Evaluation of a Radioenzymic Kit for Determination of Plasma Catecholamines

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We evaluated a commercially available reagent test-kit (Upjohn) for simultaneously determining norepinephrine, epinephrine, and dopamine in 50-μL of plasma. The three catecholamines are enzymically converted into the radioactive O-methyl derivatives and separated by thin-layer chromatography. Day-to-day precision (CV) was 11, 10, and 14% for norepinephrine, epinephrine, and dopamine, respectively. The relationship between concentration of catecholamine and radioactivity (net dpm) was linear to at least 8 ng (corresponding to 1 μmol/L in plasma). Sensitivity was approximately 2 pg for dopamine, 1 pg for each of the other two catecholamines. Under our conditions, epinephrine was not quite completely resolved from the other two fractions. Catecholamine values determined in normal humans, after 30 min supine and after normal laboratory activity, agreed well with those found by other investigators. Correlation was good between kit results and those obtained in another laboratory that used self-prepared reagents and "high-performance" liquid chromatography for the separation.

Additional Keyphrases: norepinephrine, epinephrine, and dopamine • reference intervals

A rapid, reliable method for determining plasma catecholamines has long been needed. In recent years, sensitive isotope-dilution (1, 2) and radioenzymic methods (3–7) have been developed, but their complexity has prevented widespread use. We have evaluated a commercially available kit ("Cat-A-Kit"; Upjohn Diagnostics, The Upjohn Co., Kalamazoo, MI 49001) for determination of NE, E, and DA; the procedure is based on the radioenzymic method of Passon and Peuler (3).1

Materials and Methods

Specimen Collection and Handling

Five milliliters of blood was drawn directly into evacuated tubes (Upjohn Diagnostics) containing EGTA and glutathione. The tube was immediately cooled in ice and centrifuged within 15 min after collection, in a refrigerated centrifuge. Plasma was promptly separated and stored at −25 °C until the determination was done, which was always within three months of the blood collection.

1 Nonstandard abbreviations used: NE, norepinephrine; E, epinephrine; DA, dopamine; EGTA, ethylene glycol bis(β-aminoethyl ether)N,N'·tetraacetic acid; MT, methoxytryramine; MN, metanephrine; NMN, normetanephrine; and dpm, disintegrations per minute.

Procedure

The method is applied, in duplicate, to a blank and two identical plasma samples, one of which has had known amounts of NE, E, and DA added as internal standards.

Incubate 50 μL of sample with 50 μL of a reagent mixture containing tromethamine buffer, glutathione, S-adenosyl-L-[methyl-3H]methionine, catechol-O-methyltransferase (EC 2.1.1.6), EGTA, and MgCl2, to convert NE, E, and DA to their corresponding [3H]-3-methoxy derivatives. Add a stopping solution containing borate buffer and non-radioactive MN, NMN, and MT as carriers for the [3H]-labeled reaction products and extract these three compounds with a mixture of toluene and isomyl alcohol (3/2 by vol). Separate the layers in the extraction steps by freezing, and isolate and thaw the aqueous layers. Finally, extract the three products into 100 μL of dilute acetic acid and separate them by thin-layer chromatography. Outline the zones of NMN, MN, and MT under ultraviolet light, isolate them, and place them into separate scintillation vials. Elute [3H]MT from the plate material, extract it into the scintillator, and count the radioactivity. Elute [3H]NMN and [3H]MN and convert these by oxidation with periodate to [3H]vanillin, and extract this into scintillation fluid.

Apparatus and Reagents

The kit contains an extensive instruction manual, with much useful advice on technique. Necessary chemicals for the enzymic conversion are enclosed in frozen solutions, as well as a concentrated combined-standard solution and a control plasma of known NE, E, and DA content. The recommended thin-layer chromatographic plates and thin-layer chromatographic solvent are not included. We counted radioactivity in a Packard Tricarb 2650 liquid scintillation counter.

We followed the supplier's instructions, given with the kit, except for the following:

(a) We used DC Plastifolien Kieselgel 60F254 (Merck, Darmstadt, Germany) instead of the recommended Analtech glass thin-layer plates. Although this change resulted in a longer chromatographic run (180 min), the Plastifolien gave good visual separation and simplified isolation of the fractions by cutting, a safer procedure than scraping, because the amount of radioactivity used is rather high: 5 μCi per incubation.

(b) We used a 10-channel "Multispotter" (Analytical Instrument Specialties, Libertyville, IL 60048) to apply the material to be chromatographed. This instrument was modified to apply material to the plates in 8-mm bands instead of in spots, and outfitted with 250-μL syringes (Hamilton Co., Reno, NV 89510).

(c) For internal standardization we added 500 pg each of NE, E, and DA instead of 100-pg amounts. To dilute the concentrated kit stock standard, we used distilled water, which was first deoxygenated by bubbling pure nitrogen through it,
to forestall any oxidation of the catecholamines. Reproducibility was thus improved.

Results and Discussion

Precision

Catecholamine concentrations in the control plasma supplied with the kit were determined in duplicate 17 times, at twice-weekly intervals. The mean values were 5.7, 3.7, and 5.0 nmol/L, with CV's of 10, 11, and 14% for NE, E, and DA, respectively. The supplier's specifications for this plasma were 5.2, 3.6, and 4.4 nmol/L with CV's of 14, 17, and 18% for NE; E, and DA, respectively.

The same procedure, carried out on a locally prepared pooled plasma with about the same catecholamine concentrations as the kit plasma, showed similar CV values.

The overall CV includes the deviations in three components of the determination: blank, sample, and sample with internal standard. Table 1 shows the within-run precision of each, expressed as count rate. The relatively high CV in the blanks, much lower CV in samples plus internal standard, and still lower CV in the samples were consistently observed. Count rates for the (same) sample and sample with internal standard showed substantial day-to-day variation.

Linearity and Sensitivity

Various amounts of NE, E, and DA were added to a normal plasma and these catecholamines determined. Figure 1 shows the relationship between the concentration of catecholamine and the corresponding disintegrations per minute (dpm) to be linear up to at least 8 ng (corresponding to 1 μmol/L in plasma). The differences in radioactivity of each fraction are possibly attributable to differences in methylation efficiency, extraction efficiency, or both.

The sensitivity of the assay is about 1 pg each for NE and E and 2 pg for DA, corresponding to 0.1 and 0.25 nmol/L, respectively, in plasma. Taking into consideration the blank CV, we chose the limit of detection as twice the blank count rate.

Cross Contamination

We investigated resolution of the three catecholamine-derivative fractions on the chromatographic plate by applying the method to 500-pg amounts of each single catecholamine (epinephrine, cat. no. E-4250, Sigma Chemical Co., St. Louis, MO 63178, specified 97–100% pure; norepinephrine, Nogepha BV, Herculesstraat 28, Alkmaar, The Netherlands, 98–100%; dopamine, E. Merck Nederland BV, 1005 AD Amsterdam, The Netherlands, 98%). The respective percentages of one fraction present in the zone of another (based on the total radioactivity found in the three zones) were: E in NE and DA, 5 and 4%; NE in E and DA, both 1.5%; DA in E and NE, 0.6 and 0.1%. These values are higher than those quoted in the kit manual, except for DA in NE, where the values are similar. The differences may be due to the use of other plates for thin-layer chromatography than those recommended, or to the presence of other methylated products, or both. We conclude that, in cases in which one or more of the catecholamines is strongly increased above the normal, a correction for cross contamination is necessary.

Reference Intervals

Mean concentrations (nmol/L, and SEM) of these analytes found in the plasma of ostensibly healthy laboratory staff members after 30 min in the supine position were as follows (n = 12 for NE and E, n = 8 for DA): E, 0.22 (0.07); NE, 1.13 (0.07); DA, 0.14 (0.05). After average laboratory work for at least an hour, these values were: E, 0.33 (0.09); NE, 2.80 (0.02); and DA, 0.18 (0.05). All E and NE values were lower during rest than after activity; DA values did not differ significantly.

These results agree well with those recorded in literature (2, 6–8), determined by double-isotope derivative, single-isotope radioenzymic assay, and liquid-chromatographic techniques.

Intermethod Comparison

We collected 13 samples from patients, divided each of them into two portions, and assayed them in our laboratory by the

Table 1. Day-to-Day Variation and Within-Run Precision of Count Rate (dpm above Background)

<table>
<thead>
<tr>
<th>Day-to-day variation</th>
<th>Within-run precision</th>
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<tbody>
<tr>
<td>Mean dpm</td>
<td>SD</td>
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<tr>
<td>NE</td>
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<tr>
<td>E</td>
<td>50</td>
</tr>
<tr>
<td>DA</td>
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Sample

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<td>390</td>
<td>25</td>
<td>37</td>
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</tr>
<tr>
<td>DA</td>
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<td>440</td>
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Sample plus int. std, 500 pg

<table>
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<tr>
<th>NE</th>
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<th>2850</th>
<th>16</th>
<th>1480</th>
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<tbody>
<tr>
<td>E</td>
<td>23 120</td>
<td>3100</td>
<td>13</td>
<td>2180</td>
<td>9</td>
</tr>
<tr>
<td>DA</td>
<td>34 710</td>
<td>4400</td>
<td>13</td>
<td>2770</td>
<td>8</td>
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</tbody>
</table>

* Pooled serum containing about NE 6, E 4, and DA 4 nmol/L.
kit method and also in an outside laboratory (for NE and E only) with use of self-prepared reagents for the radioenzymic reaction and extraction and "high-performance" liquid chromatography for the separation of the fractions (9). The resulting values (Figure 2) showed an abnormal distribution, which precluded the use of linear correlation statistics. Calculation of the Spearman rank-correlation coefficient resulted in values of 0.89 and 0.93 for NE and E, respectively, meaning that for both catecholamines the two sets of values are positively correlated at the $p = 0.01$ level (critical value for $n = 13$ is 0.70).

General Remarks

The procedure is not difficult to perform when all the recommended equipment is available. It is rather time-consuming, as, in our hands, a series of 10 plasma samples plus blanks and control plasma (all run in duplicate), together with the necessary preparations and calculations requires two days for one technologist (not including sample counting-time). Because the kit itself is also costly, the overall expense of performing this determination is high.

Careful attention to detail and careful avoidance of contamination were found to be essential for good results.

We conclude that this kit is useful for determining NE, E, and DA concentrations in a manner satisfactory for most studies. The precision, although not very good, is sufficient when taking into consideration the great individual and physiological variations in the concentrations.

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References