Optimization of the Hydrolysis of Conjugated *L*-DOPA, Dopamine and Dihydroxyphenylacetic Acid in Human Urine for Assay by High-Performance Liquid Chromatography with Electrochemical Detection

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Summary: Conjugates of the catechol compounds, *L*-dihydroxyphenylalanine (*L*-DOPA), dopamine and dihydroxyphenylacetic acid in human urine were analysed using the isocratic ion-pair reversed-phase HPLC method with electrochemical detection. Acid hydrolysis, using 4 mol/l HCl for 60 min, was more effective than treatment with sulphatase for the generation of free catechols. Free (non-conjugated) catechols already present, as well as those produced by either of the hydrolysis procedures, were adsorbed onto aluminium oxide and extracted in acid solution. The repeatability of the technique for within and between-batch urine analysis was less than 2% and 8%, respectively.

Free urinary dopamine (and dihydroxyphenylacetic acid) concentrations were much higher in the urine of patients treated with *L*-DOPA for *Parkinson*'s disease than in healthy volunteers. At high dopamine (and dihydroxyphenylacetic acid) levels the conjugation capacity was apparently exceeded, since the overall percent conjugation of *L*-DOPA, dopamine and dihydroxyphenylacetic acid was decreased "concentration dependently" when the concentrations of free catechols were increased. Both in the control group and *L*-DOPA-treated groups, enzymatic hydrolysis was much less effective than acid hydrolysis in generating free catechols. This indicated that there were other, non-sulphated conjugates in the urine, accounting for between 66 and 100% of total conjugates.

Introduction

Conjugation reactions play a significant role in the metabolism of catecholamines and their oxidation products (1). Different analytical techniques have been used for determinations of catecholamines. HPLC methods with electrochemical or fluorescence detection have been described (2). A gas-chromatographic/mass-spectrometric method has also been reported (3). Liquid chromatography is the method of choice for the assay of catecholamines and metabolites, having the advantages of analytical sensitivity and selectivity. To measure the total concentrations of the catechols in urine, it is necessary to hydrolyse the samples before the HPLC analysis. Both enzymatic (4) and acid hydrolysis methods (5) have been used, but there is no consensus about how these methods should be optimally applied.

We found accidentally that the percentage release of dopamine and its metabolite, dihydroxyphenylacetic acid, from urine by hydrolysis, differed greatly for patients on *L*-dihydroxyphenylalanine (*L*-DOPA) therapy and healthy subjects. It seemed that hydrolysis was inhibited either by the high catechol levels resulting from the *L*-DOPA therapy, or by dopa decarboxylase inhibitors.

These preliminary findings prompted us to study in detail some of the factors affecting enzymatic and acid hydrolysis. Free *L*-DOPA, dopamine and dihydroxyphenylacetic acid in urine, both before and after various hydrolysis procedures, were adsorbed onto aluminium oxide, then extracted into acidic solution. The concentrations of catechols were determined by ion-pair reversed-phase chromatography and detected using an electrochemical detector. After the initial validation of the hydrolysis and analysis, the overall method was applied to clinical urine samples to compare the conjugation profile of catechols in the urine of healthy volunteers (very low dopamine levels) and patients treated with *L*-DOPA and dopa decarboxylase inhibitors (moderate or high dopamine concentrations).

Materials and Methods

Reagents

Ultrapure reagent-grade water was obtained with a Milli-Q system (Millipore Corporation, Milford, MA, USA). The solvents for chromatography were HPLC-grade (Rathburn, Walkerburn, UK) and the chemicals analytical grade. Aluminium oxide (Al_2O_3), citric acid, disodium EDTA dihydrate, hydrogen chloride (HCl), perchloric acid, phosphoric acid, sodium acetate, sodium chloride, sodium dihydrogen phosphate and sulphuric

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acid were purchased from E. Merck (Darmstadt, Germany). Do-
pamine, dihydroxyphenylacetic acid, L-DOPA, dihydroxybenzyl-

amine, heptane sulphonic acid, tris(hydroxymethyl)aminomethane
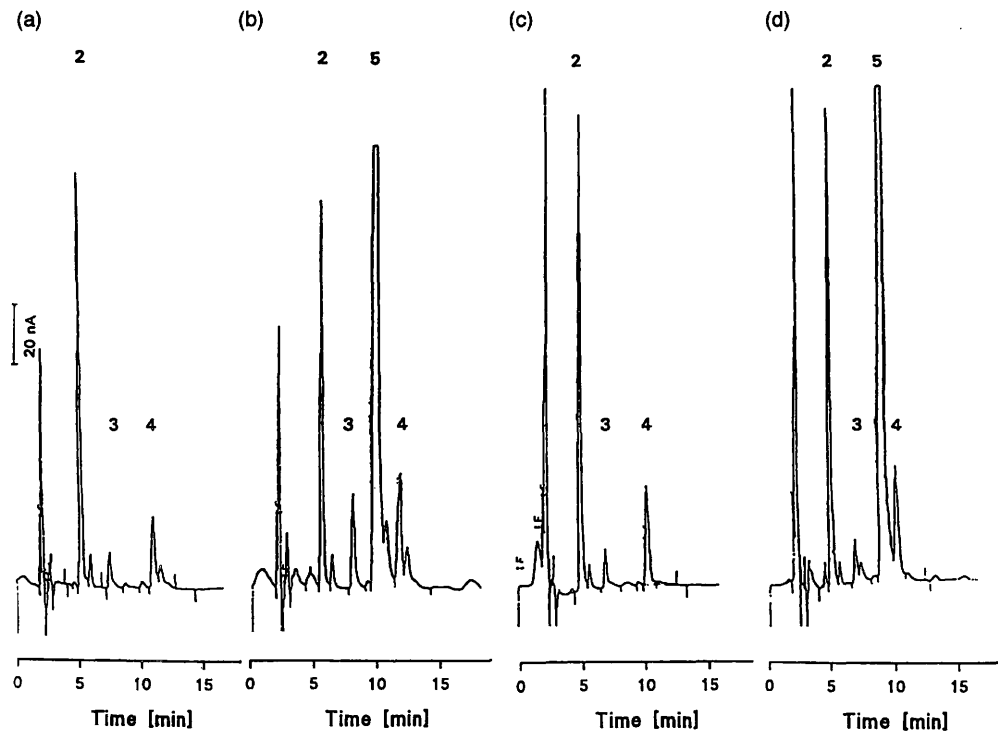hydrochloride and arylsulphatase (aryl-sulphate sulphohydrolase;

Fig. 1 Chromatograms derived from urine samples of the control
subject. Urine sample:
(a) before acid hydrolysis,
(b) after acid hydrolysis (4 mol/l HCl for 60 min at 95 °C),
(c) before enzymatic hydrolysis,

(d) after enzymatic hydrolysis (18.4 U sulphatase in 400 µl reaction
mixture for 60 min at 37 °C).
Peaks: 2 = dihydroxybenzylamine, 3 = dopamine, 4 = dihydroxy-
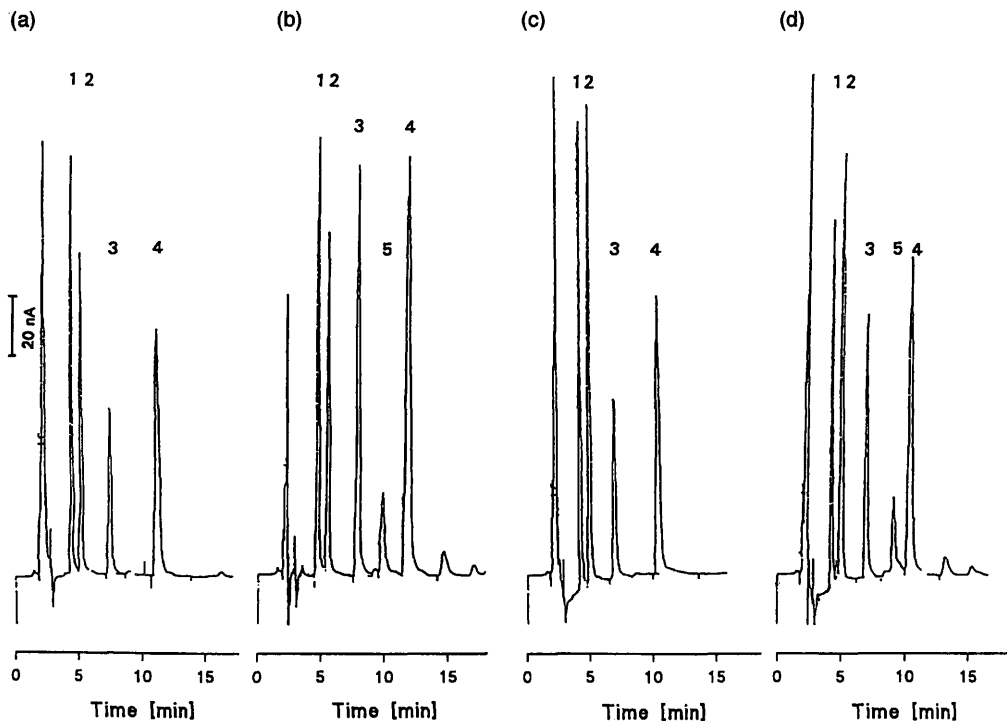phenylacetic acid, 5 = unknown extra peak.

Fig. 2 Chromatograms derived from the urine samples of the pa-
tient treated with L-DOPA. Urine sample:
(a) before acid hydrolysis,
(b) after acid hydrolysis (4 mol/l HCl for 60 min at 95 °C).
(c) before enzymatic hydrolysis,

(d) after enzymatic hydrolysis (18.4 U sulphatase in 400 µl reaction
mixture for 60 min at 37 °C).
Peaks: 1 = L-DOPA, 2 = dihydroxybenzylamine, 3 = dopamine,
4 = dihydroxyphenylacetic acid, 5 = unknown extra peak.

Tab. 1 Intra- and inter-assay precision of analysis for dopamine, dihydroxyphenylacetic acid and L-DOPA in urine samples at two concentration levels (n = 4).

	Mean (mg/l)	Intra-assay CV (%)	Inter-assay CV (%)
L-DOPA	56.9	1.6	7.7
	25.1	0.3	4.6
Dopamine	56.5	1.7	6.6
	17.7	2.0	6.7
Dihydroxyphenyl-acetic acid	38.0	1.2	8.4
	9.2	1.0	3.5

EC 3.1.6.1; type H-1 from *Helix pomatia*) were purchased from Sigma (St. Louis, MO, USA).

Standard solutions

Stock solutions (1 g/l) of L-DOPA, dopamine and dihydroxyphenylacetic acid were prepared by dissolving the compounds in 0.4 mol/l $HClO_4$ containing 1 g/l $Na_2S_2O_5$ and 150 mg/l EDTA. Working standard solutions, containing 2000 µg/l of each substance and internal standard dihydroxybenzylamine (125 µg/l), were prepared by diluting the stock solutions in the 0.4 mol/l $HClO_4$ solution. The stock solutions were stored at + 4 °C and used within 4 months. The working standard dilutions were prepared daily.

Liquid chromatography

The HPLC system consisted of an isocratic Waters Model 6000A pump with dual SSI suppressors in series, a Waters 712 Wisp auto-injector with cooler (Waters Association, Milford, MA, USA) and a Hewlett-Packard 3396 series II recording integrator (Palo Alto,

CA, USA). An analytical cell 5011 of ESA Coulochem Model 5100A coulometric detector (ESA, Inc, USA) set at + 0.10 V/− 0.30 V with conditioning cell 5021 set + 0.50 V was used. Spherex 5 C18 column (5 µm, 125 × 4.6 mm I.D., Phenomex, Torrance, CA, USA) with Separon SGX C18 precolumn was used for analytical separations.

The isocratic mobile phase contained 0.1 mol/l Na_2HPO_4, 0.15 mmol/l EDTA, 20 mmol/l citric acid, 1.0 mmol/l heptane sulphonic acid and methanol, volume fraction 0.1, pH 3.0. This filtered eluent was degassed before use. The flow rate was 0.7 ml/min. The individual compounds were screened by comparing their absolute retention times with those of the standard compounds.

Sample preparation

The urine samples from 7 patients with *Parkinson*'s disease were collected during 2−4 h after the first morning dose (6). Similarly, the urine samples of 5 healthy control subjects without drug therapy were collected. The specimens were taken into plastic bottles containing 2.5 ml of 6 mol/l HCl as a preservative and stored at −20 °C until assay.

Free catechol compounds from urine were selectively extracted onto Al_2O_3 before quantification based on the modification of a previous method (7). One millilitre of diluted urine sample (1 : 2 or 1 : 20 in healthy controls and 1 : 20 or 1 : 200 in patients on L-DOPA therapy; see below) was mixed with 600 µl of 1.5 mol/l Tris-HCl buffer pH 8.6 and placed on 50 mg of Al_2O_3, after which 10 µl of internal standard containing 125 ng of dihydroxybenzylamine was added. The pH of the sample was kept between 8.5 and 8.7. The samples were mixed in a shaker for 10 min and centrifuged (10 min at 2000 g at + 4 °C) after which the supernatants were aspirated. The Al_2O_3 was washed twice with 1.5 ml of water. L-DOPA, dopamine and dihydroxyphenylacetic acid were extracted by shaking the samples for 10 min with 1 ml of 0.4 mol/l $HClO_4$ containing 1 g/l $Na_2S_2O_5$ and 150 mg/l EDTA. After centrifugation the supernatant was filtered (Acrodisc LC filter, 0.45 µm, Gelman Sciences, Ann Arbor, MI, USA) into a HPLC tube and

Tab. 2 Enzymatic hydrolysis of urine samples taken from healthy control subjects and L-DOPA treated patients with sulphatase for 60 min at 37 °C.

	n	Control subjects		n	L-DOPA-treated patients	
		Concentration (mg/l)[a]	Efficacy of hydrolysis[b]		Concentration (mg/l)[a]	Efficacy of hydrolysis[b]
L-DOPA						
Control				2	28.6 28.2	
9.2 U sulphatase		−		5	27.4 ± 0.9	−3.2
18.4 U sulphatase		−		2	28.2 28.3	−0.5
Dopamine						
Control	4	0.34 ± 0.01		2	12.4 12.2	
9.2 U sulphatase	8	0.47 ± 0.02	40	5	19.8 ± 0.4	61
18.4 U sulphatase	4	0.52 ± 0.01	55	2	22.8 22.5	84
Dihydroxyphenylacetic acid						
Control	4	1.39 ± 0.03		2	28.7 28.4	
9.2 U sulphatase	8	1.52 ± 0.04	9	5	40.8 ± 0.7	43
18.4 U sulphatase	4	1.60 ± 0.02	15	2	40.3 39.8	40

[a] Mean ± SD

[b] $\dfrac{\text{After hydrolysis} - \text{Before hydrolysis}}{\text{Before hydrolysis}} \times 100$

stored at −80 °C for chromatography. A 20 µl aliquot of sample was injected into HPLC system.

Enzymatic hydrolysis of urine samples

To reveal the presence of sulphate conjugates of catechol compounds, aliquots (100 µl) of diluted urine (1 : 2 or 1 : 20 using 0.02 mol/l HCl in the samples of healthy controls and patients on L-DOPA therapy, respectively) were buffered with 200 µl of 0.2 mol/l acetate buffer, pH 5, and treated with arylsulphatase (9.2 or 18.4 units) dissolved in 100 µl of ice cold 2 g/l sodium chloride solution. The incubation time was 1 h at + 37 °C. The reaction was stopped placing in an ice bath. The hydrolysed sample was adsorbed onto Al_2O_3 and extracted as above.

Acid hydrolysis of urine samples

To compare the hydrolysis of conjugated catecholamines with different acids, 150 µl of either 4 mol/l HCl, 4 mol/l $HClO_4$ or 4 mol/l H_2SO_4 were added to 1 ml of a diluted urine sample (1 : 20 or 1 : 200 with the solution containing 1 g/l of $Na_2S_2O_5$ and 150 mg/l EDTA in samples of healthy controls and patients on L-DOPA therapy, respectively), and incubated for 60 min. To detect the effect of time on hydrolysis with 4 mol/l HCl, various incubation times (30, 60, 90 and 120 min) were used. This mixture was heated for 1 h at + 95 °C in an oven and then cooled on ice. Finally, it was adsorbed onto Al_2O_2 and desorbed into acidic solution as above.

Calibration and calculation

For each HPLC run the method was calibrated with 5 concentrations (10−400 µg/l) of calibration samples of L-DOPA, dopamine and dihydroxyphenylacetic acid, containing internal standard (125 µg/l of dihydroxybenzylamine). Peak-height ratios of each compound to internal standard, obtained from actual standards, were plotted against the concentrations of each substance to generate a linear least-squares regression line with QuattroPro software (Borland International, Scotts Valley, CA, USA). The reliability was assessed by calculating the intra-assay and inter-assay coefficient of variation (CV).

Results

Liquid chromatography of L-DOPA, dopamine and dihydroxyphenylacetic acid

L-DOPA, dopamine and dihydroxyphenylacetic acid were detected simultaneously in urine samples by isocratic ion-pair reversed-phase HPLC (figs. 1 and 2). Before the chromatographic separation, it was necessary to perform an alumina absorption to remove non-catechol compounds and impurities in matrices.

Because electrochemical detection was used, the calibration had to be performed separately for each analyte. The electrical responses (heights) were linear with correlation coefficients of more than 0.999 for the standard compounds from 10 to 400 µg/l. The detection limit of the assay (peak height three times the baseline noise) was 10 pg for dopamine and L-DOPA and 15 pg for dihydroxyphenylacetic acid.

The assay of free compounds indicates the repeatability of the technique for both within and between-batch urine analysis at the two concentration levels studied (tab. 1). The intra-assay CVs varied between 0.3% and 2%, whereas inter-assay CVs were between 3.5% and 8.4%.

In the acid hydrolysis using 4 mol/l HCl, analytical recoveries of 200 µg/l were 82.3%, 82.2% and 88.2% with CV% 1.5, 1.8 and 2.0 for L-DOPA, dopamine and dihydroxyphenylacetic acid standards, respectively. In the enzymatic hydrolysis, the recoveries of 200 µg/l of the three compounds were 88.5%, 89.0% and 92.6% with CV% of 9.7, 6.8 and 9.4, respectively. After spiking urine samples with 100 µg/l of each standard, the recoveries of L-DOPA, dopamine and dihydroxyphenylacetic acid were 82.6 ± 6.7% (mean ± CV%), 85.9 ± 6.7% and 91.0 ± 7.4%, respectively.

Enzymatic hydrolysis of urine samples

The effect of two arylsulphatase concentrations (9.2 and 18.4 U in two samples after 60 min incubation (30 min suggested by the supplier) is shown in table 2. Incubation times exceeding 60 min did not increase but rather decreased the amount of deconjugated compounds (data not shown). The use of a sulphatase preparation (type-1) from Helix pomatia did not interfere with the chromatography.

The enzymatic hydrolysis was found to be strongly inhibited in the presence of 1 mmol/l of $Na_2S_2O_5$. Sulphatase was also weakly active when the hydrolysis was performed in phosphate buffer (data not shown). It was not feasible to run the internal standard through the hydrolysis process since it was significantly decomposed during incubation. Therefore the internal standard was added at the alumina absorption stage, where its recovery was satisfactory (89.2%).

Acid hydrolysis of urine samples

The effects of different acids on the hydrolysis are shown in table 3. The differences were minor during 60 min incubation. The use of 4 mol/l HCl and the 60 min incubation at + 95 °C was adequate for the hydrolysis

Tab. 3 Effect of different acids on hydrolysis of dopamine and dihydroxyphenylacetic acid conjugates in urine of healthy volunteers during 60 min incubation at + 95 °C.

	n	Concentration (mg/l)[a]	Efficacy of hydrolysis[b]
Dopamine			
Control	4	0.30 ± 0.02	
4 mol/l HCl[c]	8	1.10 ± 0.04	265
4 mol/l $HClO_4$[c]	6	1.12 ± 0.02	271
4 mol/l H_2SO_4[a]	6	1.14 ± 0.04	279
Dihydroxyphenylacetic acid			
Control	4	0.96 ± 0.04	
4 mol/l HCl[c]	8	1.93 ± 0.05	101
4 mol/l $HClO_4$[c]	6	1.87 ± 0.03	94
4 mol/l H_2SO_4[c]	6	1.79 ± 0.04	86

[a] Mean ± SD

[b] $\dfrac{\text{After hydrolysis} - \text{Before hydrolysis}}{\text{Before hydrolysis}} \times 100$

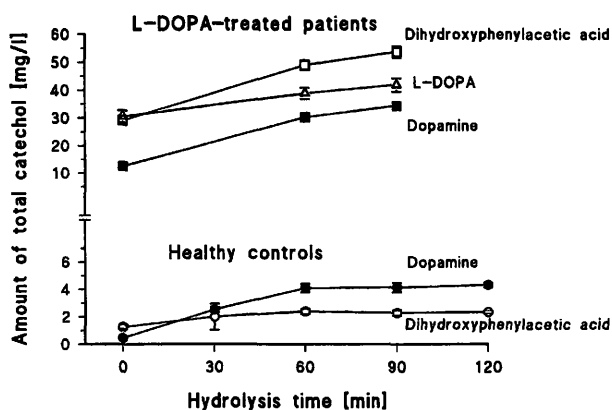[c] Final concentration in hydrolysis mixture 0.52 mol/l

Fig. 3 Effect of hydrolysis time on the liberation of free catechols (L-DOPA, dopamine, dihydroxyphenylacetic acid) as a function of time using 4 mol/l HCl at + 95 °C. Studies were done using urine samples from both healthy controls (n = 4) and from patients receiving L-DOPA (n = 4). Mean ± SD.

of conjugated L-DOPA, dopamine and dihydroxyphenylacetic acid. The effect of time (30–120 min) on the hydrolysis of urine samples is shown in figure 3. The hydrolysis was not complete after the 30 min incubation. There was no difference between 60 min and 90 min incubations. L-DOPA and dopamine liberation was slightly increased during prolonged incubation but dihydroxyphenylacetic acid levels were rather decreased. However, the compounds did not decompose during acid hydrolysis (fig. 3).

Comparison of healthy volunteers and patients with Parkinson's disease

An initial comparison was made during the validation process (tab. 2 and fig. 3). Our studies showed that enzymatic hydrolysis was equally effective for urine samples from the healthy controls and from the patients on L-DOPA therapy. However, the percentage hydrolysis by acid was much smaller in the latter than in the former urine samples.

In the more precise comparison, the urinary dopamine concentration of 5 control persons varied from 0.23 to 0.47 (0.30 ± 0.10 mg/l; mean ± SD), and dihydroxyphenylacetic acid concentrations from 1.1 to 5.1 (1.21 ± 0.66 mg/l; mean ± SD). In 7 L-DOPA treated patients the concentrations of dopamine were 50–200-fold and those of dihydroxyphenylacetic acid 30–40-fold greater than the control values. Generally, the variations between subjects was several-fold.

In healthy volunteers, with very low free dopamine levels, the quantity of dopamine was increased nearly 9-fold by acid hydrolysis, whereas that of dihydroxyphenylacetic acid was increased only 1.5-fold. Much smaller quantities of catechols were released by enzymatic hydrolysis than by acid hydrolysis, indicating that other conjugates were present in urine, in addition to sulphates. The amounts of these other conjugates showed wide variations, but they usually exceeded those of the sulphate conjugates (tab. 4; fig. 4).

We divided the 7 Parkinsonian patients into two groups based on the free dopamine concentrations before hydrolysis (moderate dopamine, < 20 mg/l, and high dopamine group, > 50 mg/l; tab. 4). In the moderate dopamine group, the proportion of dopamine after acid hydrolysis was increased by 64% during hydrolysis. Correspondingly, in the high dopamine group the proportion of dopamine was increased only by 30%. After acid hydrolysis the proportion of dihydroxyphenylacetic acid was increased by 48% in the moderate dopamine group,

Tab. 4 Effect of acid hydrolysis (4 mol/l HCl for 60 min at + 95 °C) and sulphatase treatment (18.4 U in 400 μl reaction mixture for 60 min at + 37 °C) on the liberation of free catechols from the urine of control subjects (low dopamine) and patients treated with L-DOPA (moderate and high dopamine concentrations).

	Concentration of catecholamines released by acid (= total conjugates)	Concentration of catecholamines released by sulphatase	Other conjugates	Proportion of other conjugates from total conjugates
	mg/l (Mean ± SD)	mg/l (Mean ± SD)	mg/l (Mean ± SD)	%
Control subjects (n = 5)				
Dopamine	2.6 ± 3.0	0.45 ± 0.49	2.2 ± 2.5	82.0 ± 7.1
Dihydroxyphenylacetic acid	1.6 ± 1.0	0.45 ± 0.30	1.1 ± 0.7	71.0 ± 6.5
L-DOPA treated subjects Moderate dopamine (< 20 mg/l) (n = 3)				
L-DOPA	6.3 ± 2.0	1.1 ± 2.0	5.2 ± 1.1	87.7 ± 22.5
Dopamine	10.7 ± 5.6	4.2 ± 5.0	6.5 ± 1.3	70.9 ± 27.0
Dihydroxyphenylacetic acid	16.7 ± 18.7	10.4 ± 13.8	6.3 ± 4.9	66.3 ± 37.6
High dopamine (> 50 mg/l) (n = 4)				
L-DOPA	8.8 ± 3.0	0.0	8.8 ± 3.0	100.0
Dopamine	19.7 ± 9.7	4.3 ± 3.8	15.4 ± 6.4	81.6 ± 16.2
Dihydroxyphenylacetic acid	4.9 ± 2.2	0.4 ± 0.6	4.6 ± 1.9	94.5 ± 8.6
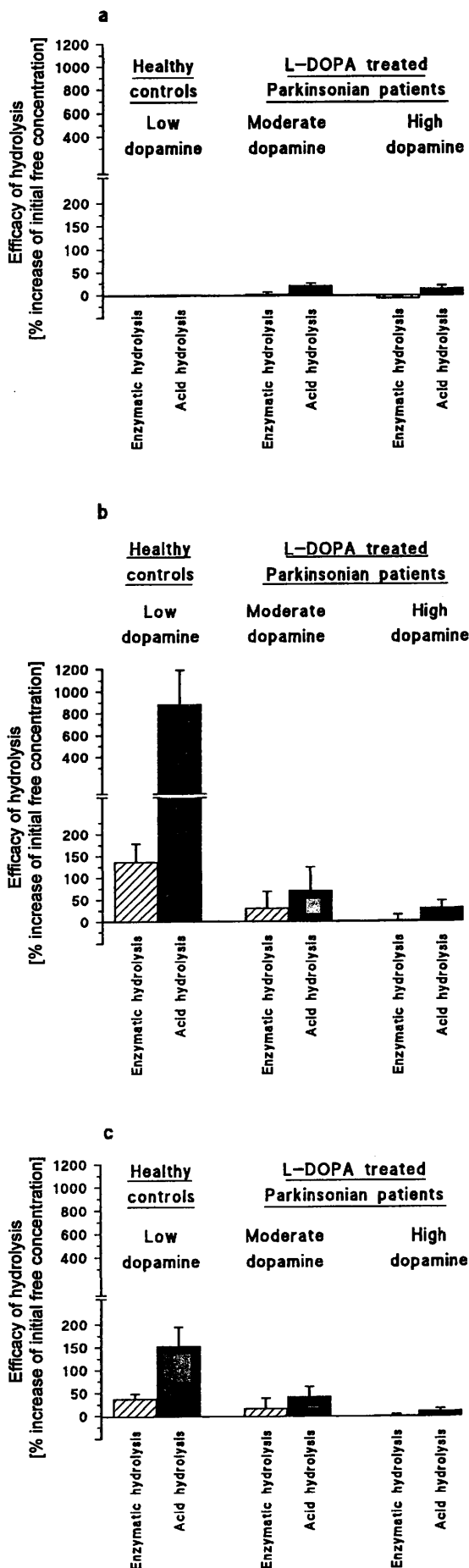
and only by 10% in the high dopamine group (fig. 4). Only minor quantities of L-DOPA conjugates were present, and they were not hydrolysed at all by sulphatase. The overall percentage conjugation of L-DOPA, dopamine and dihydroxyphenylacetic acid showed a concentration-dependent decrease, as the concentrations of the free compounds increased (fig. 4).

As in the control group, much smaller quantities of catechols were released by enzymatic hydrolysis, both in the low and the moderate dopamine groups, than by acid hydrolysis, indicating that there were other conjugates in the urine. These other conjugates accounted for 66 to 100% of the total conjugates (tab. 4; fig. 4).

Discussion

Since conjugation reactions play a significant role in the metabolism of catecholamines (1, 8) it is important to be able to quantify them. We have now compared procedures used to quantify free and conjugated forms of the catechol compounds, L-DOPA, dopamine and dihydroxyphenyl-acetic acid in urine after acid and enzymatic hydrolysis. Basal catechol levels and the quantities of conjugates display marked variations between individuals. Our conclusion is that the use of 4 mol/l HCl for 60 min at + 95 °C gives satisfactory results. The degree of hydrolysis is only slightly increased by 120 min hydrolysis. Other acids produce similar results after 60 min incubation for the hydrolysis of the dopamine conjugates, but show different efficiencies of hydrolysis for conjugates of dihydroxyphenyl-acetic acid. The studies showed that a maximum incubation time of one hour was needed for optimum hydrolysis of the analytes without decomposition. We found that at least 0.52 mol/l of $HClO_4$ was needed for adequate hydrolysis in 60 min, which differs from the conditions reported by Elchisak et al. (9). Incubation for a further hour did not increase the hydrolysis.

The enzymatic procedure using sulphatase gives a less complete hydrolysis of conjugated catecholamines. This is partially due to the fact that other conjugates are present, which are not hydrolysed by sulphatase. In fact, non-sulphate conjugates seem to be more abundant than sulphate conjugates. Enzyme preparations are not easy to use; they are sometimes sticky and quite expensive. Therefore acid hydrolysis is generally preferred from a practical point of view too.

Fig. 4 Efficacy of enzymatic (18.4 U in 400 µl reaction mixture for 60 min at + 37 °C) and acid hydrolysis (4 mol/l HCl for 60 min at + 95 °C) in healthy volunteers (low dopamine in urine) and in patients receiving L-DOPA (divided into moderate and high dopamine groups), where a = L-DOPA, b = dopamine and c = dihydroxyphenylacetic acid.
Mean ± SD. The efficacy is expressed as the percentage increase of the free catechol from the initial free concentration.

For determination of urinary catecholamines the samples must be collected in acid to avoid spontaneous oxidation (10). However, pH-values less than 1 can decrease the recoveries of catecholamine (11). We collected the urine specimens in 6 mol/l HCl, and pH remained between 1.5 and 2.0. In many laboratories antioxidants, e. g., ascorbic acid, $Na_2S_2O_5$ or dithiothreitol (8, 9) are added to prevent oxidation during the hydrolysis procedure. We found, however, that the enzyme hydrolysis was inhibited by 1.3 mmol/l of $Na_2S_2O_5$ in the incubation solution. It has been reported that the hydrolysis of noradrenaline was considerably inhibited by adding ascorbic acid (0.5−10 mmol/l) and slightly inhibited by dithiothreitol (1−10 mmol/l) in plasma (12). On the other hand, the hydrolysis of dopamine was not inhibited by ascorbic acid or dithiothreitol (12). In our hands $Na_2S_2O_5$ did not affect the acid hydrolysis at all.

Dihydroxybenzylamine is frequently used as an internal standard in catecholamine analyses. Its recovery is the same as that of endogenous catecholamines in human and rodent plasma, while the recovery in dog, horse and goat plasma is rather less than that in human plasma (13). In our hands, the recovery of dihydroxybenzylamine decreased during enzymatic hydrolysis, although the recoveries of catechol compounds did not. Therefore we added dihydroxybenzylamine only after the enzymatic or acid hydrolysis, and before the alumina procedure.

During validation of our method we accidentally found that a large unknown peak appeared during the acid hy-

drolysis. This seriously marked the detection of dihydroxyphenylacetic acid, since it was extracted in both the alumina and solid phase procedures. Its retention time was insensitive to pH changes, but when heptane sulphonic acid was used instead of octane sulphonic acid in the mobile phase, we finally succeeded in separating it from the dihydroxyphenylacetic acid peak (fig. 1 and 2). This exemplifies the practical interference problem when working with a biological matrix.

The initial dopamine (and dihydroxyphenylacetic acid) levels seemed to dictate the degree of hydrolysis. These findings are interpreted as follows. At high urinary dopamine (and dihydroxyphenylacetic acid) levels, such as those seen in the L-DOPA treated patients, the capacity of conjugation is saturated and the free dopamine (and dihydroxyphenylacetic acid) concentrations are much higher than those in the healthy volunteers. Hydrolysis releases even more dopamine (and dihydroxyphenylacetic acid) from urine of the patients whose conjugation capacity almost or totally exhausted, than from the urine of the healthy controls whose still have a relatively high conjugation capacity. The percentage hydrolysis is decreased in the samples from L-DOPA-treated patients, owing to their much higher catechol concentrations.

Acknowledgements

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