Effect of Protein Ingestion on Urinary Dopamine Excretion

Evidence for the Functional Importance of Renal Decarboxylation of Circulating 3,4-Dihydroxyphenylalanine in Man

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Abstract

Since dietary protein increases urinary dopamine (DA) excretion in animals, this study was undertaken to assess the role of DA production in the acute changes in renal function following protein ingestion in man. Excretion of DA, sodium, potassium, water, solute, and creatinine were measured in six normal men in 30-min intervals over 5 h after oral ingestion of protein and/or carbidopa, an inhibitor of DA formation from 3,4-dihydroxyphenylalanine (DOPA). Overall, protein increased urinary DA 50% ($P = 0.031$) while carbidopa reduced it 70% ($P < 0.0001$), although suppression of DA excretion by carbidopa was not uniform over the 5 h of observation. Carbidopa doubled the level of DOPA in venous plasma and greatly magnified the DOPA response to protein. Inhibition of decarboxylase activity reduced excretion of sodium, potassium, solute and water after protein ingestion. These results indicate that extraneuronal DOPA decarboxylation in kidney contributes to acute protein-induced changes in renal function in man and suggest a general role for the decarboxylation of circulating DOPA in the expression of dopaminergic effects on the kidney in vivo.

Introduction

Dopamine (DA1)[1] is known to exert several effects on renal function including, prominently, an increase in renal blood flow (1–3), a decrease in sodium reabsorption (4), and an increase in potassium and phosphate excretion (5, 6). These effects of DA to promote renal blood flow and sodium excretion contribute to its therapeutic utility in the treatment of hypotension and congestive heart failure. In addition, evidence has accumulated within the past few years implicating endogenous DA in the regulation of salt excretion (7–9), though the nature of the renal dopaminergic system remains obscure. Recent studies from this laboratory have indicated that dietary protein intake affects the urinary excretion of DA in the rat (10, 11). Because of the possibility that the relationship between protein intake and urinary DA might help elucidate the nature of the peripheral dopaminergic system, and because of the current interest in the relationship between protein intake, renal function and the pathogenesis of progressive renal failure (12), the present study was undertaken.

In this investigation the effect of a single protein meal on urinary DA excretion and renal function was determined in six normal subjects both before and after inhibition of DA biosynthesis with carbidopa. The results demonstrate that protein ingestion enhances DA formation by a mechanism involving the extraneuronal decarboxylation of circulating 3,4-dihydroxyphenylalanine (DOPA), the amino acid precursor of DA, probably within kidney. Since some of the effects of protein ingestion on renal function are antagonized by blockade of DA biosynthesis, these results support the hypothesis that renal responses to a protein meal are mediated, in part, by renal DA production and that one component of a dopaminergic regulatory system in kidney involves the extraneuronal decarboxylation of circulating DOPA to DA.

Methods

Study design. Six male subjects (ages 25–43 yr) with normal renal function and no history of hypertension were tested on the Clinical Research Center at the Beth Israel Hospital. The protocol was approved by the Committee on Clinical Investigations, New Procedures, and New Forms of Therapy at Beth Israel Hospital and informed consent was obtained from all subjects. The study employed a 2 × 2 factorial design with protein (presence or absence) and carbidopa (presence or absence) as the two factors. Subjects thus underwent a series of 4 studies with a variable sequence of testing in each subject and a 10–14-d interval between any two tests.

Protocol. Subjects were admitted to the Clinical Research Center the evening prior to testing and were studied after an overnight fast. In the morning at least 30 min elapsed between insertion of an intravenous catheter in an antecubital vein for blood sampling and collection of basal samples. Urine was obtained from spontaneous voidings. To ensure frequent voiding during the test procedure all studies were performed in the presence of a water diuresis, following an oral load of 20 ml/kg given at time zero. Urine and blood samples were collected at 30-min intervals over 5 h; pulse and blood pressure were also measured every 30 min. Although subjects stood to void, all blood samples were obtained with the subjects supine immediately before standing, ~25 min after the previous voiding.

In addition to the water load, in the two protein tests subjects consumed 60 g of protein over 15–25 min in the form of dietetic tuna (Diet chunk white tuna in water, Star-kist Foods, Inc., Terminal Island, CA); this protein meal also contained 4.3 g fat, no carbohydrate, 5 meq sodium, 15 meq potassium and ~200–400 ng free DOPA (by our own analysis of tuna DOPA content). In the two studies with carbidopa (kindly supplied by Merck, Sharp and Dohme, West Point, PA), subjects were given two 50-mg oral doses 9 and 1 h before the start of the test procedure.

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1. *Abbreviations used in this paper:* ANOVA, analysis of variance; $C_{H_2O}Cl$, free water clearance; $C_{Osmol}Cl$, osmolar clearance; CrCl, creatinine clearance, DA, dopamine; DOPA, 3,4-dihydroxyphenylalanine; E, epinephrine; NE, norepinephrine.

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Urine catecholamine analysis. Urine samples were acidified to a pH between 2 and 3 and stored at −20°C prior to assay. Unless otherwise specified below all chemicals were obtained from Fisher Scientific Co. (Fair Lawn, NJ).

Analysis of catecholamines in urine followed the method of Smedes et al. (13) as modified by Macdonald and Lake (14). To 1.0-ml aliquots of urine was added 100 μl of the internal standard (3,4-dihydroxybenzylamine, DHBA; Aldrich Chemical Co., Milwaukee, WI) in 50 mM H_3PO_4; pH was adjusted to 7.5 as necessary. This mixture was then added to 30–50 mg of Sepralyte SCX (Analytichem International, Harbor City, CA) previously activated by exposure to 1 ml of 0.2 M Na_2HPO_4, pH 7.5, and mixed vigorously. After removal of the supernatant, the SCX was washed twice with water. Catecholamines were eluted with 1 ml of 1 M NaH_2PO_4, pH 2.9. This supernatant was transferred quantitatively into screw-topped, glass tubes. Approximately 0.5 ml of NH_4Cl/NH_4OH buffer containing 0.2% (wt/vol) diphenylboric acid ethanolamine complex (Aldrich Chemical Co.) and 0.5% EDTA was added to bring pH to 8.6. After addition of 2.5 ml of *n*-heptane containing 1% (vol/vol) octanol and 0.35% (wt/vol) tetraoctylammonium bromide (Fluka AG, Buchs, Switzerland) tubes were capped and shaken. The heptane supernatant was then transferred to a centrifuge tube and 1 ml octanol and 0.2 ml of 0.4 N acetic acid containing 10–15 mg glutathione were added. After mixing and centrifugation, aliquots of the acetic acid phase were injected onto a chromatographic system for quantitation of catecholamines.

This system was composed of a pump (6000A; Millipore, Waters Associates, Chromatography Division, Milford, MA), an automatic sample injector (Waters Intelligent Sample Processor, WISP; Millipore), a reverse-phase column (250 × 4 mm, Bio-Sil ODS-5S; Bio-Rad Laboratories, Richmond, CA) and a glassy carbon amperometric detector (LC-4B/17; Bioanalytical Systems, West Lafayette, IN). The mobile phase was an acetate-citrate buffer composed of sodium acetate (100 mM), sodium hydroxide (60 mM) and citric acid (30 mM, all three from Mallinckrodt, Inc., Paris, KY) at pH 5.3 containing 4–6% methanol, 80 mM EDTA and 0.95 mM sodium octyl sulfonate (Aldrich Chemical Co. or Eastman Kodak, Rochester, NY) flowing at a rate of 1.0 ml/min. The detector potential was set at +0.65 V vs. Ag/AgCl reference electrode. Detector response was quantitated by peak height utilizing an integrating recorder (3390A, Hewlett-Packard Co., Avondale, PA). Intraassay coefficients of variation for urine samples (corrected for recovery) are 4–6% for epinephrine, norepinephrine and dopamine; interassay coefficients of variation are 6–7% for all three catecholamines.

Plasma DOPA and norepinephrine assay. Plasma for DOPA and norepinephrine (NE) determinations was collected in chilled, heparinized tubes containing reduced glutathione (5 mM; Sigma Chemical Co., St. Louis, MO), separated promptly and stored at −75°C until analysis, which followed a modification of the method of Davis et al. (15). After addition of DHBA to a plasma sample, catecholamines were isolated by adsorption onto acid-washed alumina (Woelm neutral, ICN Nutritional Biochemicals, Cleveland, OH) in the presence of 2 M tris(hydroxymethyl)aminomethane buffer (pH 8.7; Sigma Chemical Co.) containing 2% EDTA. Catecholamines were eluted from the alumina with 0.2 N perchloric acid. Adsorption onto alumina and elution with perchloric acid were repeated in order to reduce chromatographic interference from uric acid. After removal of alumina particles by filtration (microfilter; Bioanalytical Systems), aliquots of the second alumina eluate were injected onto a liquid chromatographic system for catecholamine analysis.

A system similar to that employed for urine catecholamines was used for plasma DOPA and NE determinations except for the presence of a manual injector (7125; Rheodyne, Cotati, CA) and a different pump (400/02H; Peris Industries Inc., State College, PA). The mobile phase was composed of 150 mM monochloroacetic acid (Mallinckrodt, Inc.) containing 0.5 mM EDTA, 2 mM octane sulfonic acid, 4–5 g NaOH (to adjust pH to 3.2–3.3) and 2–4% acetonitrile. Flow rate was also 1.0 ml/min. Intraassay coefficients of variation for DOPA are 4% and for NE 2–4%. In a control test of the six individuals in this study, the coefficients of variation for plasma DOPA obtained every 30 min over a 5-h period on a morning when they remained fasting ranged between 6 and 11%.

Biochemical analyses. Serum and urine levels of creatinine and of sodium and potassium were measured by computerized analyzer (SMAC II, Technicon Instruments, Inc., Tarrytown, NY). Serum and urine osmolalities were determined by freezing point depression (Osmometer, Advanced Instruments, Needham Heights, MA). Plasma aldosterone was measured by standard radioimmunoassay (Coat-a-Count, Diagnostic Products Corporation, Los Angeles, CA). Clearances for creatinine, free water and solute were calculated according to standard formulae.

Data analysis. Data are presented as means±SEM, unless otherwise stated. The study followed a 2 × 2 factorial design with protein (0/+) and carbidopa (0/+) as the two main factors. In addition, since each subject participated in all four parts of the study, "subjects" was considered a third factor crossed with both protein and carbidopa. Data analysis was therefore based upon a 3-factor analysis of variance (ANOVA), mixed model, with protein (+/0) and carbidopa (+/0) as fixed factors and "subjects," a random factor (16). ANOVA also included an additional factor "time" (fixed) for repeated measures within a test where appropriate. Homogeneity of variances within the ANOVA was assessed by Hartley's test (17) and goodness of fit of the data to the ANOVA model by the Wilk-Shapiro and Kolmogorov-Smirnov tests (16). Calculations were performed with BMDPC Statistical Software, Los Angeles, CA (18).

In analysis of correlation, correlation coefficients were calculated after determination that both variables were normally distributed (16). Following "z" transformation (with bias correction), correlation coefficients were analyzed by ANOVA. Test mean correlation coefficients represent reverse transformation of the average of z transformed data.

Results

Effect of protein and carbidopa on urinary catecholamine excretion. Urinary excretion of DA, NE, and epinephrine (E) over the 5 h collection period in each of the four tests is shown in Table I. While neither protein ingestion nor carbidopa administration affected urinary NE or E, DA excretion was altered by both. Protein increased urinary DA excretion both in the presence and absence of carbidopa ($P = 0.031$), while carbidopa lowered urinary DA excretion by >60% whether protein was present or not ($P = 0.0001$). The temporal profile of DA excretion over the 5-h test periods, illustrated in Fig. 1, provides further insight into these effects of protein and carbidopa on urinary DA excretion. As shown in Fig. 1, although urinary DA increased slightly at the end of the test period in the two studies without carbidopa, in the two carbidopa studies the rise in urinary DA was progressive over the 5 h of observation. The temporal characteristics of DA excretion in these studies thus suggest: first,

Table I. Effects of Protein and Carbidopa on Urinary Catecholamine Excretion

A	B			
Protein	Carbidopa	DA	NE	E
		μg	μg	μg
0	0	51.2±4.7	6.67±1.32	1.58±0.42
	+	11.3±2.5	6.88±0.77	1.53±0.26
+	0	68.5±7.6	7.05±0.75	1.83±0.43
	+	25.0±3.9	6.18±0.58	1.30±0.19
ANOVA Table				
A Protein		0.031	NS	NS
B Carbidopa		0.0001	NS	NS
A × B		NS	NS	NS

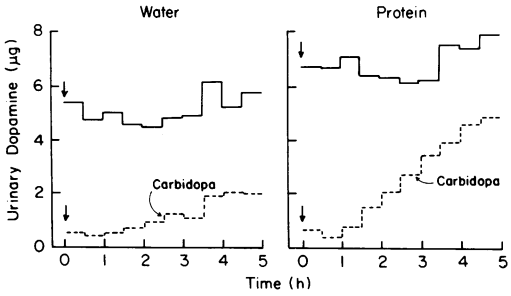

Figure 1. Time course of urinary dopamine excretion after water alone or water plus protein, each in the absence (*solid line*) and presence (*broken line*) of carbidopa pretreatment. Data are presented as the mean for the six subjects in each test condition. Vertical arrows denote time of feeding.

that the urinary DA response to protein ingestion is prompt, since a difference in DA excretion between the two studies without carbidopa was apparent even in the first 30-min urine collection; and second, that the effectiveness of carbidopa as an inhibitor of DA formation was maximal in the first 1–2 h of the test interval, but waned thereafter, especially in the presence of protein.

Effect of protein and carbidopa on plasma concentrations of DOPA and NE. Since plasma DOPA is a potential precursor of urinary DA, measurements of DOPA in venous plasma were obtained over the 5-h test interval in all studies. The results of these determinations are shown in Fig. 2. In the absence of carbidopa, DOPA levels changed very little over time and were on average increased slightly (~10%) by protein ingestion. Carbidopa administration, however, markedly increased DOPA levels both in the presence and absence of protein (main effect of carbidopa, $P = 0.0015$). Ingestion of protein following carbidopa treatment produced an even more striking elevation in plasma DOPA, achieving peak levels 2.5 times the preingestion baseline 2 h after the protein meal. Statistical analysis of the data shown in Fig. 2 demonstrated highly significant, time-dependent effects of protein and carbidopa and a time-dependent interaction between protein and carbidopa (protein × time, carbidopa × time

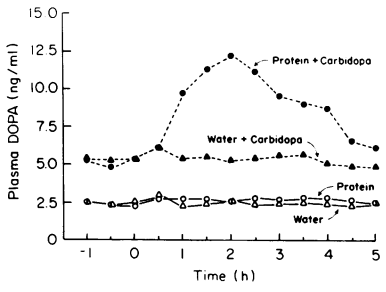

Figure 2. Effect of protein and carbidopa on plasma DOPA concentrations. Water alone (*triangles*) or water plus protein (*circles*) were ingested at time "0." Carbidopa studies are denoted by solid symbols. Data are presented as the mean for the six subjects in each test condition.

ANOVA Table	
A Protein (+/0)	0.046
B Carbidopa (+/0)	0.0015
A × B	0.071

and protein × carbidopa × time interactions, all $P < 0.00001$). Plasma NE concentrations (data not shown) were unaffected by the administration of protein or of carbidopa.

Effect of protein and carbidopa on urinary sodium and potassium excretion. Sodium and potassium excretion during each 30-min collection are shown in Fig. 3 and the total amounts excreted over the 5-h test periods are summarized in Table II. Total excretion of both sodium and potassium was higher following the protein meal, especially in the absence of carbidopa, and was lower after carbidopa, especially in the presence of protein. Although electrolyte content of the protein meal (5 meq sodium and 15 meq potassium) may have contributed to the elevation in cation excretion observed following protein ingestion, the natriuretic response to protein (~40 meq) was far greater than the increment in sodium intake and the effect of carbidopa to suppress potassium excretion was more pronounced following protein despite the additional potassium intake. Statistical analysis of the electrolyte excretion data, as summarized in Table II, revealed a highly significant antinatriuretic effect of carbidopa ($P = 0.009$), but only a marginally significant protein-induced natriuresis ($P < 0.1$). When the data were analyzed following logarithmic transformation, however, the main effects of both protein and carbidopa were statistically significant ($P < 0.05$). The explanation for this difference resides in the fact that among all subjects natriuretic responses to protein were of similar proportion, but not of similar amount. In a mixed model ANOVA, such as the one employed here, the protein variance is compared to the protein × subject variance, the measure of variability among subjects in the effect of protein. When the protein × subject variance is itself statistically significant, as in the analysis of the untransformed sodium excretion data, the

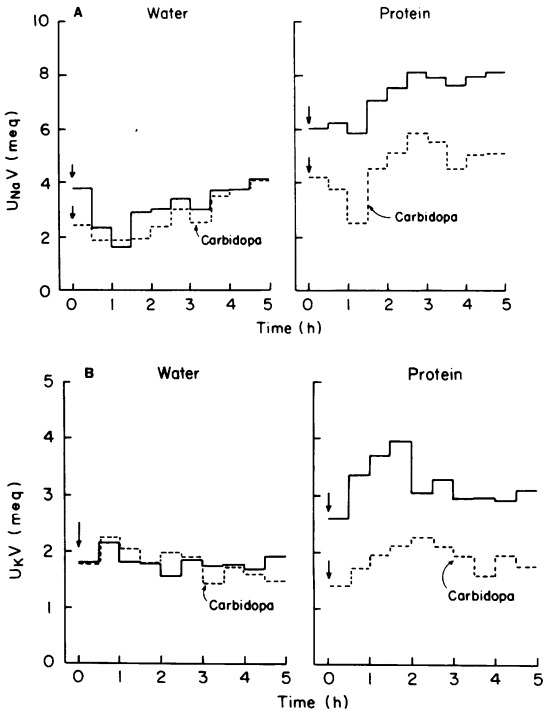

Figure 3. Time course of urinary sodium (*A*) and potassium (*B*) excretion after water alone (left) or water plus protein (right). Studies after carbidopa pretreatment are shown by broken lines. Vertical arrows denote time of feeding. Data are presented as the mean for the six subjects in each test condition.

Table II. Effects of Protein and Carbidopa on Sodium, Potassium and Water Excretion

A	B				
Protein	Carbidopa	$U_{Na}V$	U_KV	Volume	Volume
		meq	*meq*	*ml*	*% load*
0	0	32.0±9.0 (26.0)	18.3±1.1	1167±109	87.2±7.3
0	+	27.4±8.4 (21.6)	18.2±1.8	956±182	75.7±14.8
+	0	72.5±17.8 (64.0)	32.0±3.5	1448±147	107.8±10.9
+	+	46.4±15.0 (33.8)	18.9±3.3	1061±188	76.5±12.2
ANOVA Table		0.065 (0.02)	0.064	0.0028	0.005
A Protein		0.009 (0.03)	0.076	0.011	0.027
B Carbidopa		0.099 (0.13)	0.041	NS	NS
A × B					

Data in parentheses refer to the results of analysis of $U_{Na}V$ data following logarithmic transformation. Mean Na excretion in each test is presented as the antilogarithm of mean log (Na) and is, therefore, the geometric mean of Na excretion in the 6 subjects.

risk of type II (false negative) errors increases. The significance of this protein × subject variation, however, disappears with logarithmic transformation, implying that the effect of protein is not additive among all subjects, but multiplicative (i.e., proportional), a condition that favors the application of ANOVA to log-transformed data. For potassium excretion the main effects of protein and carbidopa were of borderline significance, although the interaction between protein and carbidopa was statistically significant ($P = 0.041$). (Results of ANOVA following logarithmic transformation of urinary potassium were equivalent to those of the untransformed data presented in Table II.)

One difference of note between sodium and potassium excretion is apparent from Fig. 3. While potassium excretion was relatively stable over the 5 h of observation, sodium excretion rose progressively. Since this temporal pattern in urinary excretion was common to both sodium and DA (Fig. 1), the relationship between these two was examined more closely. The correlation between sodium and DA was computed for all subjects from the 10 urine samples collected during each test and the 24 correlation coefficients (after "z" transformation) were then analyzed by ANOVA. In the absence of either protein or carbidopa the average correlation (obtained by retransformation of means from z-transformed data) between sodium and DA was only 0.2. With protein alone the mean correlation was 0.5, with carbidopa alone 0.7 and with both protein and carbidopa 0.6. Statistical analysis indicated that carbidopa, both in the presence and absence of protein, increased the correlation between urinary sodium and DA excretion ($P < 0.01$). Inhibition of DOPA decarboxylation (and the gradual disappearance of this effect over time) altered the excretion of sodium in parallel with that of DA, implying a primary role for DA in the regulation of renal sodium excretion. The lower correlation between sodium and DA in the presence of protein (compared to carbidopa) suggests that other factor(s), in addition to DA, contribute to the natriuretic response to protein ingestion.

Effect of protein and carbidopa on plasma aldosterone levels. Although the main overall effects of protein feeding and carbidopa on aldosterone concentrations were not statistically significant (105 pg/ml on average without vs. 91 with protein; 91 pg/ml without vs. 105 with carbidopa), mean levels were higher at 0 and 1 h but not at 5 h in the presence of carbidopa than in its absence (79 pg/ml without vs. 102 with carbidopa at 0 h, 119 without versus 142 with at 1 h, and 76 without versus 71 with at 5 h; carbidopa × time interaction, $P = 0.011$). Since by 5 h the effect of carbidopa on urinary DA excretion (Fig. 1) had largely, though not completely, dissipated, these data are consistent with an effect of carbidopa to increase plasma aldosterone concentrations. Similar observations were reported previously in volume-expanded dogs (19), suggesting that in these two situations the inhibitory effect of DA on aldosterone secretion (reviewed elsewhere, 20), depends upon DOPA decarboxylation to DA.

Effect of protein and carbidopa on water and solute excretion. The total volumes of urine excreted over the 5 h test periods are presented in Table II and the rates of free water and osmolar clearances for each 30-min interval are shown in Fig. 4. Urine volumes (in absolute amount or as percent of administered load) were greater following protein ingestion ($P < 0.005$) and less after carbidopa administration ($P < 0.03$). Examination of Fig.

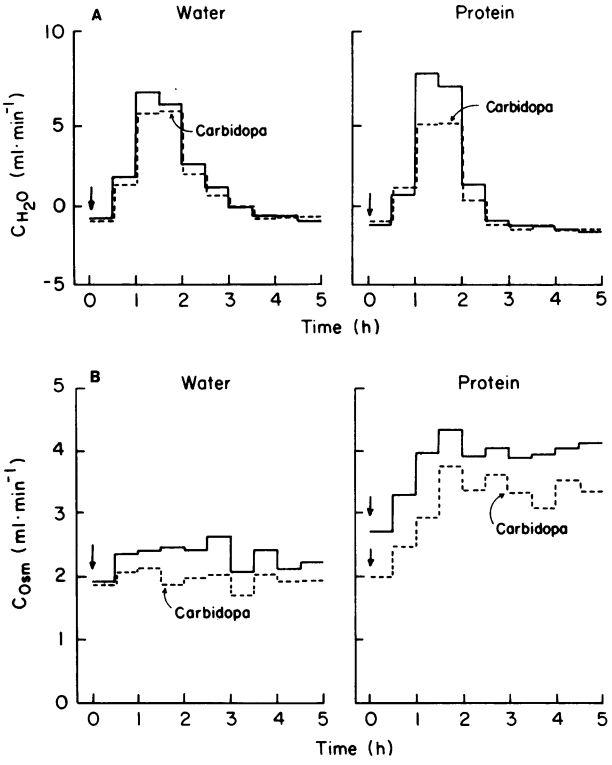

Figure 4. Effect of protein and carbidopa on free water (*A*) and osmollar (*B*) clearance. Studies after carbidopa pretreatment are shown by broken lines. Vertical arrows denote time of ingestion of water (left) or water plus protein (right). Data are presented as the mean for the six subjects in each test condition.

ANOVA Table

	$C_{H_2O}Cl$	$C_{Osmol}Cl$
A Protein (+/0)	<0.01	<0.005
B Carbidopa (+/0)	NS	<0.01
A × B	NS	NS

4 indicates that the major effect of protein was to increase osmolar clearance throughout the entire time of the study, while carbidopa lowered urine volume by diminishing both osmolar and free water clearance.

Effect of protein and carbidopa on serum creatinine, creatinine excretion, and creatinine clearance. Serum creatinine concentrations (computed for each subject from the mean of all measurements obtained during each 5-h test period) and total creatinine excretion are presented in Table III and the calculated creatinine clearance for each 30-min interval illustrated in Fig. 5. Serum creatinine levels were, on average, 30% higher following protein ingestion ($P = 0.0006$) and 7% higher after carbidopa administration ($P = 0.028$), while basal creatinine values (not shown) did not differ among the four tests. Urinary creatinine excretion was also markedly increased by protein feeding (an average elevation of 56% in the two tests, $P = 0.0003$). Carbidopa also influenced creatinine excretion, reducing it 3% in the presence of protein and elevating it 15% in its absence (protein × carbidopa interaction, $P = 0.031$). Protein ingestion raised creatinine clearance ($P < 0.05$). Although in the presence of protein, carbidopa administration increased serum and reduced urine creatinine, implying a diminution in creatinine clearance, the effect of carbidopa on creatinine clearance was not statistically significant. The nonsteady state conditions in the circulating creatinine pool during these studies, however, emphasizes the limitations inherent in the use of creatinine clearance as a measure of glomerular filtration.

Discussion

This study demonstrates that ingestion of a single protein meal acutely increases urinary DA excretion in normal human subjects (Table I, Fig. 1); NE and E excretion, in contrast, are not affected. This observation is consistent with data in the rat documenting a rise in urinary DA excretion in response to protein supplementation of a lab chow diet (10) and a diminution in urinary DA with protein restriction (11). The available evidence, therefore, indicates that in both man and rodent dietary protein exerts a specific stimulatory effect on urinary DA excretion.

The present study, moreover, provides evidence that the protein-induced augmentation in DA excretion involves the extraneuronal decarboxylation of DOPA since carbidopa reduced urinary DA excretion without affecting urinary NE (Table I, Fig. 1); intraneuronal blockade of aromatic-L-amino acid decarboxylase (the enzyme responsible for DOPA decarboxylation) would be expected to lower urinary NE as well as DA. Since aromatic-L-amino acid decarboxylase is widely distributed throughout the body (21, 22), it is probable that decarboxylation of DOPA to DA occurs in many tissues. DOPA decarboxylation in tissues apart from kidney, however, is unlikely to contribute to urinary DA excretion as evidence from micropuncture experiments (23) and from the isolated perfused rat kidney (24) demonstrates that DA presented to the kidney is extensively metabolized and not excreted as free DA. Moreover, in previous work (8), as well as in the present study (Table I), inhibition of decarboxylation sharply reduced urinary DA excretion.

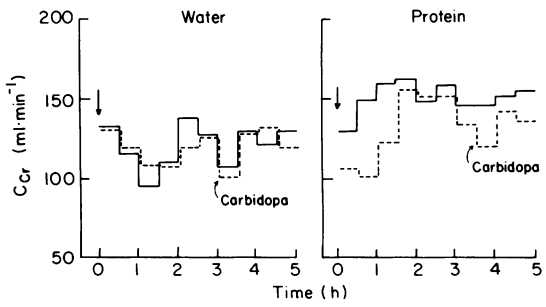

Figure 5. Effect of protein and carbidopa on creatinine clearance. Vertical arrows denote ingestion of water (left panel) or water plus protein (right panel). Carbidopa studies are denoted by broken lines. Data are presented as the mean for the six subjects in each test condition. Average CrCl for each test was: water alone, 121 ml/min (no carbidopa) and 119 (+ carbidopa); and protein meal, 151 ml/min (no carbidopa) and 132 (+ carbidopa).

The temporal sequence of changes in plasma DOPA and urinary DA in the presence of carbidopa, summarized in Figs. 1 and 2, provides additional support for the conclusion that the increment in urinary DA excretion following protein ingestion reflects decarboxylation of circulating DOPA. The changes observed in plasma DOPA concentration (Fig. 2), furthermore, suggest the existence of three distinct phases in the urinary DA response to protein after carbidopa administration: (*a*) an initial period of effective inhibition of DA formation; (*b*) a period of less effective blockade due to increased circulating levels of DOPA (since carbidopa is a competitive inhibitor of the enzyme); and (*c*) a final period of declining antagonism of DOPA decarboxylation, as manifested by increasing DA excretion despite falling plasma DOPA levels. The time course of changes in plasma DOPA and urinary DA excretion, in the presence of carbidopa blockade, thus are consistent with the hypothesis that protein induces release of DOPA into the circulation, which in turn leads to an increase in urinary DA excretion.

The finding that inhibition of DOPA decarboxylation (in the absence of protein) is associated with a doubling of the plasma DOPA concentration indicates the major role played by decarboxylation in the clearance of circulating DOPA throughout the body (Fig. 2). In addition, ingestion of the protein meal, in the setting of impaired clearance of DOPA from plasma due to carbidopa pretreatment, resulted in a further increment (+150%) in plasma DOPA concentration. This acute rise in plasma DOPA implies that protein ingestion increases entry of DOPA into the circulation which was only detectable in this study when metabolism of DOPA was blocked by carbidopa. Assuming that protein ingestion induced an equivalent release of DOPA in the absence of carbidopa, the small and statistically insignificant elevation of plasma DOPA following protein (Fig. 2) suggests that the DOPA formed in response to protein ingestion is rapidly removed from plasma and converted to DA and DA metabolites, a portion of which is excreted in the urine (Fig. 1). Because of the rapidity of this metabolic transformation, the enhanced flux of DOPA through the circulating compartment following protein, as reflected by a rise in venous plasma DOPA concentration, was demonstrable only in the presence of decarboxylase inhibition. Given the fact that in the dog arterial DOPA levels are higher than venous (25), the possibility exists that greater changes in DOPA concentration after protein may have been demonstrable in arterial plasma.

The increase in plasma DOPA noted following protein intake in carbidopa-treated subjects must originate from either exogenous or endogenous sources. The possibility that DOPA might be a constituent of the ingested protein or the product of synthesis from tyrosine within gut tissues or by intestinal microflora is unlikely since its content in canned tuna is low and the major metabolic fate for DOPA within the gastrointestinal tract is local

decarboxylation or conjugation. Moreover, if urinary DA responses to protein or tyrosine reported elsewhere are also indicative of systemic release of DOPA prior to renal decarboxylation, the demonstration that oral tyrosine reproduces in rats the protein-related rise in urinary DA and increases plasma levels and urine content of DA in human subjects (10, 26, 27), implies that the DOPA response to protein is related to the increased availability of tyrosine. This relationship, however, does not depend upon oral administration since tyrosine delivered parenterally also elevates urine and plasma DA (28, 29). Consequently, a more likely explanation for DOPA entry into the circulation is that it represents an endogenous response to ingestion of the protein load.

Whether this DOPA response to protein is secondary to mass action from the postprandial absorption of tyrosine from the gut or is due to an unidentified neurohumoral reflex must await future studies, but in either case, release of DOPA could theoretically occur from any peripheral tissues capable of converting tyrosine to DOPA. These include melanocytes in which DOPA is formed in the synthesis of melanin by the action of the enzyme tyrosinase and chromaffin tissue (adrenergic nerves, adrenal medulla, and extraadrenal chromaffin tissue, such as paraganglia, carotid body and the like) in which DOPA formation by tyrosine hydroxylase is the first step in catecholamine biosynthesis. Chromaffin tissue, in general, is the more probable source, although a connection between skin pigmentation and plasma DOPA levels was suggested in animals (30). Since preliminary evidence suggests that the DA response to protein is independent of sympathetic nerves or adrenal innervation (10), an attractive alternative is the possibility, suggested previously in a different context (31), that circulating DOPA derives principally from the extraadrenal chromaffin tissue. Identification of the tissue responsible for the DOPA response to protein ingestion clearly requires further study.

The results of studies with carbidopa, as shown in Tables II and III and in Figs. 3–5, are consistent with DA participation in some of the renal responses to protein ingestion. In particular, the protein-induced elevations in sodium excretion and in osmolar clearance were blunted by pretreatment with carbidopa. The effect on urinary sodium excretion is especially noteworthy. Rapidly accumulating evidence indicates a potentially important role for DA in the regulation of sodium excretion (9, 32), an effect that may be independent of changes in renal blood flow (4). The concordant responses of both DA and sodium to protein ingestion and to carbidopa administration and the correlation between urinary DA and sodium in the presence of carbidopa support a role for DA in the regulation of sodium excretion in general, and implicate DA in the natriuresis associated with protein ingestion demonstrated here. Although prompt augmentation of sodium and DA excretion by protein ingestion suggests an intrarenal site for both responses, the early elevations in plasma aldosterone induced by carbidopa raise the possibility that alterations in the secretion of this hormone may also contribute to dopamine-sodium interactions in the context of protein ingestion.

The effects of protein on renal function noted here, and elsewhere (33), are remarkably similar to the known effects of DA in kidney (1–3). Both protein and DA increase clearance of paraamino hippurate, inulin, creatinine and solute, as well as the excretion of sodium and potassium ([1, 3, 33, 34] Tables II and III, Figs. 3–5). Moreover, the renal effects of exogenous DOPA also resemble those associated with protein and DA (35);

Table III. Effects of Protein and Carbidopa on Serum Creatinine and Urinary Creatinine Excretion

A	B		
Protein	Carbidopa	Serum creatinine	$U_{Cr}V$
		mg/dl	*mg*
0	0	0.85±0.06	281±8
	+	0.92±0.06	323±13
+	0	1.11±0.08	480±27
	+	1.18±0.10	464±24
ANOVA Table			
A Protein		0.0006	0.0003
B Carbidopa		0.028	NS
A × B		NS	0.031

in fact, direct administration of DOPA into the renal artery of the dog exerts specific dopaminergic responses that are blocked by carbidopa (6). Thus, the changes in renal function following protein ingestion in the presence and absence of carbidopa are entirely consistent with the hypothesis that extraneuronal renal DA formation mediates some of the effects of protein on renal function. In the present study the increased DA generated from DOPA in the kidney most likely reflects enhanced delivery of DOPA via the circulation.

The present study thus illuminates two components of an endogenous renal dopaminergic system. First, alterations in the availability of the circulating precursor DOPA may participate in the renal functional responses to a physiological stimulus, protein ingestion in this case. And second, the extraneuronal decarboxylation of DOPA within renal parenchyma may play a potentially important role in mediating dopaminergic effects on renal function. Although sympathetic (noradrenergic) or specific renal dopaminergic nerves that release DA may also contribute to the dopaminergic regulation of renal function, their role is as yet undefined. While the results of the current studies do not exclude the possibility that DA from some intraneuronal source in kidney participates in the alterations in renal physiology associated with protein ingestion, they do emphasize the prominent role of extraneuronal DOPA decarboxylation in several of the postprotein responses.

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