IN VITRO NUCLEAR MEDICINE

Development of an Enzyme-Radioimmunoassay for the Measurement of Dopamine in Human Plasma and Urine

Bahjat A. Faraj, William R. Walker, Vernon M. Camp, Farouk M. Ali, and Woodfin B. Cobbs, Jr.

Emory University School of Medicine, Atlanta, Georgia

An enzyme-radioimmunoassay for the measurement of dopamine is described. It is based on the incubation of plasma or urine in the presence of catechol-O-methyltransferase and S-adenosylmethionine. The O-methylated dopamine metabolite formed (3-O-methyldopamine) was characterized by radioimmunoassay. As little as 0.5 ng of dopamine can be detected. The assay was found to be specific, since no cross-reactivity was noted for several compounds related to dopamine. The enzyme-radioimmunoassay of dopamine was used to determine the concentrations of dopamine in urine and plasma of normal volunteers. In this group, urinary dopamine averaged 182.1 ± 2.2 μg/24 hr, and the plasma concentration 0.211 ± 0.052 ng/ml. However, in children with neuroblastoma, there was a several-fold increase over controls in the average urinary and plasma levels of dopamine (8,500 μ/24 hr and 2.3 ng/ml). The assay was also used to monitor blood levels of dopamine following the administration of L-dopa and dopamine to patients with cardiomyopathy.

J Nucl Med 19: 1217–1224, 1978

Numerous studies have indicated that the catecholamines, dopamine, norepinephrine, and epinephrine, play an important role in the functioning of the peripheral and central nervous systems (*1,2*). Norepinephrine acts locally as a neurotransmitter on effector cells of vascular smooth muscle, adipose tissue, liver, heart, and brain. Epinephrine is primarily located in the adrenal medulla and acts mainly as a hormone on distant target organs after its release from the adrenal gland into the blood stream. Dopamine has two functions: it serves as a precursor of norepinephrine and also as a neurotransmitter in the areas of the brain involved in coordinating motor activity where dopamine is localized (*3,4*).

The initial step in catecholamine biosynthesis involves the hydroxylation of L-tyrosine to form L-dihydroxyphenylalanine (L-dopa), a catechol amino acid (Fig. 1). Once formed, L-dopa is rapidly decarboxylated to dopamine through the catalytic action of the enzyme aromatic L-amino acid decarboxylase (*5,6*). Then norepinephrine and epinephrine are synthesized from dopamine by a series of enzymatic steps (*2*). Catecholamines are metabolized in the liver and in the effector cells by two enzymes, catechol-O-methyltransferase and monoamine oxidase, giving rise to acidic and O-methylated products (*7,8*) (Fig. 1).

Abnormalities in the biosynthesis and metabolism of catecholamines have been associated with many disease states including neuroblastoma (*9,10*), pheochromocytoma (*11*), ganglioneuroma (*12*), melanoma (*13,14*), thyrotoxicosis (*15*), essential hypertension (*16*), hepatic encephalopathy (*17*), Parkinson's disease (*18*), and neuropsychiatric disorders (*19*).

Received Apr. 20, 1978; revision accepted June 12, 1978.

For reprints contact: Bahjat A. Faraj, Dept. of Radiology, Division of Nuclear Medicine, Emory University, Atlanta, GA 30322.

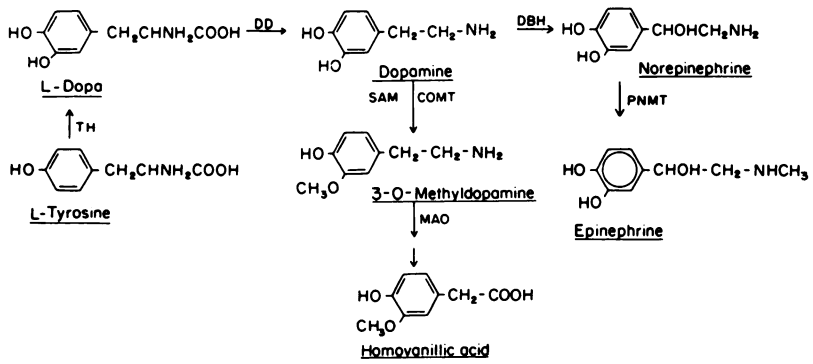

FIG. 1. Biosynthesis and metabolism of dopamine. In the enzyme systems, TH = tyrosine hydroxylase, DD = dopa decarboxylase, SAM = S-adenosylmethionine, COMT = catechol-O-methyltransferase, MAO = monoamine oxidase, DBH = dopamine beta-hydroxylase, and PNMT = phenylethanolamine-N-methyltransferase.

Dopamine's role in the genesis of extrapyramidal disease was substantiated by the demonstration of its deficiency in both the urine and brain of patients with Parkinson's disease (*20*). This led to the use of its precursor (L-dopa) in the treatment of Parkinson's disease (*21*). Furthermore, due to its unusual cardiovascular and renal effects on specific receptors (*22*), dopamine has also been used for the treatment of shock (*23,24*) and congestive heart failure (*25*).

Several methods—including fluorometric (*26–28*), ion-pair partition chromatography (*29*), liquid chromatography (*30*), and gas-chromatograpic mass-fragmentography (*31*)—have been used for the measurement of catecholamines in the biologic fluids. Most of these methods are time-consuming, however, and often present numerous problems relating to precision and accuracy. In the last few years, the development of radiometric methods (*32–34*) for the assay of catecholamines has resulted in a potentially simpler and more sensitive procedure for their analysis.

Due to the potential role of dopamine in certain metabolic diseases (*35*), we have developed an assay for its measurement in human plasma and urine using an enzyme-radioimmunoassay. It is based on the conversion of dopamine to its 0-methylated metabolite by catechol-0-methyltransferase and S-adenosylmethionine, and the formed product is estimated by our recently developed radioimmunoassay for 3-0-methyldopamine (*36*).

The availability of this assay allowed us to determine the plasma and urine levels of dopamine in normal volunteers. Furthermore, the diagnostic and clinical applicability of the enzyme-radioimmunoassay of dopamine was evaluated by measuring dopamine in children with neuroblastoma and in patients receiving L-dopa and dopamine therapeutically.

MATERIALS AND METHODS

Except for S-adenosylmethionine, DL-dithiothreitol, and tris (hydroxymethyl) aminomethane, all chemicals were obtained commercially from the same source, listed in articles by Faraj et al. (*36,37*). They were of reagent grade and were used without further purification. The antiserum against 3-0-methyldopamine was prepared according to the method of Faraj et al. (*36*).

Catechol-0-methyltransferase (COMT) preparation. COMT was purified from rat liver by a modification of the method of Axelrod and Tomchick (*38*). Wistar rats (210–250 g) were killed by suffocation in a dry-ice chamber. The livers were immediately excised and chilled on ice. All subsequent manipulations were carried out at 0–5°C. Livers (100 g) were homogenized* with isotonic KCl (400 ml) and centrifuged at 70,000 g for 30 min. The supernatant was decanted, titrated to pH 5 with *M* acetic acid, allowed to stand for 10 min, then centrifuged at 500 g for 15 min. To the supernate fraction (270 ml) was added ammonium sulfate (52 g). The solution was allowed to stand for 15 min and was centrifuged at 500 g for 10 min. The precipitate was discarded and 34 g of ammonium sulfate were added to the supernate fraction. After centrifugation (500 g), the resulting precipitate was dissolved in deionized glass-distilled water (50 ml) and dialyzed overnight (18 hr) against potassium phosphate buffer (0.01 *M*, pH 7.0; containing 0.1 m*M* dithiothreitol). Dithiothreitol (67 mg) was added to the dialyzed enzyme solution (50 ml) and the mixture was then divided into 1-ml fractions and stored at −80°C in plastic tubes until use. The final protein concentration of the enzyme solution, as determined by the Lowery method (*39*), was 27–35 mg/ml. The enzyme preparation was found to be stable for at least 3 mo.

Sample preparation. In order to determine the endogenous level of dopamine in plasma, blood samples (10 ml) were collected into chilled evacuated heparinized glass tubes containing sodium metabisulfite (0.5 mg/ml of blood). The tubes were centrifuged immediately (500 g, 10 min). The plasma was separated, deproteinized with perchloric acid (0.06 ml/ml of plasma), and then centrifuged as

above. To the supernate was added EDTA (0.1 ml; 10% w/v), sodium metabisulfite (0.1 ml; 5% w/v), and acid-washed alumina (100 mg) by the method of Anton and Sayer (*27*). The pH of the mixture was adjusted to 8.6. This was followed by centrifugation, and the supernate fraction was discarded. The alumina was washed twice with distilled water, then eluted with 0.5 *M* perchloric acid (0.6 ml). The dopamine in this fraction was analyzed as described below. In blood samples (0.5 ml) obtained from patients receiving dopamine and L-dopa for therapeutic purpose, dopamine in plasma was analyzed directly without prior extraction. In this case, blood samples were collected into chilled evacuated tubes containing EGTA and glutathione (90 mg, 60 mg/ml; 0.02 ml of blood). The plasma was separated by low-speed centrifugation of the sample at 4°C, and was frozen at −80°C until analysis. Fifty μl of this was used for the analysis of dopamine by enzyme-radioimmunoassay. For the measurement of dopamine in urine, samples were collected over a period of 24 hr into polyethylene bottles containing 30 ml of 6 *N* HCl, and were stored at −80°C prior to analysis(0.2 ml; 1:30 dilution).

Assay procedure. To determine the levels of dopamine and 3-0-methyldopamine in the sample, each one was incubated both in the presence and in the absence of COMT preparation as described below. The base for the reaction mixture was composed of tris-HCl buffer (0.4 ml, 80 m*M*, pH 8.2), distilled water (0.1 ml), $MgCl_2$ (0.2 ml, 24 m*M*), and S-adenosyl-methionine (SAM) (0.2 ml, 19.2 μM in a solution of sulfuric acid and ethanol, 9:1, pH 1–3). To this would be added one of the following: a) for the development of a standard curve, 0.5–5 ng dopamine HCl in 0.2 ml of 0.01 *N* HCl; or b) for urinalysis, 0.2 ml of a 1:30 dilution of the sample in 0.01 *N* HCl; or c) for plasma levels, 0.2 ml of the perchloric acid solution (9.5 *M*, pH 2.3) containing the dopamine extracted from plasma. The above mixture was vortex-mixed. To this was added COMT (0.05 ml). Blank tubes were prepared by substituting 0.2 ml of distilled water for the substrate or sample material. To determine the presence of 3-0-methyldopamine in the sample alone, tubes were prepared by substituting 0.25 ml of distilled water for SAM and COMT. The reaction mixture was incubated for 45 min at 37°C in a shaking water bath. Reaction was then stopped by addition of borate buffer (4 ml, 0.5 *M* sodium borate, adjusted to pH 10.5 with 10 *N* NaOH). This was followed by the addition of 1.5 g of NaCl and 0.3 g of anhydrous Na_2CO_3. The 3-0-methyldopamine generated in the reaction tubes was then extracted into ethyl acetate (30 ml). This was achieved by shaking the samples with the organic solvent for 30 min and then centrifuging (500 g) for 10 min. An aliquot (25 ml) of the ethyl acetate was removed; to the remaining two-phase mixture, 10 ml of ethyl acetate were added, and after shaking and centrifuging as above, 10 ml of solvent was withdrawn. The combined fractions of ethyl acetate (35 ml) were evaporated to dryness under N_2 at 40°C. The residue was reconstituted in 0.4 ml of sodium phosphate buffer (0.01 *M*, pH 7.4) and an aliquot of 0.3 ml was withdrawn and analyzed for 3-0-methyldopamine by radioimmunoassay, according to a modified procedure of Faraj et al. (*36*).

RIA procedure. In 12- by 75-mm plastic tubes were placed 0.3 ml of bovine serum albumin solution (0.5% w/v in 0.01 *M* sodium phosphate buffer solution, pH 7.4, containing 0.03% w/v potassium phosphate, 0.8% w/v NaCl, and 0.05% w/v human gamma globulin), 0.1 ml of antibody solution (1:2500 dilution with phosphate buffer), and 0.1 ml of [^{3}H] 3-0-methyldopamine (8000 cpm, sp. activity 17 Ci/millimol; 0.085 ng) in phosphate buffer. To obtain a standard curve, 0.05- to 1.5-ng quantities of stable 3-0-methyldopamine were added. For plasma or urine levels, one adds aliquots of the sodium phosphate buffer (pH 7.4) containing the formed 3-0-methyldopamine extracted from plasma or urine after the enzymatic incubation. The tubes were capped and incubated at 4°C for 3 hr. The free and antibody-bound [^{3}H] 3-0-methyldopamine fractions were separated from 3-0-methyldopamine by the addition of 0.5 ml of aqueous polyethylene glycol 6000 (30% w/v). The tubes were vigoroulsy vortex-mixed. The percentage of free [^{3}H] 3-0-methyldopamine was determined by measuring the radioactivity in the supernate. This was achieved by centrifugation (2000 g, 4°C) of the above mixture for 40 min. The supernates (0.2 ml) were then aspirated, placed in scintillation vials, and diluted with 14 ml of scintillation fluid before counting. Each sample was assayed in triplicate. All samples were counted to ±2% error. The dopamine level (ng/ml) in plasma or urine is given by x_1-x_2, where x_1 is the sum of the concentrations of dopamine and 3-0-methyldopamine in the sample, and x_2 is the concentration of 3-0-methyldopamine in the same sample.

Specificity of the enzyme-radioimmunoassay. To test for the specificity of the enzyme radioimmunoassay, several amino acids—metabolites and analogs of dopamine—were incubated with COMT at concentrations of 0.5–100 ng/ml and the liberated methylated products were then estimated by the radioimmunoassay of 3-0-methyldopamine as described above.

Patient population. Written informed consent was

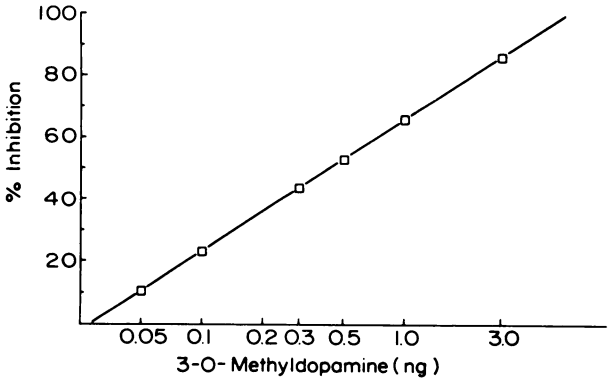

FIG. 2. Typical standard curve (semilog) showing inhibition of binding of [^{3}H] 3-O-methyldopamine to the antibody by various amounts of unlabeled 3-O-methyldopamine sample in 0.01 *M* sodium phosphate buffer. Each point represents average of sixty determinations.

TABLE 1. SPECIFICITY OF THE ENZYME RADIOIMMUNOASSAY

Substrate*	Relative activity† (%)
Dopamine (3 ng)	80
L-dopa (3 ng)	17

* Three to 100 ng of each of the following compounds were added to the standard reaction mixture and carried through the assay for dopamine: norepinephrine, epinephrine, 3,4-dihydroxyphenylacetic acid, normetanephrine, metanephrine, L-tyrosine, 3-4-dihydroxyphenethanol, 3-4-dihydroxyphenylglycol, tyramine, octopamine, 3,4-dihydroxymandellic acid, 3,4-dihydroxybenzoic acid, synephrine, and catechol. Less than 1% conversion of each to 3-O-methyldopamine was noted in the assay.

† Relative activity represents % conversion of three ng of substrate to 3-O-methyldopamine.

obtained from patients after the approval of the protocol from the University Clinical Trials Committee. The study consisted of the analysis of dopamine in urine and plasma, a) in a group of healthy normal volunteers, b) in patients with neuroblastoma, and c) in patients receiving L-dopa orally (3 g/day) and i.v. infusion of dopamine at a rate of 5 μg/kg-min therapeutically.

RESULTS

Characteristics of the assay procedure. Several buffer media with different molarity and pH were tested, and tris. HCl (80 m*M*, pH 8.2) was found to give the best enzymatic methylation efficiency for dopamine. Because the enzyme preparation might partly destroy dopamine during the enzymatic incubation (through deamination by MAO, the hepatic enzyme monoamine oxidase), we tested for this by assaying the enzyme preparation for MAO activity. It was found that the COMT preparation did not contain any MAO as determined by its inability to deaminate [^{14}C] tryptamine (*40*). In agreement with previous reports (*33,38*), Mg^{2+} was required for COMT activity at a concentration of 24 m*M*. However, levels greater than 45 m*M* inhibited the reaction considerably. Time-dependence studies of the methylation of dopamine (3 ng) with SAM (19 μM) and COMT demonstrated that the methylation was essentially complete between 40 and 50 min. The amount of protein in each COMT preparation used for the incubation was determined by ascertaining the dependence of methylation on the COMT concentration. Saturation amounts of 1.2–1.7 mg of each COMT preparation were used in each incubation. The most valid blank was found to be the one that contains COMT and SAM but no dopamine in the preparation. The stability of the enzyme was maintained at —80°C for at least 3 mo if the COMT preparation was stored over dithiothreitol.

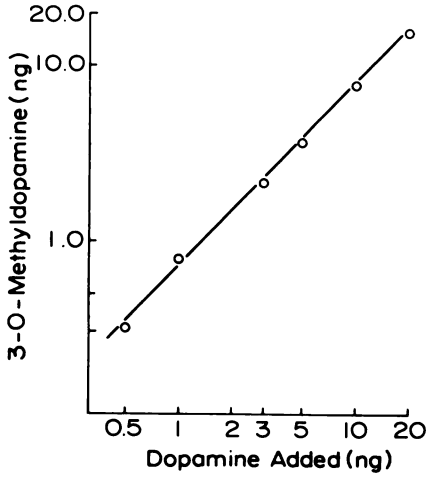

FIG. 3. Typical standard curve (log) showing unity between the amount of dopamine added (0.5–20 ng) and the 3-O-methyldopamine formed as determined by enzyme radioimmunoassay. Each point represents average of ten determinations.

Radioimmunoassay of 3-O-methyldopamine. The identity of the enzymatically formed 3-O-methyldopamine was established by our recently developed and improved radioimmunoassay for 3-O-methyldopamine. The sensitivity of the assay is illustrated in Fig. 2. A concentration of 0.5 ng/ml of 3-O-methyldopamine could be detected by the antiserum of a rabbit immunized with the 3-O-methyldopamine antigen. As previously reported, this antiserum is very specific, since several analogs and metabolites of 3-O-methyldopamine showed no appreciable cross-reactivity with antiserum.

Sensitivity and specificity. The enzyme-radioimmunoassay of dopamine exhibited linearity with amounts of dopamine added ranging from one to at

least 20 ng (Fig. 3). The sensitivity of the assays (i.e., twice blank) was at least 0.5 ng of dopamine per 1.1 ml of incubation mixture. The final overall recovery of dopamine as the 3-0-methyldopamine was 70–80%. The ability of the partially purified COMT to methylate catechol derivatives was studied. L-dopa was the only compound that cross-reacted significantly with the enzyme-radioimmunoassay of dopamine (Table 1). However, the addition of benzyloxyamine (10^{-3} *M*) to the incubation mixture completely obviated this difficulty.

Precision. Within- and between-assay variation was assessed for dopamine by assay of 24-hr urine samples in the same as well as in a different assay ($n = 15$), according to the method of Rodbard (*41*). Intra- and interassay coefficients of variation (CV) were 15.9% and 14.5%, respectively, for dopamine in urine.

Studies in humans. In this investigation, plasma and urine dopamine were determined in a group of healthy normal volunteers of both sexes (25–40 years of age). The results indicated that average plasma and urinary levels of dopamine were 0.211 ± 0.052 ng/ml and 182.1 ± 23.2 μg/24 hr (Table 2). The amounts of dopamine in the urine of patients with neuroblastoma were significantly elevated (8500 μg/24 hr, $p < 0.05$) as compared with normal subjects. In these patients, diagnosis of the tumor was established histologically. Furthermore, plasma dopamine was elevated significantly above the endogenous level following the oral administration of L-dopa and after the i.v. infusion of dopamine to patients with hypertrophic cardiomyopathy (Table 3).

TABLE 2. URINARY AND PLASMA LEVELS OF DOPAMINE

Subject* No.	Status or disease	Dopamine	
		Urine μg/24 hr	Plasma ng/ml
1	Normal	149	—
2	Normal	144	0.17
3	Normal	197	—
4	Normal	192	0.21
5	Normal	159	0.25
6	Normal	175	—
7	Normal	185	0.15
8	Normal	210	0.275
9	Normal	188	—
10	Normal	221	—
	Average ± s.d. (range)	182.1 ± 23.2 (144–221)	0.211 ± 0.052 (0.15–0.275)
11	Neuroblastoma	11400	4.21
12	Neuroblastoma	5600	2.3

* Average urinary excretion of 3-0-methyldopamine in normal subjects was found to be 62.8 ± 43.70 μg/24 hr; it was 1100 μg in patients with neuroblastoma.

TABLE 3. PLASMA DOPAMINE CONCENTRATIONS FOLLOWING I.V. INFUSION OF DOPAMINE AND THE ORAL ADMINISTRATION OF L-DOPA TO PATIENTS WITH CARDIOMYOPATHY

Subject	Disease status	Day (hr)	Dose/day		Plasma levels dopamine (ng/ml)
			Dopamine μg/kg-min	L-dopa g	
1	Hypertrophic cardiomyopathy	1	13.3	—	
		(8 a.m.)			81.4
		(4 p.m.)			55.4
		2	17.8	—	
		(8 a.m.)			104.5
		(1 p.m.)			136.9
		3	10→12	3	
		(12 noon)			206.3
		4	11.1→8.3	6	
		(8 a.m.)			323
		(4 p.m.)			415.6
		5	—	8	
		(8 a.m.)			8.1
		(4 p.m.)			29.1
2	Hypertrophic cardiomyopathy	1	—	6	
		(11:30 a.m.)			36.9
		2	—	4	
		(10 a.m.)			15.2

DISCUSSION

The need for a fast, reliable procedure for the measurements of catecholamines and their metabolites is increasingly apparent as more correlations become established between their concentrations in body fluids and disease states (*35,42*).

The purpose of this communication is to report a sensitive method for the determination of dopamine in plasma and urine by an enzyme-radioimmunoassay technique. Catechol-0-methyltransferase (*43*), an important enzyme in the metabolism of catecholamines in the central and peripheral nervous system, was isolated from rat liver and partially purified through repeated ammonium sulfate precipitation steps. The method used for the determination of catechol-0-methyltransferase activity was based on the principle that the enzyme catalyzes the transfer of methyl groups to substrates containing the catechol grouping in the presence of S-adenosylmethionine as the methyldonor and magnesium ions as the activator. In this case, dopamine was employed as the substrate for the enzymatic step. The

end product of the enzymatic reaction, 3-0-methyldopamine, was measured by radioimmunoassay.

During determination of catechol-0-methyltransferase activity, it is of paramount importance to pay sufficient attention to the kinetics of the enzyme reaction. Linearity between catechol-0-methyltransferase activity and incubation time, and between enzyme activity and amount of enzyme preparation, must be observed in any given experimental condition. Creveling and Daly (*44*) point out that regardless of the type of assay employed for catechol-0-methyltransferase, the final concentration of substrate, as well as Mg^{2+} and S-adenosylmethionine, should be such that maximal reaction rates are obtained. Since the optimum concentrations of these needed substances will depend on a number of factors—such as type and kind of preparation of tissue, incubation medium, ionic strength and composition, pH, temperature, etc.—they must be determined experimentally under the prevailing conditions. For example, we found higher activities with dopamine as substrate for catechol-0-methyltransferase in tris buffer (pH 8.2) than in the usual pH 7.0 phosphate buffer, and also that maximum conversion of dopamine (20 ng) was achieved at saturating concentration of SAM (19 μM).

In spite of the broad substrate specificity of catechol-0-methyltransferase, all known substrates are characterized by a catechol configuration regardless of other substituents in the aromatic ring. The list includes endogenously occurring compounds such as catecholamines and their metabolites (e.g., dopamine, norepinephrine, epinephrine, 3,4-dihydroxyphenylacetic acid, 3,4-dihydroxyphenylethanol, L-dopa, 3,4-dihydroxyphenylglycol), as well as ascorbic and enediol steroids and foreign catechols such as 3,4-dihydroxycinnamic acid (*43*).

The unique feature of the enzyme-radioimmunoassay of dopamine is its ability to identify the 0-methylated dopamine product directly in the reaction mixture by our specific radioimmunoassay (*36*) without prior chromatographic separation of 3-0-methyldopamine from the rest of the methylated catechol derivatives. Furthermore, this method allows the simultaneous determination of both dopamine and its metabolite, 3-0-methyldopamine in the same sample. Among the analogs that were employed to determine the specificity of the enzyme-radioimmunoassay of dopamine (Table 1), only L-dopa exhibited considerable interference with the assay. The problem was apparently due to contamination of our partially purified preparation of catechol-0-methyltransferase by aromatic amino acid decarboxylase activity. The addition of benzyloxyamine, a decarboxylase inhibitor, completely obviated this difficulty.

The enzyme-immunoassay of dopamine represents an accurate, reproducible, and sensitive method for the quantification of dopamine in plasma and urine. With this procedure, it was possible to determine as little as 0.5 ng of dopamine in urine by direct analysis. Below this level, a biologic fluid (such as plasma) would have to be extracted and concentrated for analysis. However, the ease and the simplicity of the extraction method (alumina) facilitated the processing of multiple plasma samples.

The availability of the enzyme-radioimmunoassay of dopamine has allowed us to use this technique to determine the endogenous level of this catecholamine in urine and plasma in a group of adult volunteers. In this respect, only limited results of quantitative urinary dopamine analysis have been published. A comparison of our data with those reported in the literature demonstrates that our findings for free dopamine in the urine are similar to those reported by Crout (*45*), Hoeldtke and Sloan (*46*), and Hornykiewicz (*21*). The urinary excretion of dopamine in man is much higher than that of norepinephrine and epinephrine (*45,46*). These differences indicate a fairly high rate of formation and metabolism (turnover) of dopamine in the whole organism. We note, however, that dopamine levels in plasma are very low compared with the urinary levels. The large amounts of the amine in the urine suggested that most of the urinary dopamine is actually formed in the kidney, which is very rich in L-dopa decarboxylase.

The diagnostic applicability of the enzyme-radioimmunoassay of dopamine was evaluated by confirming the biochemical diagnosis of patients with neuroblastoma (a dopamine-secreting tumor). In these patients, there was a several-fold increase in plasma and in the 24-hr excretion of dopamine and its metabolite 3-0-methyldopamine, compared with the levels in normal volunteers. The high output of the 0-methylated metabolite of dopamine in neuroblastoma is in agreement with what we have previously reported (*36*). These patients, however, did not excrete appreciable amounts of homovanillic acid (HVA), the terminal acidic metabolite of dopamine (Fig. 1) in their urine. Of particular relevance here is our conclusion that no single metabolite alone serves as a reliable diagnostic marker for neuroblastoma, and that the entire metabolic pattern of catecholamines must be examined.

The assay was also used to monitor blood levels of dopamine following the oral administration of L-dopa to patients with cardiomyopathy. Studies of the pharmacokinetics of dopamine after L-dopa was

found to be essential in optimizing the dosage schedule of dopa for individual patients.

CONCLUSION

In summary, the quantitative enzyme-radioimmunoassay approach presented here for the determination of dopamine and its metabolite, 3-0-methyldopamine, is rapid and reliable. In addition to the evaluation of HVA and VMA in urine, the analysis of dopamine and 3-0-methyldopamine may provide a more specific and comprehensive test for the detection of neuroblastoma. The test may also be helpful in monitoring the course of this disease and in the assessment of the efficacy of treatment. Furthermore, the enzyme-radioimmunoassay of dopamine may also provide a sensitive technique for studying the kinetics of dopamine after the administration of L-dopa. There are at least two advantages to kinetic investigations rather than single measurements of amine concentration in blood and urine. The studies of kinetics are more likely to be sensitive to small but physiologically important alterations, and careful analysis of data might pinpoint the cases of a lesion (*47*).

FOOTNOTE

* Polytron, Brinkman Instruments, Westbury, N.Y.

ACKNOWLEDGMENTS

This work was presented in part at the 24th Annual Meeting of the Society of Nuclear Medicine, in June, 1977. It was supported in part by U.S. Public Health Service grant GM 2242-02.

REFERENCES

1. INNES IR, NICKERSON M: In *The Pharmacological Basis of Therapeutics.* Goodman LS, Gilman A, eds. New York, MacMillan Company, 1970, pp 478–500

2. AXELROD J, WEINSHILBOUM R: Catecholamines. *New Engl J Med* 287: 237–242, 1972

3. THORNER MO: Dopamine is an important neurotransmitter in the autonomic nervous system. *Lancet* 1: 662–664, 1975

4. MOSKOWITZ MA, WURTMAN RJ: Catecholamines and neurological diseases. *New Engl J Med* 293: 274–280, 1975

5. WURTMAN RJ, LAVIN F, MOSTAFAPOUR S, et al: Brain catechol synthesis: Control by brain tyrosine concentration. *Science* 185: 183–184, 1974

6. WEINER N: Regulation of norepinephrine biosynthesis. *Ann Rev Pharmacol* 10: 273–290, 1970

7. BOHUON C, ASSICOT M: In *Frontiers in Catecholamine Research.* Usdin E, Snyder S, eds. New York, Pergamon Press, 1973, pp 107–112

8. MUSACCHIO JM: In *Biochemistry of Biogenic Amines, Vol. 3.* Iverson LL, Iversen S, Snyder SH, eds. New York, Plenum Press, 1975, pp 20–24

9. POCHEDLY C: *Neuroblastoma.* Acton, Mass, Publishing Sciences Group, 1976, pp 115–154

10. IMASHUKU S, INUI A, NAKAMURA T, et al: Catecholamine metabolism in tissue culture cells of neuroblastoma. *J Clin Endocrinol Metab* 36: 931–936, 1973

11. ANTON AH: In *The Heart.* Hurst JW, Logue RB, Schlant RC, et al., eds. New York, McGraw-Hill, 1978, pp 1428–1430

12. BOLANDE RP: The neurocristopathies. *Human Path* 5: 409–429, 1974

13. AUBERT C, LAGRANGE C, RORSMAN H, et al: Catechols in primary and metastatic human malignant melanoma cells in monolayer culture. *Europ J Cancer* 12: 441–445, 1976

14. HINTERBERGER H, FREEDMAN A, BARTHOLOMEW RJ: Precursors of melanin in the blood and urine in malignant melanoma. *Clin Chim Acta* 39: 395–400, 1972

15. WURTMAN RJ: In *Frontiers in Catecholamine Research.* Usdin E, Snyder S, eds. New York, Pergamon Press, 1973, pp 781–785

16. SENER PS, OSIKOWSKA B, BIRCH M: Plasma-noradrenaline in essential hypertension. *Lancet* 1: 1078–1081, 1977

17. FISCHER JE: False neurotransmitters and hepatic coma. *Res Publ Assoc Res Nerv Ment Dis* 53: 53–73, 1974

18. GOODALL McC, ADTON H: Dopamine (3-hydroxytyramine) metabolism in parkinsonism. *J Clin Invest* 48: 2300–2307, 1969

19. SCHILDKRAUT JJ, ORSULAK PJ, GUDEMAN JE, et al: In *Neuroregulators and Psychiatric Disorders.* Usdin E, Hamburg DA, Barchas JD, eds. New York, Oxford University Press, 1977, pp 122–128

20. HORNYKIEWICZ O: Dopamine in the basal ganglia: Its role and therapeutic implications (Including the clinical use of L-DOPA). *Br Med Bull* 29: 172–178, 1973

21. HORNYKIEWICZ O: In *The Role of Amines and Membranes in Drug Action.* Biel JH, Abood LG, eds. New York, Marcel Dekker, 1971, pp 173–258

22. GOLDBERG LI: Cardiovascular and renal actions of dopamine: Potential clinical applications. *Pharmacol Rev* 24: 1–29, 1972

23. GOLDBERG LI, TALLEY RC: Current therapy of shock. *Adv Int Med* 17: 363–378, 1971

24. REID PR, THOMPSON WL: The clinical use of dopamine in the treatment of shock. *Johns Hopkins Med J* 137: 276–279, 1975

25. GOLDBERG LI: Use of sympathomimetic amines in heart failure. *Amer J Cardiol* 22: 177–182, 1968

26. PRASAD ALN, FAHNS S, ISGREEN WP: Dopamine: A sensitive automated assay and examples of Levadopa therapy. *Biochem Med* 14: 24–32, 1975

27. ANTON AH, SYRE DF: The distribution of dopamine and dopa in various animals and a method for their determination in diverse biological material. *J Pharmacol Exptl Ther* 145: 326–336, 1964

28. LAVERTY R, TAYLOR KM: The fluorometric assay of catecholamines and related compounds: Improvements and extensions to the hydroxyindole technique. *Anal Biochem* 22: 269–279, 1968

29. PERSSON BA, KARGER BL: High performance ion pair partition chromatography: The separation of biogenic amines and their metabolites. *J Chromat Sci* 12: 521–528, 1974

30. KISSINGER PT, FELICE LJ, RIGGIN RM, et al: Electrochemical detection of selected organic components in the eluate from high-performance liquidchromatography. *Clin Chem* 20: 992–997, 1974

31. MUSKIET FA, FREMOUW-OTTEVANGERS DC, MEULEN J, et al: Determination of some L-3,4-dihydroxyphenylalanine and dopamine metabolites in urine by means of mass fragmentography. *Clin Chem* 24: 122–127, 1978

32. SOLE MJ, HUSSAIN MN: A simple specific radioenzymatic assay for the simultaneous measurement of picogram quantities of norepinephrine, epinephrine and dopamine in plasma and tissues. *Biochem Med* 18: 301–307, 1977

33. PASSON PG, PEULER JD: A simplified radiometric assay for plasma norepinephrine and epinephrine. *Anal Biochem* 51: 618–631, 1973

34. BEN-JONATHAN N, PORTER JC: A sensitive radioenzymatic assay for dopamine, norepinephrine, and epinephrine in plasma and tissue. *Endocrinology* 98: 1497–1507, 1976

35. HOELDTKE R: Catecholamine metabolism in health and disease. *Metabolism* 23: 663–683, 1974

36. FARAJ BA, CAMP VM, PRUITT AW, et al: The measurement of 3-0-methyldopamine in urine and plasma by a rapid and specific radioimmunoassay. *J Nucl Med* 18: 1027–1033, 1977

37. FARAJ BA, MU JY, LEWIS MS, et al: Determination of plasma and tissue levels of tyramine by radioimmunoassay. *Proc Soc Exp Bio Med* 149: 664–669, 1975

38. AXELROD J, TOMCHICK R: Enzymatic O-methylation of epinephrine and other catechols. *J Biol Chem* 233: 702–705, 1958

39. LOWRY OH, ROSEBROUGH NJ, FARR AL, et al: Protein measurement with the folin Phenol reagent. *J Biol Chem* 193: 265–275, 1951

40. WURTMAN RJ, AXELROD J: A sensitive and specific assay for the estimation of monoamine oxidase. *Biochem Pharmacol* 12: 1439–1441, 1963

41. RODBARD D: Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays. *Clin Chem* 20: 1255–1270, 1974

42. MROCHEK JE, DINSMORE SR, OHRT DW: Monitoring phenylalanine-tyrosine metabolism by high-resolution liquid chromatography of urine. *Clin Chem* 19: 927–936, 1973

43. GULDBERG HC, MARSDEN CA: Catechol-O-methyl transferase: Pharmacological aspects and physiological role. *Pharmacol Rev* 27: 135–206, 1975

44. CREVELING CR, DALY JW: In *Analysis of Biogenic Amines and their Related Enzymes.* Blick D, ed. New York, Interscience, 1971, pp 169–173

45. CROUT JR: Sampling and analysis of catecholamines and metabolites. *Anesthesiology* 29: 661–669, 1968

46. HOELDTKE RD, SLOAN JW: Acid hydrolysis of urinary catecholamines. *J Lab Clin Med* 75: 159–165, 1970

47. FARAJ BA, DAYTON PG, CAMP VM, et al: Studies of the fate of tyramine in dogs: The effect of monoamine oxidase inhibition, portafemoral shunt and coronary artery ligation on the kinetics of tyramine. *J Pharmacol Exp Ther* 200: 384–393, 1977