We describe a new method for the simultaneous assay of 3,4-dihydroxyphenylalanine (L-dopa) and dopamine in serum. Both compounds have been determined quantitatively at concentrations as low as 10 μg/liter with a coefficient of variation of less than 4%. Peak heights were linearly related to concentration up to 10 mg/liter for each compound. Assay of human sera gave within-run and day-to-day coefficients of variation of 2.8% and 3.1% for L-dopa, and 2.3% and 3.7% for dopamine. The linear relationship between readings (nA, y-axis) and concentration (μg/liter) is described by the following equations: 

\[ y = (21.0 \pm 0.082)x + (0.46 \pm 0.31 \text{ SD}) \] 

for L-dopa and 

\[ y = (17.0 \pm 0.10)x + (0.08 \pm 0.04 \text{ SD}) \] 

for dopamine. The procedure combines liquid/solid extraction, liquid chromatography, and controlled-potential electrochemistry. The simplicity, sensitivity, and accuracy of this method make it suitable for routine clinical analysis of serum samples to optimize bioavailability for individual patients. After the extraction procedure is completed, samples can be analyzed at the rate of 10/h.

Additional Keyphrases: monitoring efficacy of dopa decarboxylase inhibitors • electrochemical detection of eluate • dopamine sulfate esters

The importance of L-dopa (3,4-dihydroxyphenylalanine) in the treatment of Parkinson's disease is well established (1). Therapy with L-dopa consists of gradually increasing the daily dosage until the optimum response is reached, usually within two to three weeks (2). The amount of drug required can be reduced significantly by concomitant dosing with extracerebral dopa decarboxylase (EC 4.1.1.28) inhibitors such as carbidopa (3). The ratio of dopamine to L-dopa in the serum indicates the effectiveness of the dopa decarboxylase inhibitor. The bioavailability of the unaltered drug and the effectiveness of the inhibitor are not routinely evaluated for individual patients because of the lack of a satisfactory assay. There is a definite need for a clinically feasible simultaneous method for L-dopa and dopamine in serum.

The most widely used methods of analysis for catecholamines have been variations of the classical fluorometric approach (4–7). These procedures involve conversion of the previously isolated and separated catecholamines to fluorescent derivatives. Simultaneous assay of the individual catecholamines by these methods is difficult and not well suited to routine clinical application. Most commonly, "total catecholamines" are measured, referring only to norepinephrine and epinephrine, but not L-dopa and dopamine. Recently, other techniques for catecholamine analysis have been introduced. Various groups have advocated gas chromatography with flame ionization (8), electron capture (9), or mass spectrometric detection (9, 10).

Because of the similarity of the molecules, many of the classical methods for L-dopa analysis will work equally well for the hypertensive drug α-methyl dopa. The fluorescent and colorimetric methods are not highly selective, and therefore any L-dopa present, if not first separated, may interfere with the α-methyl dopa analysis. Gas chromatographic (11) and electrochemical methods (12) are superior in this regard.

This paper describes an assay for L-dopa and dopamine (3,4-dihydroxyphenethylamine) in serum by high-performance liquid chromatography with electrochemical detection (13). Our approach consists of isolating and preconcentrating the catechols by liquid/solid extraction, then separating them on a high-performance strong cation-exchange resin (14). L-Dopa and dopamine are determined in a single chromatogram in 6 min. The dopa decarboxylase inhibitor, carbidopa, does not interfere in the analysis.

Materials and Methods

Reagents

- Hydrochloric acid, 6 mol/liter, and 370 ml/liter.
- Disodium ethylenediaminetetraacetate, 100 g/liter.
- Sodium metabisulfite, 50 g/liter. Store refrigerated and prepare fresh every two weeks.
- Phosphate buffer, pH 7.4. Dissolve 1.18 g of potassium monobasic phosphate and 8.16 g of sodium dibasic phosphate heptahydrate in 1 liter of distilled water.
- Sodium hydroxide, 500 g/liter, 3 mol/liter, and 1 mol/liter.
- Acetic acid, 1 mol/liter.
- Mobile phase, 10 mmol/liter H2SO4 and 40 mmol/liter Na2SO4.
- Alumina. Acid wash Woelm W200 neutral alumina, according to the method of Anton and Sayre (15).

Aqueous standards. Dissolve 61.6 mg of dopamine hydrochloride and 50.0 mg of L-dopa in individual
500-ml volumetric flasks. Dilute to the mark with 0.1 mol/liter HClO₄. These solutions are stored refrigerated. No degradation was detected after 10 weeks.

Serum pool. Collect about 100 ml of blood from healthy humans. Store aliquots of the serum in 5-ml vials at −35 °C so that only the required amount of serum need be thawed for each analysis. Working standards are prepared daily by adding 10-μl aliquots of the aqueous standards of dopamine and L-dopa (100 mg/liter, in 0.1 mol/liter HClO₄) to 1 ml of the serum pool. The working standard will then contain 1 mg of each compound per liter.

Apparatus

The liquid chromatographic system was identical to that previously described (13), with the addition of a 2-cm precolumn (15) and a pneumatically actuated 20-μl slider valve for sample injection (Laboratory Data Control, Riviera Beach, Fla.; Model CSVA-20). We used a commercial electrochemical detector based on our earlier work (Bioanalytical Systems Inc., P.O. Box 2206, West Lafayette, Ind. 47906; Model LC-2). The strong cation-exchange resin was dry packed in a 50 cm × 2 mm i.d. glass column (Altax Scientific, 1450 Sixth St., Berkeley, Calif. 94710; Model 251-02). The pellicular resin used was a Vydac bonded-phase cation-exchange resin (The Separations Group, 8738 Oakwood Ave., Hesperia, Calif. 92345; Model 232CX). Water was circulated through a jacket (Altax, Model 251-10) surrounding the column to maintain a temperature of 28 °C. The mobile phase was pumped at a rate of 0.40 ml/min. The detector potential was set at +0.72 V vs. an Ag/AgCl reference electrode.

Procedure

Place 1 ml of blood serum, 3.5 ml of saturated sodium chloride solution, and 100 μl of concentrated hydrochloric acid in a 15-ml polypropylene centrifuge tube. Shake well and centrifuge for 15 min at 15 000 × g to precipitate the protein. Pour the supernate into a 12-ml glass centrifuge tube. Hydrolyze sulfate conjugates by heating in a 90 °C water bath for 20 min. Cool the tubes and then add 0.5 g of ammonium sulfate. Extract twice with ethyl acetate and once with hexane (5-ml portions of each). Centrifuge briefly, aspirate, and discard the nonaqueous layers.

Place the aqueous layer in a 10-ml beaker with a magnetic stirrer. Add 100 μl of both the ethylenediaminetetraacetate and the sodium metabisulfite solutions. While stirring the mixture, adjust the pH to 8.5 ± 0.1 by rapid addition of 3 mol/liter sodium hydroxide and then 1 mol/liter sodium hydroxide. Shake with 60 mg of acid-washed alumina in a 5-ml conical screw-cap vial (Pierce Chemical Co., Box 117, Rockford, Ill. 61105; Reacti-Vials) for 12 min. Allow the alumina to settle and then aspirate and discard the serum solution. Wash the alumina once with the phosphate buffer and twice with distilled water. Dry the alumina in a vacuum oven for 10 min at 40 °C.

Elute the L-dopa and dopamine from the alumina with 300 μl of 1 mol/liter acetic acid. Gently shake the vials for 10 min on a mechanical shaker. Transfer about 200 μl of the eluent to a second vial. Inject 20 μl of this solution onto the chromatographic column. Measure the peak heights and calculate the concentrations by comparison with the peak heights obtained for the working standard containing a known concentration (1 mg/liter) of L-dopa and dopamine.

Results and Discussion

Figure 1 depicts a chromatogram for a sample isolated from the serum of a patient undergoing L-dopa therapy. This example was chosen because the concentration of L-dopa (140 μg/liter) is lower than is normally expected for a therapeutic dose and because most previous methods have a detection limit of about 1 mg/liter. Because the amount of L-dopa present in the blood of Parkinson's disease patients on L-dopa therapy is frequently about 1 mg/liter (2), it is desirable to be able to measure lower concentrations, particularly for pharmacokinetic studies. The present method permits measurements down to 10 μg/liter with a precision of better than ±4%. In addition, dopamine can be quantitated simultaneously down to the same limit, which makes the method well suited to following the efficacy of decarboxylase inhibitors. This latter capability is unique to the present assay. The two dopamine sulfate esters cannot yet be monitored directly and thus a hydrolysis procedure is necessary, to establish the total dopamine present. These esters are known to constitute a major part of the serum dopamine (17).

We know of no probable endogenous interferences with this assay, because of the highly specific combination of liquid chromatography and electrochemistry.
We are now using the method routinely for clinical samples. Very similar procedures have been devised for monitoring other catechol-based drugs in serum (e.g., α-methyl dopa and carbidopa). We believe that the approach described here has general applicability to compounds of this type, although slight modifications in conditions may be necessary to optimize the assay for a particular drug. Other commercial column packings (e.g., Du Pont “Zipax SCX”) than that used here will improve the resolution in certain situations. For example, when both carbidopa and L-dopa are administered together, they are better resolved with Zipax SCX as the stationary phase.

Table 1 summarizes precision and recovery data for the assay. The linear relation between readings and concentration was tested by adding standard amounts of each compound to the serum pool over a range from 50 μg/liter to 10 mg/liter and analyzing. The linear response in nanoamperes (y) vs. μg/liter (x) is described by the following equations:

\[
y = (21.0 \pm 0.082)x + (0.46 \pm 0.31 \text{ SD}) \quad \text{for L-dopa}
\]
\[
y = (17.0 \pm 0.10)x + (0.08 \pm 0.04 \text{ SD}) \quad \text{for dopamine.}
\]

After the extraction procedure is completed, samples can be analyzed at the rate of 10 per hour.

This investigation was supported by grants from the NIGMS, NIH (grants GM21580-01 and GM22713-01), the National Science Foundation (grant GP-42452X), and the Showalter Trust Fund. We thank Dr. David C. Wenke (Erie County Laboratory, Buffalo, N.Y.) for helpful discussion and exchange of samples.

References


