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## Note

# Determination of L-tyrosine in rat brain by reversed-phase liquid chromatography with electrochemical detection

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Liquid chromatography with electrochemical detection (LC–ElCD) has been widely exploited for highly sensitive determination of both catecholamines and 5-hydroxytryptamine (5-HT) and their metabolites in brain tissue[1]. Where such techniques are applied to *in vivo* and *in vitro* studies of the storage, metabolism and release of the biogenic amines, determination of the precursor amino acids, tyrosine and tryptophan, is also likely to be of interest. While a number of LC–ElCD assays for 5-HT and metabolites permit concurrent estimation of tryptophan[1], LC–ElCD measurement of brain tyrosine in conjunction with the catecholamines has not been reported. A recently reported LC–ElCD assay for tyrosine, associated with the measurement of mouse brain tyrosine hydroxylase activity[2], involves a lengthy preparative sequence.

We present herein a rapid LC–ElCD assay for L-tyrosine in rat brain, involving a simple extension of existing catecholamine sample preparation procedures, and requiring no additional reagents or hardware.

## MATERIALS AND METHODS

Octyl sodium sulphate was obtained from Kodak (Kirby, Liverpool, Great Britain). All other reagents and standards were purchased from Sigma London (Poole, Great Britain) or from Fisons (Loughborough, Great Britain) and were of analytical or HPLC grades.

The LC system consisted of a Constametric IIG solvent delivery pump (Laboratory Data Control, Stone, Great Britain), a 3 cm × 4.6 mm I.D. stainless-steel precolumn, dry-packed with Whatman Co:Pell ODS (Shandon Southern Products, Runcorn, Great Britain), a Rheodyne 70-10 injector, and a 25 cm × 4.6 mm I.D., stainless-steel, Altex Ultrasphere-ODS (5 μm) column (Anachem, Luton, Great Britain). The amperometric detector was composed of a Bioanalytical Systems LC-2A electrochemical controller, and a TL-5A detector cell (Anachem), the latter consisting of a glassy carbon working electrode and an Ag/AgCl reference electrode.

Male Sprague-Dawley rats (Charles River U.K., Margate, Great Britain) were group housed and maintained on rat cake diet 86 (E. Dixon and Sons, Ware, Great Britain) and water *ad libitum*. Animals (250–300 g) were stunned and decapitated between 09.00 h and 10.00 h, and brains (1.9–2.1 g) were rapidly excised. Whole

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brains were stored in polyethylene centrifuge tubes on dry ice for 10–20 min. Where individual brain parts were required, the tissues were rapidly dissected prior to storage on dry ice[3]. Each whole brain or individual region was weighed, and then homogenised on ice, for 1 min (Ultra-Turrax TP 18/10) in 6.5 volumes of ice-cold 0.2 *M* $HClO_4$. Individual aliquots of homogenate from each brain then received internal standards and/or "spikes" of authentic tyrosine, in a further 7.5 volumes of ice-cold 0.2 *M* $HClO_4$, to give a final volume of 15-fold the tissue weight.

Catecholamines were isolated from standard mixtures or supernatants of tissue homogenates by alumina extraction[4,5]. After the initial extraction, an aliquot of the Tris buffer supernatant was acidified with 0.25 volumes of 1 *M* $HClO_4$ (final pH 1.8), and stored at −20°C for the assay of tyrosine. The extraction procedure was otherwise as described.

The catecholamines, dopamine (DA) and noradrenaline (NA) were assayed in acid eluates from the alumina by a modification of the technique of Mefford *et al.*[6], at an applied potential of +0.65 V. The mobile phase was 0.05 *M* sodium acetate–0.01 *M* citric acid, pH 5.1, containing 100 mg $l^{-1}$ octyl sodium sulphate, 50 mg $l^{-1}$ EDTA and 15% methanol. Under these conditions, tyrosine, injected in the acidified Tris buffer supernatants, eluted in the solvent front, but could be resolved at an applied potential of +1.0 V, under the following mobile phase conditions: 0.1 *M* sodium acetate–0.1 *M* citric acid, pH 4.2, containing 100 mg $l^{-1}$ octyl sodium sulphate, 50 mg $l^{-1}$ EDTA and 5% methanol.

The catecholamine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were assayed in untreated supernantants of whole brain homogenates by the LC–ElCD technique of Mefford[1].

## RESULTS AND DISCUSSION

A typical chromatogram for an untreated standard mixture of tyrosine and catecholamine metabolites is illustrated in Fig. 1. Mean retention time for 4 injections of tyrosine was 7.91 ± 0.09 min, while those for 3,4-dihydroxyphenylethyleneglycol (DHPG), 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) and DOPAC were 3.65 ± 0.07, 7.35 ± 0.08 and 14.98 ± 0.30 min, respectively. These metabolites would not, therefore, be likely to interfere with the tyrosine peak. Interference from other catechols is also unlikely, since DA, NA and adrenaline (A) show even longer retention times on $C_{18}$ columns. Furthermore, catechols are, to a large extent (approx. 60%), adsorbed onto alumina[6] and any unadsorbed compounds would be greatly diluted (25-fold) in the acidified Tris buffer. No consistent peaks corresponding to these compounds have been detected in tissue extracts assayed for tyrosine. Fig. 2 shows chromatograms for an extracted tyrosine standard and an extract from a whole rat brain homogenate.

It was also important to determine that, under the conditions of the assay by which the catecholamines were isolated, tyrosine was not adsorbed onto the alumina. Fig. 3 shows a typical plot of detector output (nA) for increasing concentrations of tyrosine standards which were either carried through the acidified Tris buffer and alumina extraction or were not. Values for the "extracted" samples were corrected for dilution. The results of three such experiments (six duplicate points per experiment) consistently showed that the responses to tyrosine injected in concentrations from 0–

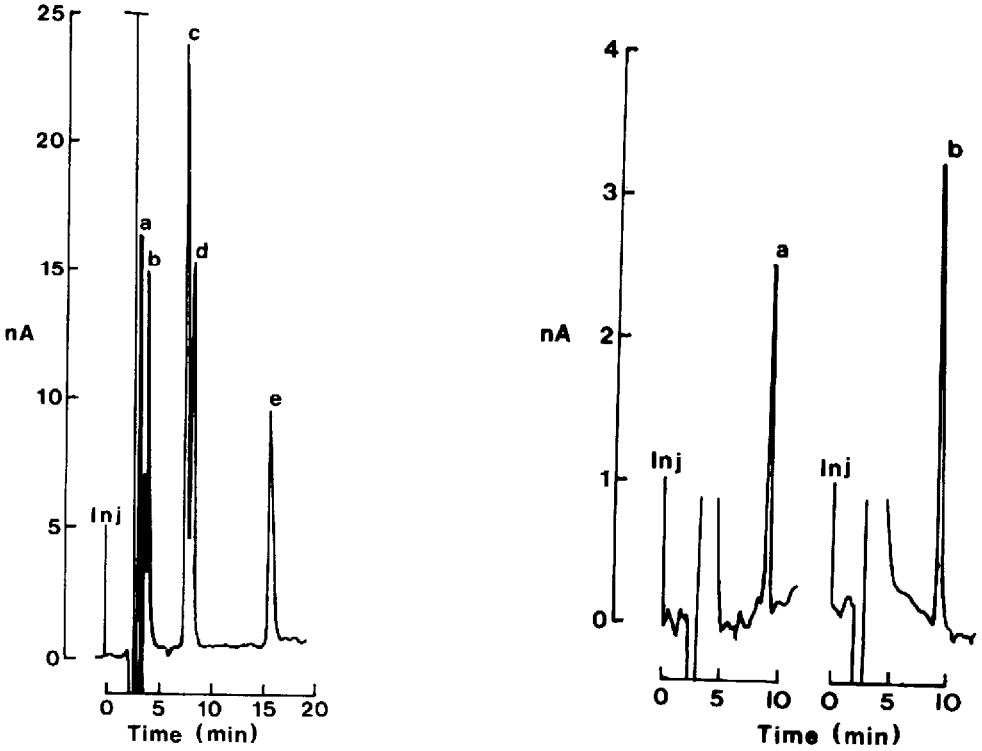


Fig. 1. Chromatographic separation of an untreated mixture (10 ng each) of authentic ascorbic acid (a), DHPG (b), MHPG (c), tyrosine (d) and DOPAC (e). Mobile phase: 0.1 *M* sodium acetate–0.1 *M* citric acid, pH 4.2, containing 100 mg $l^{-1}$ octyl sodium sulphate, 50 mg $l^{-1}$ EDTA and 5% methanol. Flow-rate: 1.0 ml/min. Applied potential: +1.0 V. Back pressure: 2450 p.s.i. Injection volume: 100 $\mu$l.

Fig. 2. Chromatograms of (a) an extracted tyrosine standard (40 ng) and (b) an extract from a 1:14 homogenate of whole rat brain. Conditions as in Fig. 1.

100 ng were linear. Further, the mean recovery for tyrosine in "extracted" samples was 93.1 ± 2.8%, suggesting that there was very little adsorption of tyrosine onto the alumina.

The apparent recovery of tyrosine spikes from rat whole brain homogenates was lower and more variable. Mean recovery of 40 ng from homogenates of 3 separate brains (6 replicates per brain) was 73.7 ± 3.8%. Presumably some tissue component, probably protein, promotes "trapping" of tyrosine. Thus, for any experiment, the calculated tissue concentrations of tyrosine were corrected for recovery using values obtained from contemporary tissue samples spiked with tyrosine. Two possible internal standards, 3-nitro-L-tyrosine[7] and α-methyl-L-*p*-tyrosine, proved unsuitable, owing to their lengthy retention times (approx. 24 and 19 min, respectively) and their poor electrochemical activities under these chromatographic conditions. 1.64 and 0.86 $\mu$g respectively were required to produce a signal equivalent to 10 ng tyrosine.

Table I shows values for tyrosine, catecholamines and metabolites estimated in the same homogenates from three separate whole rat brains. The tyrosine value of 19.51 ± 0.93 $\mu$g $g^{-1}$ wet weight is consistent with previous reports for whole brain concentrations in rat[8,9]. Table II shows the mean tyrosine content of four whole rat

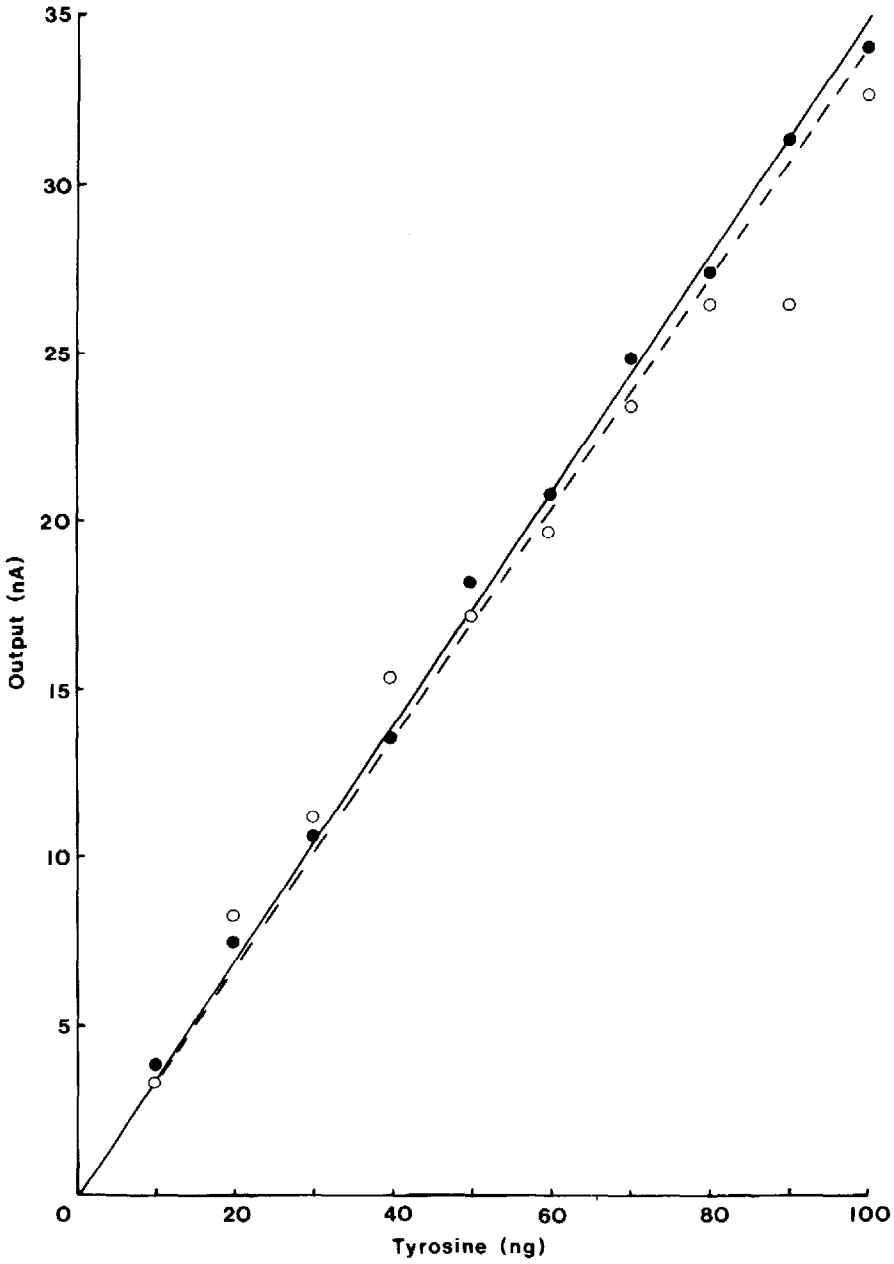


Fig. 3. Detector output plotted against amount of tyrosine injected for LC–ElCD estimation of both untreated standards (●—●) and acidified Tris buffer extracts of standards (○– – –○), the latter having been subjected to the alumina extraction procedure and corrected for dilution. Points represent means of duplicate estimations.

TABLE I

CONCENTRATIONS OF TYROSINE, CATECHOLAMINES AND METABOLITES IN RAT WHOLE BRAIN

Values represent means ± S.E. for groups of six replicate estimations from each of three individual brains.

| *Compound* | *Concentration ($\mu g\ g^{-1}$ wet tissue)* |
|---|---|
| Tyrosine | 19.51 ± 0.93 |
| DA | 1.024 ± 0.024 |
| NA | 0.361 ± 0.010 |
| HVA | 0.065 ± 0.004 |
| DOPAC | 0.110 ± 0.010 |

TABLE II

CONCENTRATIONS OF TYROSINE IN RAT WHOLE BRAIN AND SMALL BRAIN REGIONS

Values represent means ± S.E. for estimations from each of four individual whole brains and a further four dissected brains.

| *Tissue* | *Tyrosine concentration ($\mu g\ g^{-1}$ wet tissue)* |
|---|---|
| Whole brain | 15.42 ± 0.38 |
| Olfactory bulb | 24.00 ± 2.10 |
| Hypothalamus | 21.56 ± 1.73 |
| Hippocampus | 20.35 ± 1.34 |
| Striatum | 19.40 ± 1.38 |

brains, as compared to contemporary values for small regions of a further four brains. Since the assay requires only 50 $\mu$l of a 1:14 brain homogenate, concurrent estimation of catecholamines and tyrosine may be accomplished in as little as 4 mg of tissue.

In summary, the simple modification of existing techniques described allows tyrosine to be assayed by LC–ElCD in the same samples of brain homogenates as catecholamines. Rapid elution of tyrosine allows a high rate of sample turnover, and sensitivity is sufficient to allow estimation in small brain regions.

ACKNOWLEDGEMENT

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REFERENCES

1 I. N. Mefford, *J. Neurosci. Methods*, 3 (1981) 207; and references cited therein.
2 K. Oka, G. Ashiba, B. Kiss and T. Nagatsu, *Neurochem. Internat.*, 4 (1982) 375.
3 R. B. Holman, P. Angwin and J. D. Barchas, *Neuroscience*, 1 (1976) 147.
4 A. H. Anton and D. F. Sayre, *J. Pharmacol. Exp. Ther.*, 138 (1962) 60.
5 R. Keller, A. Oke, I. Mefford and R. N. Adams, *Life Sci.*, 19 (1976) 995.
6 I. N. Mefford, M. Gilberg and J. D. Barchas, *Anal. Biochem.*, 104 (1980) 469.
7 R. C. White and T. E. Nelson, *J. Chromatogr.*, 176 (1979) 430.
8 A. A.-B. Badawy, D. L. Williams, B. M. Snape and M. Evans, *Biochem. J.*, 204 (1982) 622.
9 A. Carlsson, *Ciba Found. Symp.*, 22 (1974) 117.