preliminary experiments were carried out in rats fed the high-protein diet to demonstrate the dependence of arginine and ornithine catabolism on their concentrations. The half-maximal rates of catabolism occurred at 3.4 and 3.3 mM, respectively, for arginine and ornithine. Therefore, at the physiological portal vein concentrations of these amino acids, in the fed state [−0.1 and 0.2 mM for ornithine and arginine, respectively (31)], these catabolic processes operate well below their maximum capacity. The half-maximal substrate concentrations for these processes are similar to those described for the known transporter for these amino acids into the liver [Michaelis-Menten constant for this transporter is in the range of 2–5 mM (7)]. Administration of gabaculine (see MATERIALS AND METHODS), a known inhibitor of OAT, markedly reduced the catabolism of these amino acids by >80% (data not shown), as was shown previously by us in rats fed a normal protein diet (26), supporting the key role of OAT in the catabolism of these amino acids.

**Chronic adaptation to varying dietary protein.** The effect of dietary protein on ornithine catabolism was determined in perfused livers from rats which were fed either the normal protein diet (15% casein) or the high-protein diet (60% casein) for a period of 3–4 days. Figure 1 shows that, at physiological portal vein concentrations of ornithine (0.1 mM), the livers from rats fed the high-protein diet catabolize ornithine at a rate fourfold greater than those from rats fed a normal protein diet (63.6 ± 22.6 vs. 14.4 ± 5.3 nmol CO₂·min⁻¹·g wet liver⁻¹ at the 29-min time point). Figure 2 demonstrates that the oxidative catabolism of arginine (0.2 mM) also increases with increasing dietary protein about fivefold (13.8 ± 4.9 vs. 74.5 ± 22.5 nmol CO₂·min⁻¹·g wet liver⁻¹ at the 29-min time point). The marked stimulation in the rates of arginine and ornithine catabolism observed upon feeding rats a high-protein diet could occur as a result of either enzyme or transporter induction. For example, it is known that both OAT (29) and the irreversible enzyme 1-pyrroline-5-carboxylate dehydrogenase (24) are induced in rat liver under conditions of high dietary protein. In this study, the OAT activities in livers from rats fed normal protein and high-protein diets were found to be 4.12 ± 0.76 and 17.32 ± 3.45 µmol·min⁻¹·g liver protein⁻¹, respectively. The relatively low clearance of the cationic amino acids during a single circulatory pass of the liver (27) is thought to be due to the rather low activity of the high-capacity low-affinity y⁺ transporter (42) recently characterized as the MCAT2A transporter (7). Regulation of this transporter may also be an effective means of increasing flux through the catabolic process. It is possible that the raised circulating levels of glucagon observed in rats fed a high-protein diet may be responsible for such an alteration, as Handlogten and Kilberg (16) demonstrated that intraperitoneal administration of glucagon to rats stimulates y⁺ transporter activity in isolated rat hepatocytes. Increased dietary supply of amino acids such as arginine and glutamine over a 3-day period has been shown to increase y⁺ transporter activity as much as fourfold (10).

**Acute regulation by glucagon infusion.** The short-term effect of a glucagon infusion was investigated (Fig. 3). A typical experiment on ornithine catabolism (0.1 mM) shows that the infusion of glucagon does not alter
the rate of CO$_2$ production from this amino acid (Fig. 3A), whereas it stimulates arginine catabolism (Fig. 3B). We carried out four such experiments with ornithine or arginine and analyzed them statistically by comparing the values at 39 min (before glucagon infusion) with those at 60 min. In the ornithine experiments, the results were 94.4 $\pm$ 25.3 nmol·min$^{-1}$·g liver$^{-1}$ at 39 min and 96.1 $\pm$ 29.5 nmol·min$^{-1}$·g liver$^{-1}$ at 60 min (not significant). In the arginine experiments the results were 64.9 $\pm$ 15.3 nmol·min$^{-1}$·g liver$^{-1}$ at 39 min and 92.6 $\pm$ 24.5 nmol·min$^{-1}$·g liver$^{-1}$ at 60 min ($P < 0.05$). Control experiments in which the glucagon vehicle alone was infused demonstrated no changes.

We also carried out a series of experiments in livers from gabaculine-treated rats that were perfused with 0.2 mM arginine. We measured ornithine production in the perfusate effluent. In the control experiments, in which saline was infused from 39 to 60 min, ornithine production was unchanged (63.1 $\pm$ 7.0 nmol·min$^{-1}$·g liver$^{-1}$ at 39 min compared with 64.0 $\pm$ 8.5 nmol·min$^{-1}$·g liver$^{-1}$ at 60 min; n = 4). However, when glucagon (10$^{-7}$ M) was infused from 39 to 60 min, there was a significant increase in ornithine production ($P < 0.05$) with 72.6 $\pm$ 31.8 nmol·min$^{-1}$·g liver$^{-1}$ at 39 min and 116.2 $\pm$ 30.3 nmol·min$^{-1}$·g liver$^{-1}$ at 60 min. This experiment confirms that glucagon stimulates arginine catabolism at a site before ornithine aminotransferase.

Glucagon is well established as a regulator of amino acid metabolism in the liver, both chronically and acutely. Acutely it stimulates the flux through the glycine cleavage system in isolated hepatocytes (20); chronically it coordinately induces all five urea cycle enzymes (37). The acute stimulation in arginine catabo-
lism, coupled with the absence of any effect of glucagon on the rate of CO₂ production from ornithine, suggests that the site at which glucagon is exerting its effect may be arginase. The arginase reaction is the only step unique to the catabolism of arginine; all other steps in its catabolism, including transport, are shared with ornithine. Many of glucagon’s effects [e.g., stimulation of hepatic phenylalanine hydroxylase (12)] are brought about by an increase in intracellular cAMP, which in turn stimulates cAMP-dependent protein kinases to phosphorylate various target enzymes. To determine whether glucagon’s stimulation of CO₂ production from arginine could be due to an increase in intracellular cAMP, dibutyryl cAMP (0.1 mM) was included in the perfusion medium (Fig. 4). The data demonstrate a marked increase in the rate of CO₂ production from arginine. We carried out five such experiments. The mean rate of ¹⁴CO₂ production at 34 min (before the arginine) was 74.2 ± 27.1 nmol·min⁻¹·g liver⁻¹ and 115.6 ± 49.4 nmol·min⁻¹·g liver⁻¹ at 60 min (P < 0.05). Ten minutes of exposure to dibutyryl cAMP was sufficient to produce a significantly different rate of CO₂ production (P < 0.05) determined by Student’s paired t-test. Control experiments in which butyrate (0.1 mM) was added showed no alteration in the rate of CO₂ production from arginine (data not shown). Dibutyryl cAMP (0.1 mM) had no effect on the rate of catabolism of ornithine in perfused rat livers (data not shown).

Localization of the glucagon effect on the catabolism of arginine. In vivo, rat liver is supplied with blood from the portal vein and the hepatic artery; this blood then flows through the liver and leaves through the central vein. The cells surrounding the portal inflow are known as periportal cells, and those surrounding the central vein are called perivenous cells (30). Previous studies carried out in our laboratory (26) involving retrograde perfusions have shown that the processes of arginine and ornithine catabolism in the rat liver can be carried out in their entirety in the cells of the perivenous region and, therefore, that an arginase must exist in those cells. Our next experiments were designed to determine whether glucagon’s effect on hepatic arginine catabolism was occurring in the perivenous region or whether glucagon was exerting an effect in the upstream periportal region that could then be communicated to the downstream perivenous region. In the perfusions described so far, the direction of perfusion has been in the physiological antegrade direction (i.e., from the periportal to the perivenous regions). It is, however, possible to perfuse from the perivenous to the periportal regions [retrograde perfusion (17)]. If glucagon must first exert an effect in the cells of the periportal region, there will be no stimulation of arginine’s catabolism by glucagon in a retrograde perfusion, because the signal will occur after this catabolic process. Figure 5 shows that infusion of glucagon stimulates the production of CO₂ from arginine in the perfusions carried out in the retrograde direction. The data demonstrate a marked increase in the rate of CO₂ production from arginine. We carried out four such experiments and analyzed them statistically. The mean rate of ¹⁴CO₂ production was 41.9 ± 23.0 nmol·min⁻¹·g liver⁻¹ at 29 min (before glucagon was infused) and 74.5 ± 23.3 nmol·min⁻¹·g liver⁻¹ at 49 min. Five minutes of exposure to glucagon was sufficient to produce a significantly different rate of CO₂ production (P < 0.05) as determined by Student’s paired t-test. Control experiments, in which the vehicle was infused, produced no alteration in the rate of catabolism of arginine (data not shown); thus glucagon can stimulate perivenous cells to give an enhanced rate of catabolism of the amino acid arginine. Because perivenous cells are well supplied with glucagon receptors (2), the most likely mechanism would be a direct effect of glucagon on these cells that results in arginase stimulation. However, we cannot exclude the possibility that glucagon may act on periportal cells that may transmit signals to the perivenous cells via gap junctions. It has been shown that two forms of arginase are present in rat liver, A₁ and A₂. A₁ is known to be present in the perivenous region, and it is possible that this enzyme is subject to acute regulation by glucagon in vivo. This requires further investigation.

In summary, catabolism of arginine and ornithine within the rat liver is increased in animals that ate the 60% casein diet. Both our own and Young’s groups have shown in whole body studies carried out in rats (9) and in humans (4, 5) that the catabolism, but not the de novo synthesis, of arginine is regulated to maintain whole body arginine levels. Our studies indicate that with increased intake of dietary protein (and therefore of arginine) the liver alters its rate of catabolism such that the flux through this catabolic process increases, thus contributing to the maintenance of whole body arginine levels in vivo. In addition, we have demonstrated the ability of arginine catabolism to respond immediately to an infusion of glucagon, and this may permit rapid alterations in the rate of disposal of this amino acid under physiological conditions such as ingestion of a high-protein meal.

Dan O’Sullivan thanks the School of Graduate Studies, Memorial University of Newfoundland, for a graduate fellowship. Technical assistance provided by B. Hall is gratefully acknowledged.

This work was supported by grants from the Medical Research Council of Canada.

Address for reprint requests and other correspondence: M. Brosnan, Department of Biochemistry, Memorial University of Newfoundland, St. John’s, Newfoundland, Canada A1B 3X9 (E-mail: mbrosnan@morgan.ucs.mun.ca).

Received 14 December 1998; accepted in final form 25 October 1999.

REFERENCES


2. Berthoud, V. M., V. Iwanij, A. M. Garcia, and J. C. Sáez. Connexins and glucagon receptors during development of rat...