Intravenous γ-Glutamyl-Tyrosine Elevates Brain Tyrosine But Not Catecholamine Concentrations in Normal Rats

Donna C. Berger, Mary A. Hilton, Frederick K. Hilton, Scott D. Duncan, Paula G. Radmacher, and Susan M. Greene

A number of clinical situations may benefit from intravenous supplements of tyrosine (Tyr). In total parenteral nutrition (TPN), the supply of Tyr is limited by its poor solubility. In both rats and infants maintained on pediatric TPN, plasma Tyr levels are approximately 30% of normal, and in rat brains Tyr concentrations are similarly reduced. We reported previously that supplementing a TPN solution with the soluble peptide, γ-glutamyl-Tyr [Glu(Tyr)], normalizes plasma Tyr and doubles brain Tyr in rats. To assess more fully the behavior of intravenous Glu(Tyr) in vivo, 20 mmol/L Glu(Tyr) was infused into the inferior vena cava of rats at rates increased every 2 hours over an 8-hour period (300 to 450 μmol Glu(Tyr)/kg body weight/h). The surgical procedure for catheterization is described. At the maximum rate of infusion, plasma Tyr and Glu(Tyr) concentrations reached mean plateau values of 326 and 252 μmol/L, respectively. Brain Tyr concentrations were 71 and 264 nmol/g wet weight in control rats infused with heparinized saline (SAL group) and rats infused with Glu(Tyr) (PEP group) respectively. No differences were found in concentrations of norepinephrine (NE), dopamine (DA), or homovanillic acid (HVA) in prefrontal cortex (PFC), striatum (STR), or remaining brain (RB) tissue in PEP and SAL rats. We did not detect undegraded Glu(Tyr) in the brain, and less than 0.5% of infused Glu(Tyr) appeared in the urine.

COMMERCIAL MIXTURES of amino acids used for total parenteral nutrition (TPN) contain little or no tyrosine (Tyr) because of the limited solubility of this amino acid. In an attempt to compensate for the deficiency of Tyr in these mixtures, manufacturers include high concentrations of phenylalanine (Phe), the metabolic precursor of Tyr. A pediatric TPN mixture (Aminosyn-PF 10%; Abbott Laboratories, N Chicago, IL) contains a 10:1 molar ratio of Phe to Tyr; when this is used in TPN to maintain infants or rats, plasma concentrations of Tyr are significantly less than normal and levels of Phe are above normal, indicating limited conversion of Phe to Tyr during parenteral administration.[1]

In addition to its important role in protein synthesis, Tyr is a precursor of other important metabolites, including catecholamines, which serve hormonal and neurotransmitter functions. We previously proposed use of the soluble peptide, γ-glutamyl-Tyr [Glu(Tyr)], as an intravenous source of Tyr. γ-Glutamyl transferase ([GGT]EC 2.3.2.2), an enzyme that is highly active in endothelial cells,[2] should hydrolyze Glu(Tyr) in the circulation. Glu(Tyr) need not be regarded as a foreign peptide; it can also be synthesized by GGT, which catalyzes transfer of the γ-glutamyl group of glutathione to Tyr.[3] When mice were given an intravenous injection of Glu(Tyr), Tyr concentration in plasma increased rapidly, and this increase was blunted by acivicin, an inhibitor of GGT.[4] We then substituted Glu(Tyr) for half the Phe in a pediatric TPN mixture. When young adult rats were maintained on this modified TPN, plasma concentrations of Phe and Tyr were restored to normal.[1]

The present study explores the disposition in rats of Glu(Tyr) infused intravenously in increasing concentrations. The capacity of this peptide to elevate Tyr concentrations in both plasma and brains of young rats was assessed. We also investigated the extent of urinary excretion of the infused peptide and whether intact Glu(Tyr) crosses the blood-brain barrier in detectable amounts. Finally, we studied the effect in brain of elevated levels of Tyr on ambient concentrations of catecholamines. A surgical procedure was developed to provide for infusion into the inferior vena cava, which we reasoned would tolerate the increasing rates of infusion better than the more commonly accessed superior vena cava. The rat maintains complete freedom of movement in the metabolism cage without the use of a harness,[5] which is assumed to contribute to the stress experienced by the animal.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) were housed individually upon receipt. On the day of infusion, body weights were 171 to 220 g. The protocol for the use of rats in these experiments was approved by the Institutional Animal Care and Use Committee of the University of Louisville.

Surgical Procedure

Catheters were assembled as shown in Fig 1. The 22-gauge, small-animal, single-channel infusion swivel, spring, and button for attachment to the animal were obtained from Harvard Apparatus (South Natick, MA). The assembled catheter apparatus, surgical drapes, and 5-0 silk ties (Ethicon, Somerville, NJ) were gas-sterilized in ethylene oxide. Surgical instruments were sterilized by autoclave.

Each rat was anesthetized by intramuscular injection of rodent anesthesia (ketamine hydrochloride 37.5 mg/mL and xylazine 5 mg/mL; Aveco, Fort Dodge, IA) at a dose of 1 mL/kg body weight. The upper-back and groin areas were shaved and cleansed with 70% ethanol. A 2- to 3-cm longitudinal incision was made in the groin (Fig 1). Subcutaneous tissues were divided by blunt dissec-

From the Departments of Anatomical Sciences and Neurobiology, Biochemistry, and Pediatrics, University of Louisville School of Medicine, Louisville, KY.

Submitted February 27, 1995; accepted May 16, 1995.

Supported by Research Corporation Technologies, Tucson, AZ, and by the Alliant Community Trust Fund, Louisville, KY.

Address reprint requests to Mary A. Hilton, PhD, Department of Biochemistry, School of Medicine, University of Louisville, Louisville, KY 40292.

0026-0495/96/4501-0019$03.00/0

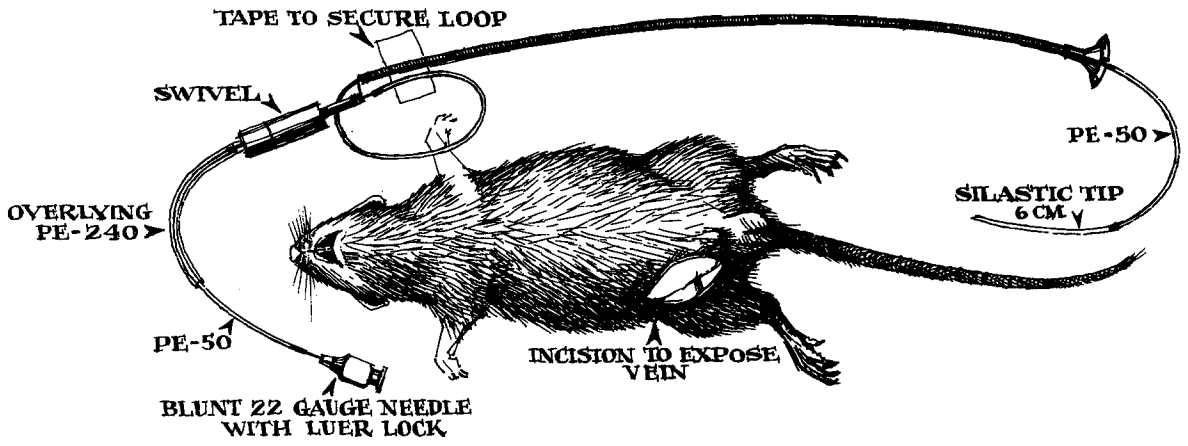

Fig 1. Position of incision in the groin area of the rat and detail of catheter assembly. Catheter tip consists of medical-grade silastic tubing (0.025 in ID × 0.047 in OD), chosen to minimize the possibility of puncturing blood vessels. The remaining 45 cm of the catheter is PE50 tubing (0.58 mm ID × 0.965 mm OD; Clay Adams, Parsippany, NJ) sealed circumferentially to the silastic tip with cyanoacrylate glue. Rigidity of the polyethylene tubing prevents occlusion during normal activity of the rat. This assembly is threaded through the spring toward the swivel. The tubing is looped as shown and connected to the outlet nipple of the swivel. A 15-cm length of PE50 tubing is joined to the inlet nipple of the swivel, overlaid with 10 cm of protecting PE240 tubing (0.066 in ID × 0.095 in OD; Clay Adams), and fitted with the needle as shown. All junctions of PE50 with the swivel and needle are sealed with cyanoacrylate glue.

tion at the incision site and posterolaterally along the abdominal wall. A 2- to 3-cm longitudinal incision was then made in the interscapular region. Subcutaneous tissues were divided as before around the incision and anterolaterally. A 12-cm piece of fire-polished and sterilized glass tubing (5 mm ID × 7 mm OD) was tunneled laterally and inferiorly from the back to the groin. The catheter, filled with heparinized saline with 1 U heparin/mL (sodium heparin injection USP; SoloPak Laboratories, Franklin Park, IL), was passed from the back to the groin through the glass tubing. The tubing containing the catheter was removed through the incision in the groin. The incision in the back was covered with saline-soaked sterile gauze, and the animal was moved to a supine position and draped. The tubing containing the catheter tip was positioned on the drape, away from the operating field, to maintain sterility of the catheter. The skin and musculature at the incision in the groin were held by gentle four-point retraction while the femoral vein was catheterized as shown in Fig 2.

After catheterization, the incision site was flushed with heparinized saline, followed by 1 mL aqueous solution containing 40 mg ampicillin (Polycillin-N; Apothecon, Princeton, NJ). The incision site was closed with 9-mm wound clips (Clay Adams, Parsippany, NJ) and treated with providone-iodine ointment (Acme United, Fairfield, CT). The animal was then returned to a prone position. Slack in the catheter was taken up by gentle pressure at the loop connected to the swivel (Fig 1). The button was placed subcutaneously in the interscapular incision and sutured to the skin using 3-0 silk sutures (CE-4; American Cyanamid, Danbury, CT). After irrigation with heparinized saline and ampicillin solution, the skin was closed with wound clips and treated with providone-iodine ointment as above.

Animal Care

After surgery, each rat was placed in an individual metabolism cage, modified by a slit opening in the central portion of the support serving as the roof of the cage. The spring could move within this slit, permitting complete freedom of movement of the rat within the cage. The spring-guarded PE50 tubing, secured by a clamp holding the swivel device, was out of reach of the rat. A Lifecare micropump equipped with a microdrip pump set-SL (Abbott Laboratories) was connected to the needle in the catheter apparatus via the Luer lock, taking care to exclude air from the line. Heparinized saline was infused at 2 mL/h. Rats had free access to water and rodent chow (Purina Mills, St Louis, MO). The animal room was on a 6 AM to 6 PM light cycle. Rats had recovered presurgery body weights in 48 hours. On the third day postsurgery, approximately 3 hours after the start of a light cycle, food was removed from the cages and an initial (time 0) blood sample was collected from the tail vein and centrifuged in heparinized hematocrit tubes at 2,000 × *g* for 6 minutes (Microcapillary Centrifuge Model MB; International Equipment, Needham Heights, MA). Plasma was removed and frozen at −80°C until analyzed. Infusion was then begun as described later. Blood was collected and samples were treated as above at the end of each 2-hour infusion period. Urine was collected in graduated cylinders set in ice below the outlet from each metabolism cage. Separate urine samples were collected from peptide-infused (PEP) rats for each 2-hour period of infusion; a single 8-hour sample was collected from heparinized saline–infused control (SAL) rats. Efforts were made to allow rats to empty their bladders as they were being removed from the cages for collection of blood samples; if rats did void during blood collection, these losses were noted. After final blood samples were taken at 8 hours, rats were killed by decapitation. The brain was rapidly removed, rinsed in cold saline, blotted on filter paper, and dissected over ice to obtain prefrontal cortex (PFC) and striata (STR). These specimens and the remaining brain (RB) tissue were frozen rapidly in liquid nitrogen and stored at −80°C until the time of analysis. Urine remaining in the bladder at necropsy was added to the final urine sample.

Analytical Methods

Blood samples were analyzed by high-performance liquid chromatography (HPLC) for Tyr, Phe, tryptophan (Trp), and Glu(Tyr) as described previously.[1] Each urine sample was lyophilized and reconstituted to one sixth of the original volume in 1N perchloric acid (PCA). The resultant suspension was mixed thoroughly and centrifuged. The pellet was discarded, and a 1,000-μL aliquot of the supernate was treated with 70 μL 10N KOH to precipitate excess perchlorate, set on ice for 10 minutes, and filtered by centrifugation at 2,000 × *g* in a BAS microfilter assembly (Bioanalytical Systems, West Lafayette, IN) fitted with a 0.45-μm Nylon-66

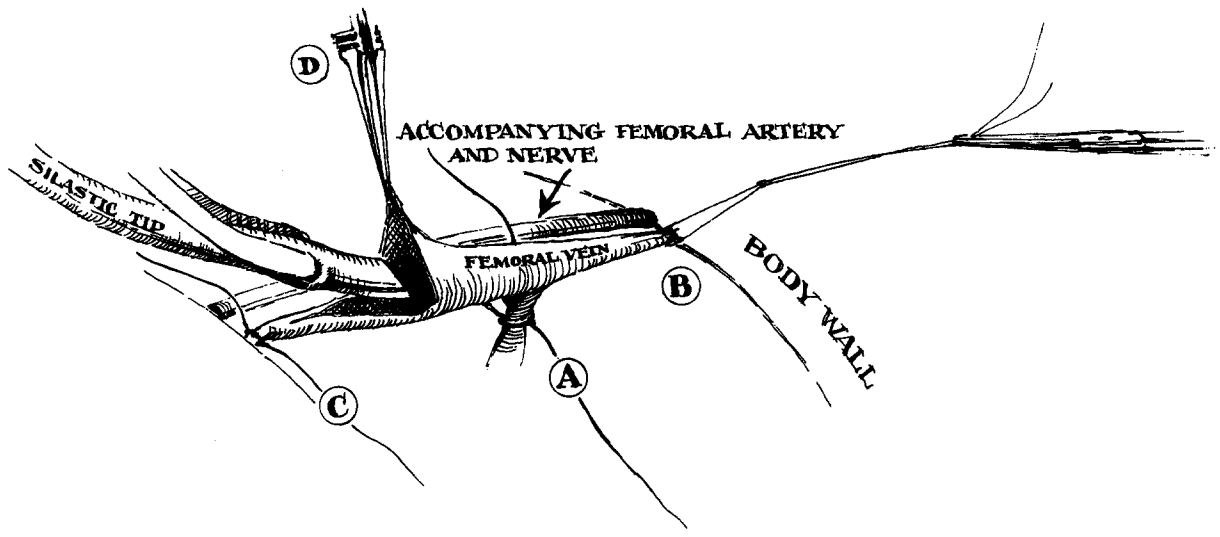

Fig 2. Insertion of the catheter. Under magnification, the femoral vein is carefully separated by blunt dissection from the accompanying artery and nerve, and the deep muscular branch of the femoral vein is identified. A 5-0 silk tie with a single overhand knot is placed around this branch of the femoral vein, and the vessel is ligated. A free end of the tie is brought under the femoral vein and over the artery and nerve (A) and left in place. Another 5-0 silk tie is placed proximally around the femoral vein and tied with a double overhand knot, leaving a 1-cm noose. The free ends are gripped with a hemostat, positioned so that the noose exerts a tourniquet effect on the vessel (B). A 5-0 silk tie is placed around the femoral vein at its most distal exposure, and the vessel is ligated (C). The vessel is gently raised with forceps close to the distal ligature, and a venotomy is made, using straight Vannas-style spring scissors with a 3-mm cutting edge (Fine Science Tools, Belmont, CA). The lip of the venotomy is lifted using Dumont microsurgery forceps (Fine Science Tools) (D). The catheter is placed in the vessel and advanced using curved forceps to the proximal tourniquet. The hemostat is repositioned to permit passage of the catheter. The catheter is advanced until the junction of the silastic tip with the PE50 tubing is within the vessel. The proximal tourniquet is tied at the junction, securing the catheter. The ligature around the deep vein and the distal ligature is tied securely around the PE50 portion of the catheter, and blood is drawn into the catheter to verify patency.

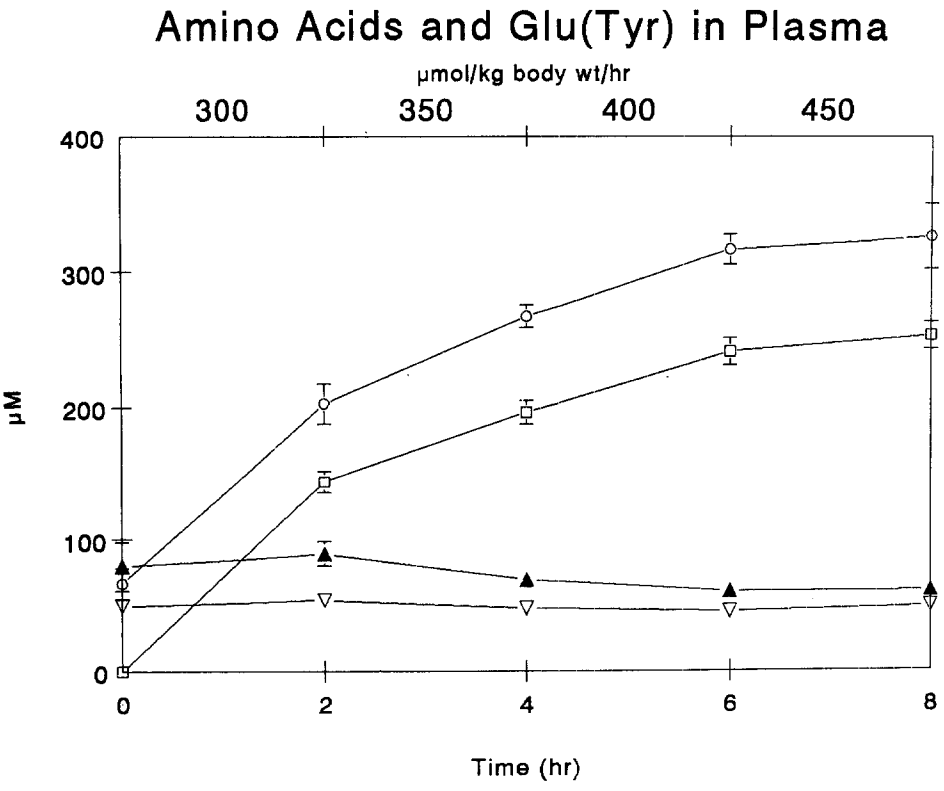

Fig 3. Concentrations of amino acids and Glu(Tyr) in plasma of PEP rats during infusion of peptide. (○) Tyr; (□) Glu-(Tyr); (▲) Trp; (▽) Phe. Rates of peptide infusion are shown on the top x-axis, corresponding to time on the lower x-axis. Mean ± SEM values for 4 rats are shown; some errors are small enough to be obscured by the symbols (see Table 1).

membrane (Rainin Instruments, Woburn, MA). Aliquots of the filtrate were analyzed for Glu(Tyr) by the HPLC method used for plasma, except that elution was performed with 12% methanol in 0.095% trichloroacetic acid and detection was at 275 nm to minimize absorbance of interfering substances.

Brain tissue was homogenized in 0.2N PCA containing dihydroxybenzylamine as an internal standard. Final concentrations were 100 mg PFC/500 μL PCA, 25 mg STR/100 μL PCA, and 1 g RB/2.5 mL PCA. Specimens were kept on ice during homogenization. RB was homogenized with an Ika-Werk Ultra-Turrex Tissuemizer (Tekmar, Cincinnati, OH) at a setting of 50; PFC and STR were homogenized with a Ten Broeck glass tissue grinder driven by a Precision Homogenizer (Precision Scientific, Chicago, IL) at a setting of 1. Homogenates were centrifuged at 4°C for 20 minutes at 13,000 × *g* in a Sorvall (Wilmington, DE) RC2-B refrigerated centrifuge. The supernate was removed and treated with 10N KOH at 1 μL/100 μL supernate and set on ice for 10 minutes. Further treatment was as described for plasma and urine. Levels of the catecholamines, norepinephrine (NE) and dopamine (DA), and the DA metabolite, homovanillic acid (HVA), were measured in brain tissue by HPLC with electrochemical detection, and a Catecholamine column (100 × 4.6 mm; Alltech Associates, Deerfield, IL) was used in the HPLC apparatus described previously.[4] The eluting buffer was prepared by dissolving 6.8 g sodium acetate trihydrate (Baker Analyzed; J.T. Baker, Phillipsburg, NJ), 6.3 g citric acid monohydrate (Sigma), and 50 mg disodium EDTA dihydrate (International Biotechnologies, New Haven, CT) in approximately 900 mL glass-filtered deionized water and adjusting the pH to 4.0 with glacial acetic acid (Fisher Scientific, Pittsburgh, PA). Sodium octyl sulfate (232 mg; Sigma) was then added, and the volume was made to 1 L; 45 mL was removed and replaced with 45 mL HPLC-grade methanol (Fisher). Brain tissue extracts were diluted appropriately (1:3 to 1:8) with eluting buffer, and 20-μL samples were injected in running solvent, sparged with helium. Elution (1.3 mL/min) was monitored by an electrochemical detector (Model LC-4B; Bioanalytical Systems) set at +750 mV. Reference standards of NE, DA, HVA, and dihydroxybenzylamine were obtained from Sigma.

Infusion

γ-L-Glutamyl-L-tyrosine [Glu(Tyr)] was obtained from Peptides International (Louisville, KY). The peptide was dissolved in heparinized saline, and pH was adjusted to 6.6 with NaOH. The final solution, made isotonic with NaCl, contained 20 mmol/L Glu(Tyr). The solution was sterilized by filtration through a sterile 0.22-μm cellulose acetate membrane filter unit (Lida Manufacturing, Bensenville, IL) into sterile Viaflex bags (Baxter Healthcare, Deerfield, IL) from which pump sets were filled. Target infusion rates for four experimental rats (PEP group) began at 15 mL (300 μmol peptide)/kg body weight/h for the first 2 hours and increased by 2.5 mL (50 μmol peptide)/kg body weight/h at 2-hour intervals to a maximum of 22.5 mL (450 μmol peptide)/kg body weight/h during the 6- to 8-hour interval. Four control rats (SAL group) were infused with heparinized saline at equivalent rates.

Statistical comparisons were based on Student's *t* test using Epistatistic software (Gustafson, Richardson, TX).

RESULTS

Concentrations of aromatic amino acids and Glu(Tyr) in plasma of PEP rats during the 8-hour infusion of Glu(Tyr) are shown in Fig 3. Plasma concentrations of Tyr and Glu(Tyr) increase steadily through the first 6 hours of infusion, with no further statistically significant increase at

Table 1. Concentrations (μmol/L) of Amino Acids in Plasma During Intravenous Infusion in Rats

	Period of Infusion (time of day)																	
	0 h (8:40-9:40 AM)			2 h (10:40-11:40 AM)			4 h (12:40-1:40 PM)			6 h (2:40-3:40 PM)			8 h (4:40-5:40 PM)		
Group	Tyr	Phe	Trp	Tyr	Phe	Trp	Tyr	Phe	Trp	Tyr	Phe	Trp	Tyr	Phe	Trp
SAL (n = 4)	89.6 ± 9.5	55.3 ± 5.3	89.9 ± 7.2	68.7 ± 5.8	62.6 ± 3.2	88.5 ± 9.3	61.8 ± 4.3	57.5 ± 2.4	69.0 ± 6.3	82.5 ± 13.6	83.9 ± 24.0	93.8 ± 14.6	53.4 ± 2.5†	59.2 ± 1.6	63.2 ± 3.6
PEP (n = 4)	76.1 ± 10.4	49.5 ± 6.3	79.9 ± 18.4	202.8 ± 14.9*†‡	54.1 ± 1.6	89.3 ± 9.2	267.2 ± 8.3*†‡	47.6 ± 3.2	69.0 ± 4.6	316.3 ± 11.2*†‡	44.9 ± 3.1	60.2 ± 4.2	325.8 ± 23.8*†	48.8 ± 2.7	60.9 ± 4.2

NOTE. Results are the mean ± SEM.
*Difference from corresponding value for SAL rats at $P < .01$.
†Difference from time 0 value within the same group at $P < .01$.
‡Difference from value in immediately preceding infusion period in rats within the same group at $P < .01$.

Table 2. Amino Acids in Rat Plasma and Brain

	Plasma (μmol/L)			Brain (nmol/g wet weight)			Plasma to Brain Ratio		
Group	Tyr	Phe	Trp	Tyr	Phe	Trp	Tyr	Phe	Trp
SAL (n = 4)	53.4 ± 2.5	59.2 ± 1.6	63.2 ± 3.6	71.0 ± 3.8	67.3 ± 2.0	13.0 ± 0.8	0.74	0.88	4.86
PEP (n = 4)	325.8 ± 23.8*	48.8 ± 2.7	60.9 ± 4.2	263.5 ± 21.0*	56.8 ± 1.8*	11.8 ± 0.9	1.24	0.86	5.16

NOTE. Amino acid levels were measured in plasma prepared from blood samples collected at 8 hours, immediately before rats were killed, and in RB from which PFC and STR had been removed. Results are the mean ± SEM.

*$P < .01$ *v* SAL.

8 hours. Concentrations of Tyr throughout remain higher than those of Glu(Tyr). Plasma concentrations of Tyr, Phe, and Trp in SAL and PEP rats are shown for comparison in Table 1. In each group, there were no significant differences in the concentrations of Phe or Trp at the end of any infusion period from the values at time 0 or from values in the preceding infusion period. Thus, there is no evidence of a reported diurnal rhythm[7] in the concentrations of these amino acids during this 8-hour experiment (~9 AM to 5 PM). In SAL rats, mean Tyr levels at 8 hours were significantly decreased as compared with time 0, but not compared with levels at the end of any infusion period. In PEP rats, infusion of Glu(Tyr) produced a marked increase in plasma Tyr, with the final concentration greater than four times the value at time 0 and over six times the final concentration in SAL rats.

Concentrations of aromatic amino acids in RB and their plasma to brain ratios in PEP and SAL rats are shown in Table 2. In brains of PEP rats, the mean concentration of Tyr is 3.7 times greater than control values, whereas the concentration of Phe is decreased significantly from that in SAL rats. However, plasma to brain ratios of Phe and Trp are similar in PEP and SAL rats, which suggests that transport of these amino acids from plasma to brain is not significantly impaired in PEP rats by the high plasma concentrations of Tyr. The plasma to brain ratio of Tyr is 67% higher in PEP than in SAL rats, which indicates that transport of Tyr between plasma and brain is affected by the observed high plasma and brain concentrations of Tyr and perhaps also by the high plasma concentration of Glu(Tyr).

Concentrations of NE, DA, and HVA in brain tissue of PEP and SAL rats are shown in Table 3. No significant differences result from the 3.7-fold higher concentrations of Tyr in brains of PEP versus SAL rats.

Excretion of Glu(Tyr) in the urine is reported in Table 4. Body weights were recorded just before the experiment started. For each rat, the actual amount of Glu(Tyr) infused was calculated from the measured concentration and volume of infusate delivered. When significant amounts of urine were lost, no values are listed. Small amounts of peptide were found in urine samples collected during the first 2 hours of infusion (at 300 μmol peptide/kg body weight/h) and in all subsequent samples. Even though urine collection from metabolism cages is not strictly quantitative, final samples from the four rats indicate that even during the period of maximum infusion (~450 μmol Glu(Tyr)/kg body weight/h), the estimated excretion of Glu(Tyr) remains less than 0.5% of the amount infused.

DISCUSSION

We have explored the behavior in young rats of intravenously administered Glu(Tyr) as a vehicle for elevating concentrations of Tyr in plasma and brain. A surgical procedure is described that permits delivery of infusate at increasing rates into the inferior vena cava with exteriorization of the catheter in the interscapular region. In other experiments in this laboratory, rats catheterized in this manner have been successfully infused for 14 days. At necropsy, the only finding attributable to the surgery was the presence of granulation tissue at the site of the button in the interscapular region of some animals. Other published methods for accessing the inferior vena cava involve catheterization via the femoral vein with exteriorization through the tail[8,9] or require opening of the abdominal cavity.[10]

The data in Fig 3 define the upper limit of plasma Tyr concentrations that can be achieved under the conditions of this experiment. The early sharp increase in plasma Tyr illustrates the inability of catabolic processes (eg, hepatic Tyr aminotransferase) to maintain normal plasma Tyr concentrations. Plateau concentrations of Tyr and Glu(Tyr) observed at 6 and 8 hours may be affected by the capacity of GGT to catalyze synthesis and hydrolysis of Glu(Tyr).[3]

The data in Table 4 show that excretion of Glu(Tyr) in urine begins in the initial infusion period. In our earlier study with rats maintained on TPN supplemented with Glu(Tyr), no Glu(Tyr) appeared in the urine.[1] In those studies, the peptide was infused at approximately 250

Table 3. Catecholamines and HVA in Rat Brain Tissue

	NE (pmol/mg)			DA (pmol/mg)			HVA (pmol/mg)		
Group	RB	PFC	STR	RB	PFC	STR	RB	PFC	STR
SAL (n = 4)	2.52 ± 0.12	1.72 ± 0.20	3.02 ± 0.50	2.38 ± 0.50	1.36 ± 0.82	45.1 ± 4.9	0.23 ± 0.02	0.24 ± 0.05	1.51 ± 0.19*
PEP (n = 4)	2.64 ± 0.12	1.98 ± 0.10	4.17 ± 0.33	2.17 ± 0.16	0.76 ± 0.45	57.4 ± 4.0	0.27 ± 0.01	0.25 ± 0.03	1.72 ± 0.13

NOTE. Values are based on wet weight of tissue, and are the mean ± SEM.

*Mean value for 3 rats.

Table 4. Urinary Excretion of Infused Glu(Tyr) in Rats

Rat No.	Body Weight (g)	Time (h)	Glu(Tyr) Infused (μmol/2 h)	Urine Collected (mL)	Glu(Tyr) in Urine (nmol)	Excretion of Infused Glu(Tyr) (%)
1	220	0-2	132	3.0	187	0.1
		2-4	156	5.9	252	0.2
		4-6	175	4.6	294	0.2
		6-8	199	10.2	321	0.2
2	209	0-2	127	5.4	234	0.2
		2-4	151	—	—	—
		4-6	172	5.2	499	0.3
		6-8	192	6.9	665	0.3
3	171	0-2	101	—	—	—
		2-4	117	5.2	157	0.1
		4-6	132	5.2	216	0.2
		6-8	148	7.1	118	0.1
4	201	0-2	122	6.3	182	0.2
		2-4	143	—	—	—
		4-6	163	3.6	143	0.1
		6-8	183	5.2	154	0.1

μmol/kg body weight/h and plasma concentrations were 55 μmol/L. In the present experiment, Glu(Tyr) was infused initially at 300 μmol/kg body weight/h and plasma concentration at 2 hours was 144 μmol/L. The high activity of GGT in renal tubules[3] can be expected to limit the concentration of intact peptide in the lumenal fluid; the capacity of this enzyme to hydrolyze lumenal Glu(Tyr) appears to be exceeded at plasma concentrations of 55 to 144 μmol/L Glu(Tyr). Renal transport of certain γ-glutamyl peptides has been demonstrated in mice.[11] If Glu(Tyr) is reabsorbed in rat kidney, the consistently low percentage of infused Glu(Tyr) excreted in the urine (Table 4) indicates that this transport capacity has not been exceeded in this experiment.

We were unable to detect Glu(Tyr) in brain homogenates, even though brain tissue was not entirely free of adventitious blood plasma containing 144 μmol/L Glu(Tyr). The limit of detection of the peptide in this experiment was estimated to be 20 pmol/mg tissue. The high activity of GGT in the choroid plexus[2] should minimize entry of intact Glu(Tyr) into the brain.

Several other derivatives of Tyr, including *N*-acetyl-Tyr and neutral α-carboxy–linked dipeptides of Tyr, have been used as intravenous sources of Tyr.[12,13] Certain commercial mixtures of amino acids for clinical use in TPN (eg, Aminosyn-II, 10%; Abbott Laboratories) contain *N*-acetyl-Tyr as a putative source of Tyr. However, *N*-acetyl-Tyr has poor bioavailability in humans, due to limited hydrolysis and also to significant urinary loss.[14] Maher et al[15] have used acute injections of several neutral dipeptides of Tyr (Tyr-Tyr, Tyr-Pro, Tyr-Ala, or Ala-Tyr) to produce transient elevations of brain Tyr in rats. The most soluble of these peptides is Ala-Tyr, with a solubility of approximately 0.2 mmol/L.[12,15] Short-term intravenous administration of Ala-Tyr has also been shown to increase plasma Tyr concentrations in patients in renal failure, for whom Tyr may be considered a conditionally essential amino acid.[16] In these patients, infusion of *N*-acetyl-Tyr did not increase plasma Tyr concentrations.

The relative insolubility of Tyr presents problems in vivo and in vitro. Precipitation of Tyr in rat tissues by feeding diets high in Tyr was reported many years ago.[17] Plasma concentrations of Tyr were not reported in these studies. In an adult patient with impaired ability to catabolize Tyr because of defective activity of hepatic Tyr aminotransferase (tyrosinemia type II), crystallization of Tyr in eyes and skin and massive tyrosinuria was noted at a plasma Tyr concentration of 1,847 μmol/L; skin lesions cleared and tyrosinuria disappeared when the plasma Tyr concentration was decreased to 459 μmol/L by a diet low in Tyr and Phe.[18] In another patient undergoing dietary treatment for tyrosinemia type II, crystallization of Tyr in skin and eyes was not observed at a plasma Tyr concentration of 680 μmol/L.[19] The maximum plasma Tyr concentration (381 μmol/L) produced in a single rat in the present study would thus not be expected to exceed the solubility of Tyr in tissues.

A number of studies, summarized by Milner and Wurtman,[20] have suggested that Tyr supplementation is effective in increasing brain stores of catecholamines depleted by rapid firing of neurons, by the use of drugs, in cold-stressed rats and in spontaneously hypertensive rats. These and other investigators suggest that catecholamine production is controlled by the activity of the rate-limiting enzyme, Tyr hydroxylase, and by concentrations of the substrate, Tyr. Tyr supplements given orally have been reported to reduce adverse reactions to environmental stress in humans.[21] In the present study, chronically elevated concentrations of Tyr were produced in plasma and brain. No differences were found between the ambient concentrations of NE, DA, and HVA in brains of rats with normal (71 nmol/g) and with markedly elevated (264 nmol/g) brain Tyr levels. This is consistent with effective feedback control by catecholamines of the activity of Tyr hydroxylase[22] and with the view of some investigators that Tyr hydroxylase activity is not limited by the concentration of Tyr in normal rat brain.[23]

Besides serving as a precursor of catecholamines in neural tissue, Tyr is also incorporated into a neuroactive dipeptide, L-tyrosyl-L-arginine (kyotorphin), which has been identified in mammalian brain[24] and in human cerebrospinal fluid.[25] Kyotorphin, administered centrally to mice, produces naloxone-inhibitable analgesia, presumably acting through an opioidergic system. This peptide is synthesized from Tyr and arginine in the brain by kyotorphin synthetase.[26] Patients with persistent pain were given arginine intravenously in an effort to produce analgesia by stimulating kyotorphin synthesis in the brain.[25] They reported relief from pain and also a warm feeling, ascribed to increased formation of nitric oxide from arginine in the endothelium. More recently, intraperitoneal administration of the methyl ester of Tyr has been shown to produce dose-dependent antinociception in mice,[27] presumably by providing sufficient Tyr to stimulate kyotorphin synthesis in the brain.

As a potential intravenous source of Tyr for clinical use,

Glu(Tyr) has several attributes: (1) Glu(Tyr) is highly soluble in water at room temperature; a concentration of 1.6 mol/L can be achieved when pH is adjusted to 5 by addition of NaOH.[1] (2) GGT, the enzyme that catalyzes hydrolysis of Glu(Tyr), is well characterized, widely distributed in endothelial cells, and active in human fetal and neonatal,[28] as well as adult, tissues. (3) Glu(Tyr), administered here in high concentration to rats, is not lost in appreciable amounts in the urine. And (4) Glu(Tyr) may be an endogenous metabolite, since Tyr is a substrate for transpeptidation by GGT.[3] Seven other acidic γ-glutamyl dipeptides, presumably synthesized in situ by GGT, have been identified in monkey brain in concentrations as high as 100 nmol/g wet tissue.[29] Thus, even if intravenously administered Glu(Tyr) is not totally excluded from the brain, it would seem unlikely to be associated with neurotoxicity.

ACKNOWLEDGMENT

We appreciate the sponsorship of Dr David Adamkin, Department of Pediatrics, in obtaining funding from the Alliant Community Trust Fund. We thank Greg Niznik, Department of Anatomical Sciences and Neurobiology, for help in development of the surgical procedure. We appreciate the assistance of Dr Agnes Jimenez, formerly of the Department of Physiology and Biophysics, in development of the electrochemical method for detection of catecholamines and HVA.

Portions of this study were performed in partial fulfillment of the requirements for the MS degree in Anatomical Sciences and Neurobiology awarded to D.C.B. in August 1994.

REFERENCES

1. Radmacher PG, Hilton MA, Hilton FK, et al: Use of the soluble peptide, gamma-L-glutamyl-L-tyrosine, to provide tyrosine in total parenteral nutrition in rats. JPEN 17:337-344, 1993

2. Meister A: Glutathione metabolism and its selective modification. J Biol Chem 263:17205-17208, 1988

3. Meister A: On the enzymology of amino acid transport. Science 180:33-39, 1973

4. Hilton M, Hilton F, Montgomery W, et al: Use of the stable peptide, γ-L-glutamyl-L-tyrosine, as an intravenous source of tyrosine in mice. Metabolism 40:634-638, 1991

5. Neuhäuser M, Göttmann U, Bässler KH: Experimentelles modell für langzeitversuche zur parenteralen ernährung an der wachsenden ratte. Infusionstherapie 11:175-180, 1982

6. Warnhoff M: Simultaneous determination of norepinephrine, dopamine, 5-hydroxytryptamine and their main metabolites in rat brain using high-performance liquid chromatography with electrochemical detection. J Chromatogr 307:271-281, 1984

7. Eriksson T, Wiesel K, Voog L, et al: Diurnal rhythms in rat plasma amino acids. Life Sci 45:979-986, 1989

8. Jones PA, Hynd JW: Continuous long-term intravenous infusion in the unrestrained rat—A novel technique. Lab Anim 15:29-33, 1981

9. Moslen MT, Kanz MF, Bhatia J, et al: Two cannula method for parenteral infusion and serial blood sampling in the freely moving rat. JPEN 12:633-638, 1988

10. Kimura RE, LaPine TR, Gooch WM III: Portal venous and aortic glucose and lactate changes in a chronically catheterized rat. Pediatr Res 23:235-240, 1988

11. Anderson ME, Meister A: Transport and direct utilization of γ-glutamylcyst(e)ine for glutathione synthesis. Proc Natl Acad Sci USA 80:707-711, 1983

12. Stegink LD: Parenteral amino acid requirements: Special problems and possible solutions, in Fomon SJ, Heird WC (eds): Energy and Protein Needs During Infancy. San Diego, CA, Academic, 1986, pp 183-206

13. Grimble GK: The significance of peptides in clinical nutrition. Annu Rev Nutr 14:419-447, 1994

14. Magnusson I, Ekman L, Wangdahl M, et al: *N*-Acetyl-L-tyrosine and *N*-acetyl-L-cysteine as tyrosine and cysteine precursors during intravenous infusion in humans. Metabolism 38:957-961, 1989

15. Maher TH, Kiritsy PJ, Moya-Huff FA, et al: Use of parenteral dipeptides to increase serum tyrosine levels and to enhance catecholamine-mediated neurotransmission. J Pharm Sci 79:685-687, 1990

16. Druml W, Roth E, Lenz K, et al: Phenylalanine and tyrosine metabolism in renal failure: Dipeptides as tyrosine source. Kidney Int 36:S282-S286, 1989 (suppl 27)

17. Schweizer W: Studies on the effect of L-tyrosine on the white rat. J Physiol 106:167-176, 1947

18. Hunziker N: Richner-Hanhart syndrome and tyrosinemia type II. Dermatologica 160:180-189, 1980

19. Goldsmith LQ, Judith Thorpe AB, Roe CR: Hepatic enzymes of tyrosine metabolism in tyrosinemia II. J Invest Dermatol 73:530-532, 1979

20. Milner JD, Wurtman RJ: Catecholamine synthesis: Physiological coupling to precursor supply. Biochem Pharmacol 35:875-881, 1986

21. Banderet LE, Lieberman HR: Treatment with tyrosine, a neurotransmitter precursor, reduces environmental stress in humans. Brain Res Bull 22:759-762, 1989

22. Okuno S, Fujisawa H: A new mechanism for regulation of tyrosine 3-monooxygenase by end product and cyclic AMP-dependent protein kinase. J Biol Chem 260:2633-2635, 1985

23. Westerink BHC, Wirix E: On the significance of tyrosine for the synthesis and catabolism of dopamine in rat brain: Evaluation by HPLC with electrochemical detection. J Neurochem 40:758-764, 1983

24. Takagi H, Shiomi H, Ueda H, et al: Morphine-like analgesia by a new dipeptide, L-tyrosyl-L-arginine (Kyotorphin) and its analogue. Eur J Pharmacol 55:109-111, 1979

25. Tagaki J, Harima A, Shimizu H: A novel clinical treatment of persistent pain with L-arginine. Eur J Pharmacol 183:1443, 1990

26. Ueda H, Yoshihara Y, Fukushima N, et al: Kyotorphin (tyrosine-arginine) synthetase in rat brain synaptosomes. J Biol Chem 262:8165-8173, 1987

27. Kawabata A, Kasamatsu K, Takagi H: L-Tyrosine–induced antinociception in the mouse: Involvement of central δ-opioid receptors and bulbo-spinal noradrenergic system. Eur J Pharmacol 233:255-260, 1993

28. Vanden Broeck MC, Geubelle F: Activité de la gamma-glutamyl transférase sérique dans une population pédiatrique. Ann Biol Clin (Paris) 40:567-572, 1982

29. Reichelt KL: The isolation of gamma-glutamyl peptides from monkey brain. J Neurochem 17:19-25, 1970