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Note

Simplified determination of the brain catecholamines norepinephrine, 5-hydroxyindoleacetic acid, dopamine and 5-hydroxytryptamine by high-performance liquid chromatography using electrochemical detection

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A variety of methods for the extraction of tissue catecholamines and their biogenic metabolites have been described for high-performance liquid chromatography (HPLC) using electrochemical detection (ED). For the rapid analyses of multiple samples it is necessary to combine an easy, yet accurate, dependable preparation with an inexpensive means of production and reliable reproducibility. We have successfully utilized the described procedure for the extraction and quantitation of brain tissue catecholamines in hamsters, rats and mice. Several methods have been reported which allow for the direct injection of the supernatant obtained after sonication and centrifugation of the tissue sample [1—5], but in general these tend to require immediate chromatographic processing and vary greatly in their degrees of sensitivity. The present technique allows for storage of dried samples until a time when it is convenient

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for chromatographic analyses. Sensitivity of detection under these conditions has been found to be in the order of 15 pg for norepinephrine (NE) and variably higher for the other compounds listed. Accurate calculation of NE, 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA) and 5-hydroxytryptamine (5-HT) tissue content is determined by ratio comparison with dihydroxybenzylamine (DHBA) as internal standard. This ratio value is then calculated into the corrected tissue standard curve (see Fig. 2) for derivatization of tissue content. Other compounds of potential interest which may be quantitated by this technique are 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), epinephrine (E) and L-tryptophan.

EXPERIMENTAL

Apparatus

The chromatograph consisted of a Model M6000A solvent delivery system, a Model U6K injector, a pre-column using Bondapak C_{18}/Corasil (Waters Assoc., Milford, MA, U.S.A.) and an Ultrasphere-ODS 5-μm, 15.0 cm × 4.6 mm (Beckman, Fullerton, CA, U.S.A.) reversed-phase column. Electrochemical detection incorporated a TL-5 glassy carbon electrode and LC-4 amperometric controller from Bioanalytical Systems (West Lafayette, IN, U.S.A.). The detector potential was maintained at 0.65 V vs. an Ag/AgCl reference electrode. A linear-strip recorder was used in parallel with a 3390 A integrator (Hewlett-Packard, Avondale, PA, U.S.A.).

Chromatography

The isocratic buffer made in deionized distilled water consisted of 10 m*M* sodium octanesulfonate, 100 m*M* potassium phosphate, 87 m*M* acetic acid, 1 m*M* EDTA (free acid), and was determined to be optimal at pH 4.0. Following filtration through an HA 0.45-μm filter (Millipore, Bedford, MA, U.S.A.), acetonitrile (FH 0.5-μm filter) was added to a final volume concentration of 7%. This mixture was degassed under vacuum while magnetically stirred, then enclosed and maintained by the slow bubbling of helium gas.

Sample preparation

Brain samples were dissected from rats (Sprague–Dawley) or hamsters (Golden Syrian) following decapitation. Each median eminence (ME) (weighing 1.0 ± 0.2 mg) was removed and placed into cold, 500-μl polypropylene tubes (No. 2072, West Coast Scientific, Berkeley, CA, U.S.A.) containing 5 ng DHBA per 100 μl of 70% ethanol. The preoptic region (POA) and medial basal hypothalamus (MBH) were dissected out, weighed and placed individually into cold tubes with 5 ng DHBA per 100 μl of 70% ethanol per 10 mg tissue. These were sonicated for 30–45 s using a micro-ultrasonic cell disrupter (Kontes, Vineland, NJ, U.S.A.) and placed in −25°C for 48 h. Following brief vortexing, samples were centrifuged at −4°C for 20 min at 12 000 *g*. The supernatant was transferred to new tubes and evaporated in the dark by a gentle stream of dry nitrogen gas. Dried samples could then be stored at −25°C until convenient for HPLC–ED. Before reading, samples were taken up in 0.2 *M* formic acid (50 μl for ME; 100 μl for MBH and POA), vortexed and covered over ice for a

minimum of 20 min before chromatographic analysis. For ME samples, 10–20 μl out of 50 μl of this eluent were injected onto the column and 10 μl out of 100 μl for the MBH and POA samples.

Standards

Stock NE, 5-HIAA, DA, 5-HT and DHBA (Sigma) were dissolved at a concentration of 1 mg/ml and stored (for a maximum time of two months) in 70% ethanol. These standards were diluted with 0.2 *M* formic acid to give working standards prepared fresh daily. Of diluted samples 10 μl were injected onto the column to derive 750, 500 and 250 pg of each in mixture (see Fig. 1A and B).

RESULTS AND DISCUSSION

An example of a standard mixture and two chromatograms from the hamster hypothalamic samples are shown in Fig. 1. Samples were overlapped between the elution of dopamine and serotonin to accommodate the injection of another sample in that time-frame. Standard curves were used to calculate actual tissue content derived from the ratio of the catechol with the internal standard (Fig. 2). Similar curves were produced for the rat. Pooled tissue homogenate was distributed in aliquots (equal to the mean individual tissue

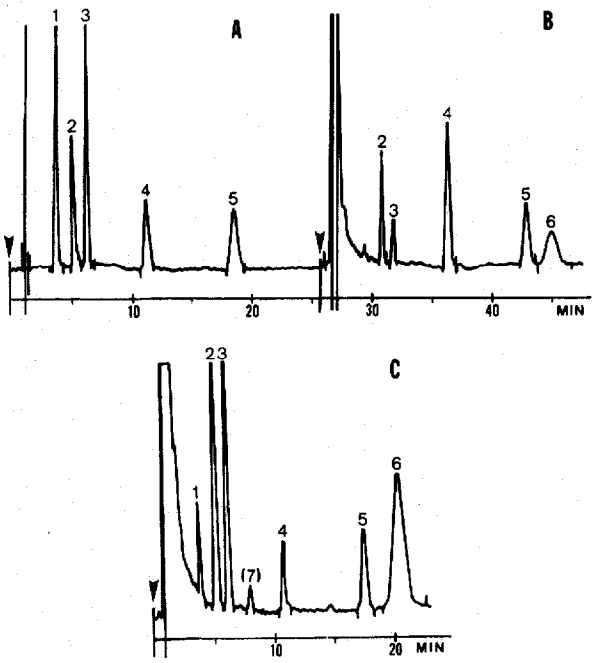

Fig. 1. Integrated HPLC–ED profiles of (A) 500 pg of each catecholamine standard (in 5 μl of 0.2 *M* formic acid); (B) a hamster median eminence sample (10 μl injected out of 100 μl eluent); (C) a hamster hypothalamic sample (5 μl injected out of 100 μl eluent) shown with the serotonin (5-HT) from the previously injected hypothalamic sample. All runs were made at 1 nA full scale and 0.3 cm/min chart speed. See text for other conditions. For peak identificaton and retention times, see Table I.

TABLE I

RETENTION TIMES OF THE CATECHOLAMINES INVESTIGATED

Peak No.	Compound	Retention time (min)
1	3,4-Dihydroxyphenylacetic acid	3.9
2	Norepinephrine	5.3
3	5-Hydroxyindoleacetic acid	6.4
4	Dihydroxybenzylamine	11.1
5	Dopamine	18.1
6	5-Hydroxytryptamine	44
(7)	Epinephrine	8.1

mass) into vials containing known amounts of standard amines in 70% ethanol. These were processed as described.

Present studies are concerned with the comparison of regional brain catecholamine content as well as turnover rates in hamsters exposed to varying

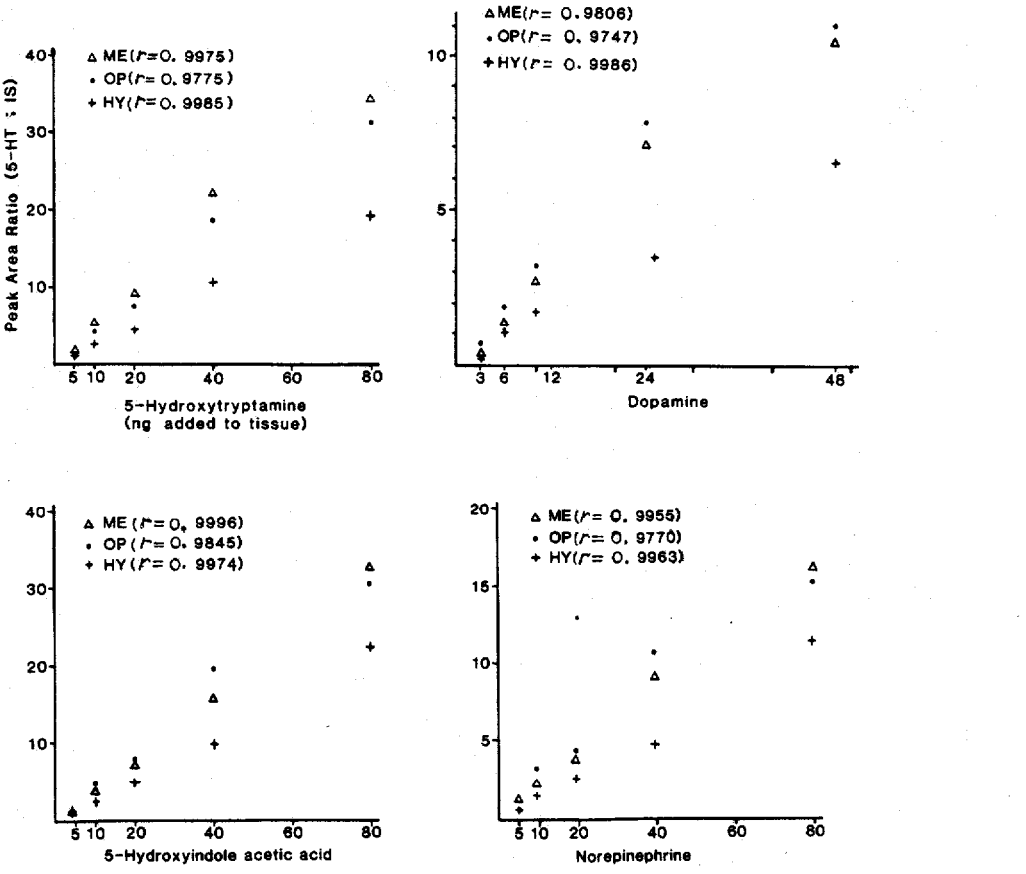

Fig. 2. Tissue standard curves for primary compounds of interest added in this study (see text) using median eminence (△), preoptic area (•) and hypothalamus (+) for hamster. Each point represents the added amount quantitated minus the endogenous amount present in the tissue. Correlation coefficients (r) are given for each curve.

photoperiods. Correlations of gonadotropin and prolactin secretion and other reproductive effects will be reported elsewhere.

Dried samples have been stored at −25°C for up to two months without significant change or variance from identical samples run within two days after drying. Since a great deal of time and solvent is required for column priming and equilibrium, it is beneficial and convenient to accumulate a number of samples prior to chromatographic analysis. Parameters of solvent molarity, pH, organic modifier, ion-pairing reagent and column temperature were extensively investigated to validate this present report. This procedure has demonstrated an effective means of analyzing large numbers of samples in a relatively short time and with minimal sample preparation.

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