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Note

Simultaneous determination of endogenous catechols and exogenous 2- and 6-fluorinated catechols in tissue and plasma using liquid chromatography with electrochemical detection

GRAEME EISENHOFER*,*

Clinical Neuroscience Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20817 (U.S.A.)

KENETH L. KIRK

Laboratory of Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20817 (U.S.A.)

IRWIN J. KOPIN

Clinical Neuroscience Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20817 (U.S.A.)

and

DAVID S. GOLDSTEIN

Hypertension-Endocrine Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20817 (U.S.A.)

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Catecholamines and their amino acid precursor dihydroxyphenylalanine (DOPA), labeled with a fluorine atom at the carbon-2 or carbon-6 position on the catechol nucleus, may have a number of applications as false neurotransmitters. In particular, DOPA labeled with positron-emitting fluorine (^{18}F) at the carbon-6 position is proving useful for positron emission tomographic imaging of central nervous system dopaminergic neurons [1]. The fluorinated analogues of dopamine (DA) are taken up by sympathetic nerve endings and there β-hydroxylated to the fluoronorepinephrine derivatives, which have similar turnovers to that of the endogenous compound [2]. Thus, DA labeled with ^{18}F at the carbon-

*Present address: Baker Medical Research Institute, Commercial Road, Prahran, Victoria 3181, Australia.

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2 or carbon-6 position can be used for positron emission tomographic imaging of sympathetically innervated tissues and may prove useful for quantifying norepinephrine (NE) turnover non-invasively in clinical settings.

Critical to examination of the potential of ^{18}F-labeled DA for such a purpose and for interpretation of the images obtained by positron emission tomography is an understanding of the fate of the fluorinated catecholamine inside the body. This requires the measurement of parent compounds and metabolites in tissue and plasma samples. The present report describes a method using liquid chromatography (LC) with electrochemical detection (ED) for the simultaneous determination of tissue and plasma concentrations of the endogenous catechols DOPA, DA, NE, epinephrine (E), dihydroxyphenylacetic acid (DOPAC) and dihydroxyphenylglycol (DHPG) and of the exogenous fluorinated catechols 2-fluorodihydroxyphenylalanine (2F-DOPA), 6-fluorodihydroxyphenylalanine (6F-DOPA), 2-fluorodopamine (2F-DA), 6-fluorodopamine (6F-DA), 2-fluoronorepinephrine (2F-NE), 6-fluoronorepinephrine (6F-NE), 2-fluorodihydroxyphenylacetic acid (2F-DOPAC) and 6-fluorodihydroxyphenylacetic acid (6F-DOPAC). Adequate resolution of all these compounds requires careful attention to the constitution of the mobile phase and other aspects of the chromatographic conditions

EXPERIMENTAL

Catechols

Fluorinated catechols were synthesized by K.L.K. according to previously established methods [3,4]. All other catechols were purchased from Sigma (St. Louis, MO, U.S.A.).

Apparatus and chromatographic conditions for liquid chromatography

The apparatus included a Waters Model 590 programmable solvent-delivery system on line with a Model 710B automated sample processor, the latter connected to a 30 cm $\times$ 3.9 cm, 10 μm particle size, μBondapak C_{18} reversed-phase column (Waters Assoc., Milford, MA, U.S.A.). The catechols were quantified by ED using a series electrode system (Environmental Sciences Assoc., Bedford, MA, U.S.A.). The first electrode in the series was set at 0.30 V (conditioning cell), whereas the second and third electrodes of the analytical cell were set at 0.15 and -0.35 V, respectively. These potentials are only applicable to the instrument used here. The output of the third electrode was recorded using a Waters Model 730 data module.

The mobile phase, filtered and degassed before use, was pumped through the system at 1.0 ml/min and contained (per liter) 13.8 g of NaH_2PO_4, 100 mg of EDTA, 0.15–0.45 g of sodium octane sulfonate (PIC B8-low UV, Waters), 10–30 ml of acetonitrile and phosphoric acid to adjust the pH to between 3.0 and 4.0. Various octane sulfonate concentrations and pH values were examined to establish chromatographic conditions for the separation of endogenous catechols and exogenous fluorinated catechols. The required amount of acetonitrile in the mobile phase depended on the octane sulfonate concentration (higher octane sul-

fonate concentrations necessitated higher acetonitrile concentrations) and the condition of the column (with repeated use, columns required lower acetonitrile concentrations due to decreased retention).

Sample collection and handling

Tissue (0.3–0.6 g left ventricle, spleen, submaxillary gland) and blood (1 ml from femoral artery) were obtained from pentobarbital-anaesthetised rats at various intervals after intravenous administration of 250 μg/kg 2F-DA or 6F-DA (1 mg/ml saline) or an equivalent volume of saline. Blood was centrifuged immediately and plasma (0.5 ml) frozen at −70°C until assayed. Tissue samples were rinsed briefly in normal saline and frozen quickly in isopentane supercooled on dry ice, and then homogenised in 2 ml of 0.4% perchloric acid. After centrifugation, supernatants were frozen at −70°C until assayed.

After addition of 1 ng of the internal standard, dihydroxybenzylamine (DHBA), samples (0.2 ml tissue supernatants or 0.5 ml plasma) were adsorbed onto alumina according to a previously described method [5], and the catechols eluted from the alumina using mobile phase adjusted to pH 2.2 with phosphoric acid.

RESULTS AND DISCUSSION

The validity of the assay for endogenous catechols together with potential sources of interference have been described previously [5]. Separation of fluorinated catechols from endogenous catechols and from DHBA, the internal standard, required careful attention to pH and to the concentration of the ion-pairing reagent, octane sulfonate, in the mobile phase. The effects of varying the octane sulfonate concentration or pH on the elution times of the various catechols relative to the internal standard are shown in Fig. 1. Increasing the octane sulfonate concentration prolonged the times at which the amines (NE, E, DA, DHBA, 2F-NE, 6F-NE, 2F-DA and 6F-DA) were eluted from the column but had no effect on or decreased the times at which the carboxylic acids (DOPAC, 2F-DOPAC and 6F-DOPAC) were eluted. The effect of octane sulfonate on retention was larger for the amines than for the amino acids (DOPA, 2F-DOPA and 6F-DOPA) and larger for the primary amines (e.g. NE) than for the secondary amines (e.g. E). Chromatographic peaks corresponding to E and 6F-NE were resolved best at octane sulfonate concentrations above 0.35 g/l, but even at these concentrations complete baseline separation of E and 6F-NE was difficult (Fig. 2). Since concentrations in tissue and plasma generally are low, the similarity of retention times of E and 6F-NE was rarely a problem.

Changing the pH of the mobile phase provided another means to separate the various substances. Increasing the pH decreased the retention times of the carboxylic acid and amino acid catechols, whereas the retention times of the amines were unaffected (Fig. 1). For the 6-fluorocatechols, optimal separation was obtained at a mobile phase pH of 3.6 and an octane sulfonate concentration of 0.4 g/l. These conditions allowed the simultaneous separation of 2-fluorocatechols (Fig. 2), although for these substances an octane sulfonate concentration of 0.25 g/l and a pH of 3.45 was also suitable.

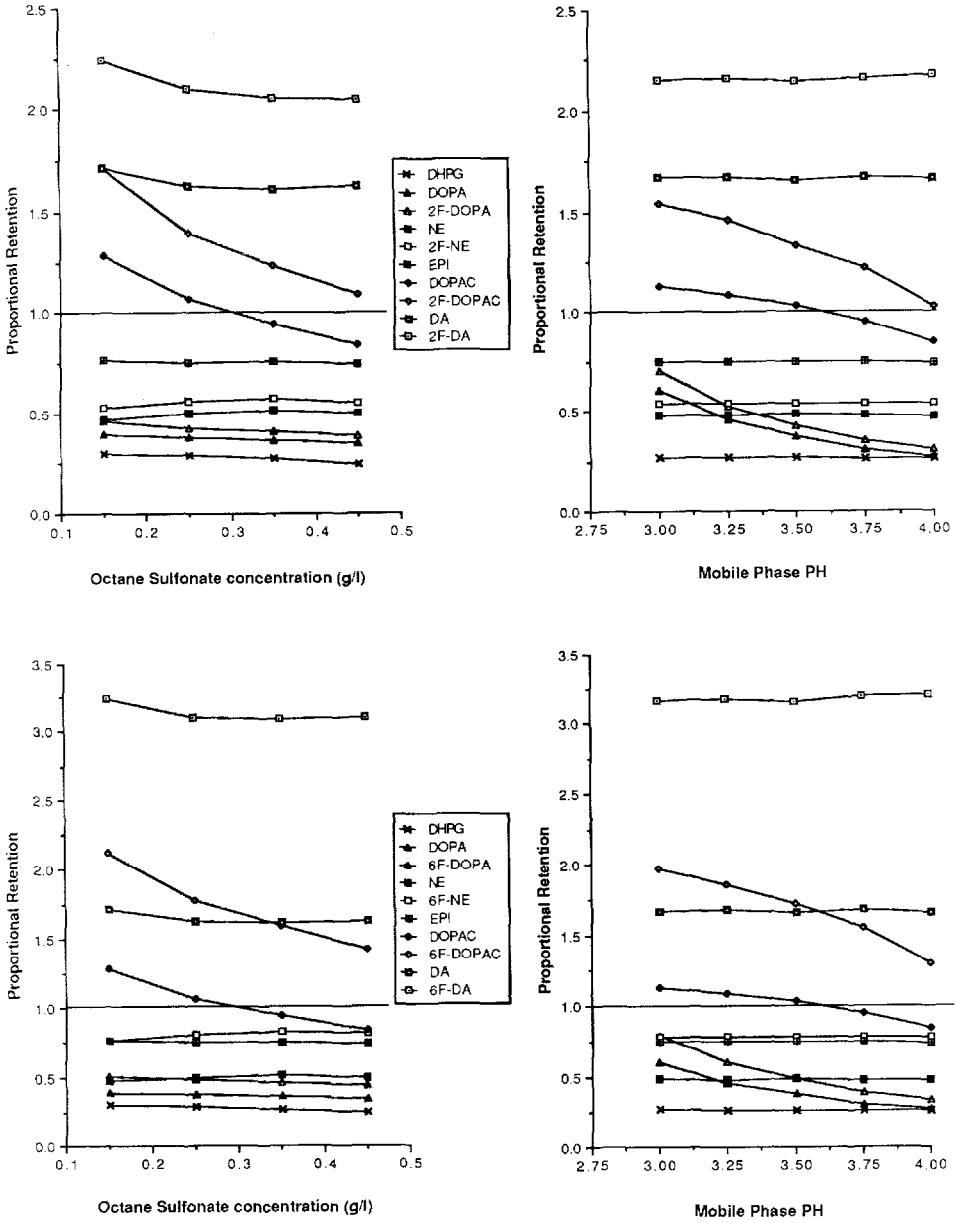

Fig. 1. Effects of octane sulfonate concentration and mobile phase pH on elution times of endogenous catechols, 2-fluorinated catechols (above) and 6-fluorinated catechols (below) relative to elution times of the internal standard, DHBA. Proportional retention times are the elution times (times from injection to detection) of each catechol divided by the elution time of the internal standard, DHBA (18-20 min). For experiments in which the octane sulfonate concentration was varied, the pH was maintained at 3.5. For experiments in which the pH was varied, the octane sulfonate concentration was maintained at 0.25 g/l.

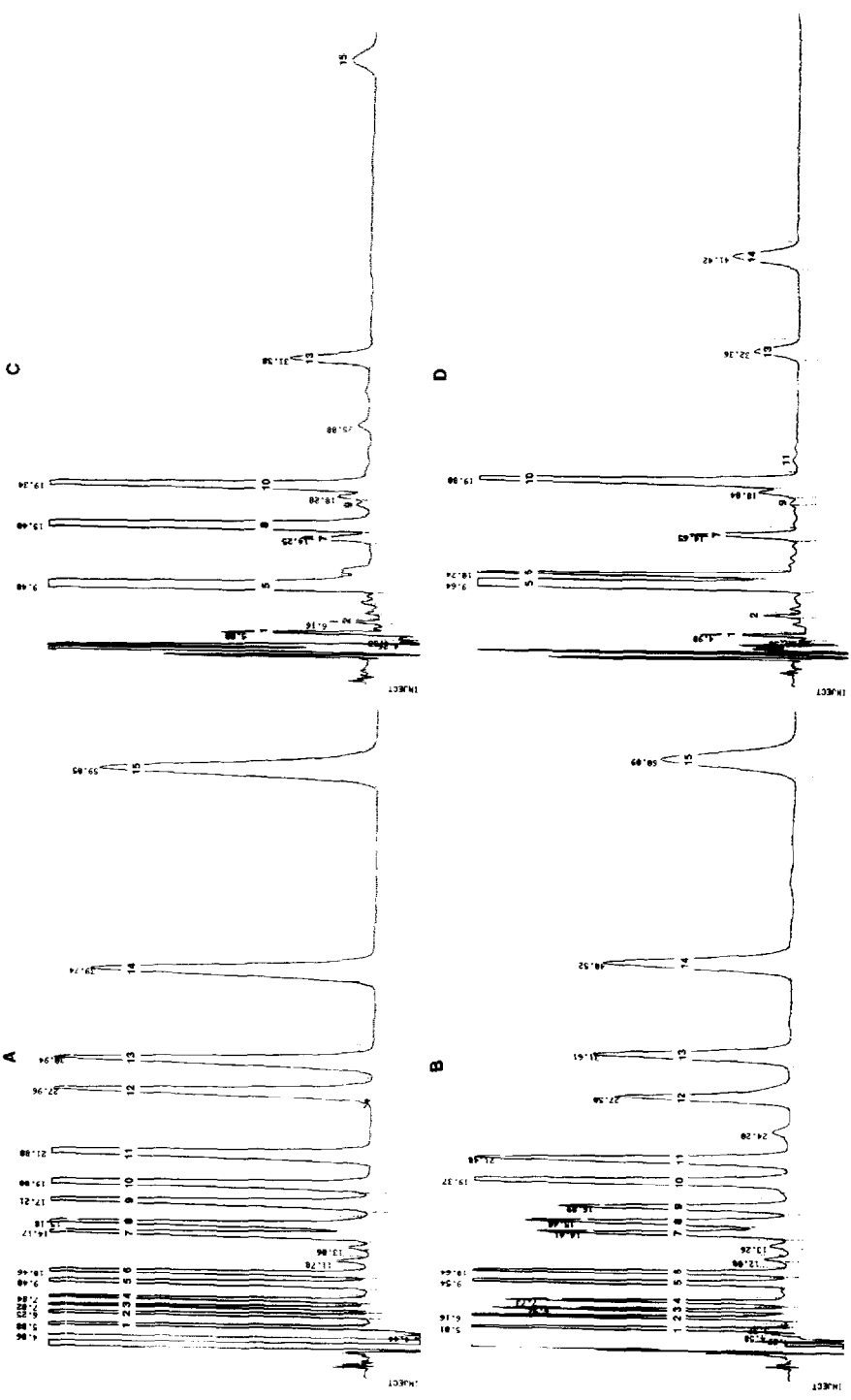

Fig. 2. Chromatographic recordings (*y*-axis, integrator response in arbitrary units; *x*-axis, time after injection given by the numbers at the top of each peak) of DHPG (1), DOPA (2), 2F-DOPA (3), 6F-DOPA (4), NE (5), 2F-NE (6), E (7), 6F-NE (8), DOPAC (9), DHBA, internal standard (10), 2F-DOPAC (11), 6F-DOPAC (12), DA (13), 2F-DA (14) and 6F-DA (15) after injection of (A) a standard solution containing 1 or 2 ng each of the compounds, (B) an alumina extract of the standard solution, (C) an alumina extract of supernatant from rat submaxillary gland obtained 60 min after intravenous administration of 250 μg/kg 6F-DA, and (D) an alumina extract of supernatant from rat submaxillary gland obtained 60 min after intravenous administration of 250 μg/kg 2F-DA. Mobile phase: pH 3.6, 0.4 g/l octane sulfonate, 2.0% acetonitrile, 12.8 g/l NaH PO_4, 100 mg/l EDTA.

TABLE I

INTRA- AND INTER-ASSAY COEFFICIENTS OF VARIATION, DETECTION LIMITS, EXTRACTION EFFICIENCIES AND TYPICAL RETENTION TIMES OF EACH OF THE ENDOGENOUS AND FLUORINATED CATECHOLS

Mobile phase: pH 3.6, 0.4 g/l octane sulfonate, 2.0% acetonitrile, 13.8 g/l NaH_2PO_4, 100 mg/l EDTA.

Catechol	Retention time (min)	Coefficient of variation (%)		Extraction efficiency (mean ± S.D.) (%)	Detection limit (3× baseline noise) (pg)
		Intra-assay	Inter-assay		
DHPG	5.0	3.3	13.0	77 ± 2	5
DOPA	6.5	1.4	13.5	55 ± 7	10
2F-DOPA	7.25	0.7	4.9	61 ± 7	10
6F-DOPA	8.0	3.3	6.7	55 ± 6	10
NE	9.5	2.3	2.1	65 ± 4	10
2F-NE	10.5	0.4	5.1	67 ± 6	25
E	15.0	10.0	15.3	63 ± 3	20
6F-NE	16.0	7.6	11.2	61 ± 6	20
DOPAC	17.5	2.0	15.0	58 ± 3	20
DHBA	20.0	–	–	69 ± 5	–
2F-DOPAC	22.5	2.3	4.9	67 ± 3	30
6F-DOPAC	28.5	1.1	13.6	57 ± 2	45
DA	33.0	4.3	14.8	62 ± 3	25
2F-DA	42.0	1.5	4.4	65 ± 5	50
6F-DA	60.0	2.0	7.9	58 ± 5	60

Use of mobile phase, adjusted to pH 2.2 with phosphoric acid, to elute the catechols from the alumina resulted in recoveries of between 55 and 77% for all catchols (Table I). This was an improvement over the previously described use of 0.2 *M* acetic acid [5] which does not elute carboxylic acid and amino acid catechols from alumina with the same efficiency as the DHBA internal standard. Use of phosphoric acid, however, can result in elution of contaminating compounds. While this is not a problem for the extraction of supernatants of tissue homogenates (Fig. 2) the presence of these contaminating substances in plasma extracts can occasionally present problems which may be best avoided using 0.2 *M* acetic acid to elute catechols. Under these conditions one of each of the fluorinated DOPA or DOPAC analogues not present in the sample can be used as internal standards to correct recoveries of the carboxylic acid and amino acid catechols.

Plasma concentrations of 2F-DOPAC could be detected at all time points within 60 min of 2F-DA administration, whereas 2F-DA was only detected within the first 30 min and plasma 2F-NE was not detected. Studies of tritium-labeled DA have indicated that 30 min after its intravenous injection, alumina-extractable catechols account for less than 2% of the total plasma tritium, whereas in sympathetically innervated tissues they account for greater than 75% of the tritium.

Thus, application of the present method to examination of the fate of fluorinated DA in plasma may be limited, whereas in sympathetically innervated tissues the method should prove useful.

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