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## Note

# Simplified liquid chromatographic—electrochemical determination of norepinephrine and dopamine in rat brain

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Recently developed liquid chromatographic—electrochemical (LC—EC) methods [1—5] for the determination of norepinephrine (NE) and/or dopamine (DA) in brain tissue have provided advantages in speed, sensitivity, and cost. However, all of the methods still employ a preliminary purification step. We have found that the compounds can be determined in rat brain by the direct injection of the supernatant obtained after sonication and centrifugation of the tissue.

## EXPERIMENTAL

### *Materials*

The LC—EC system consisted of an Altex 110A pump, a Rheodyne 70-10 injection valve, and a stainless steel 500 × 1.0 mm column dry-packed with pellicular Vydac SC cation-exchange resin (Rainin Instrument Co., Brighton, Mass., U.S.A.). A Model LC-4 electrochemical controller was used with a CP-S carbon paste electrode (Bioanalytical Systems, West Lafayette, Ind., U.S.A.). The potential was set at +0.5 V with respect to a Ag/AgCl reference electrode. A citrate—acetate buffer solvent system [2] was delivered at a flow-rate of 1.0 ml/min.

### *Method*

Weighed whole rat brains (1—2 g) were placed in polycarbonate centrifuge tubes containing 4.0 ml of 0.1 *M* $HClO_4$ (with 400 $\mu$l of 1 *M* $NaHSO_3$ per liter). After the addition of 500 ng of dihydroxybenzylamine (DHBA) (5.0 $\mu$l of 10.0 mg DHBA per 100 ml of 0.1 *M* $HClO_4$), the brain was sonicated at a medium setting for two 30-sec periods using a Branson Polytron sonicator (Branson Sonic Power Co., Danbury, Conn., U.S.A.). After adding 0.5 ml of 3.4 *M* $HClO_4$ and vortex mixing, the samples were centrifuged at 10,000 *g* for 10 min

and a portion of the supernatant stored in a small polyethylene tube. The catecholamines (NE and DA) were determined by injecting 20 $\mu$l of the supernatant into the LC—EC system. The NE and DA peak heights were ratioed to the DHBA peak height, and the concentrations (ng/g brain) calculated knowing the relative response of the standards, the amount of DHBA added, and the brain weight. When determining NE and DA in brain punches and areas [6] weighing 2—10 mg each, the tissue was sonicated in 200 $\mu$l of 0.1 *M* $HClO_4$ after the addition of 10 ng of DHBA, and then centrifuged and determined as above.

## RESULTS AND DISCUSSION

A chromatogram of catecholamine standards and two different rat brain samples is shown in Fig. 1. Up to 36 samples can be easily analyzed in 8 h. The standards and samples were determined with typical coefficients of variation (C.V.) of less than 5% and with absolute detection limits of ca. 10—20 pg. The internal standard (DHBA) was well recovered [74.9 ± 12% (mean ± S.D.), $n$ = 110] from whole brains. Mean recoveries of DHBA from particular brain areas (whole areas and punches) ranged from 84 to 99% with C.V. values of 4—9%. Split samples which were analyzed using both this method and a procedure with an alumina absorption step [2] showed excellent agreement ($r > 0.99$). The low oxidation potential and the cation-exchange resin combined to give sufficient selectivity for injection of the unpurified and unconcentrated sonicate. When compared to a recent reversed-phase LC—EC method [1] a saving in time in the sample preparation and chromatography steps is apparent. We are

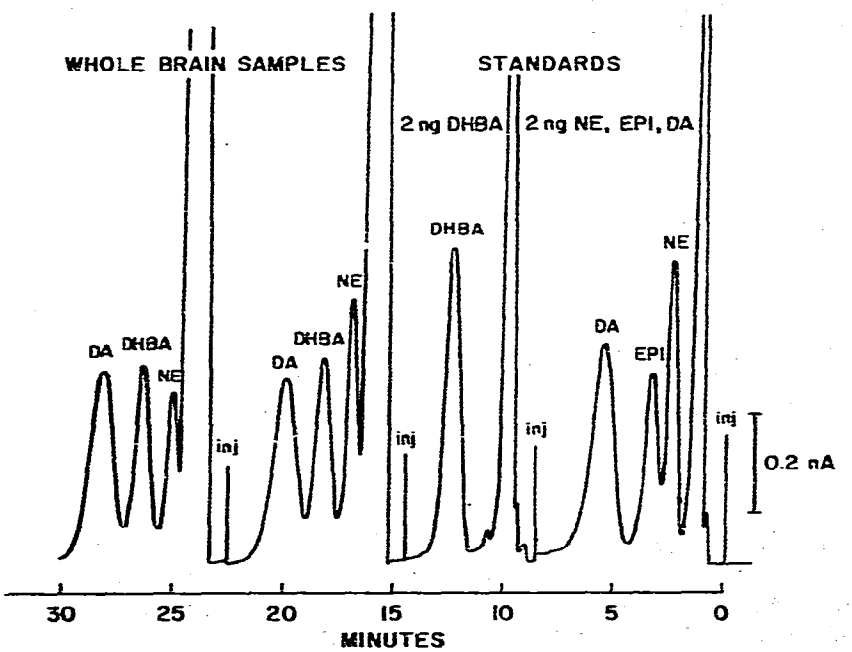


Fig. 1. Chromatogram of catecholamine standards and two different rat brain samples ( ca. 1.4 g), all run at 2 nA full scale. Dihydroxybenzylamine (DHBA) was added to the brains as an internal standard (500 ng/brain). DA, dopamine; NE, norepinephrine; EPI, epinephrine.

presently developing methods involving the direct injection of the same supernatant into a reversed-phase LC—EC/fluorimetric system [7] in order to determine a variety of indolic and catechol metabolites.

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