and NT were quantitated twice, once using TR as the internal standard and once with PT as the internal standard. Figure 2 shows the four calculated drug values after 30-min post-dryness. Calculated AT and NT values based on TR as an internal standard are very different from those calculated with PT. The AT values are more consistent (CV = 2.5% (TR as internal std.) vs 7.2% (PT as internal std.), n = 5), but the NT values decrease with time and are more variable than those calculated with NT/PT ratios. These findings indicate that TR adequately compensates for AT losses, but is less efficient than PT in compensating for NT losses. For assays in which AT and NT are measured simultaneously, it is thus important that both internal standards be used.

Although we did not test these experimental procedures with other tricyclic drugs (imipramine, desipramine, doxepin, and desmethyl doxepin), they include tertiary and secondary amines and can be expected to react similarly. For standard curves calculated with both TR and PT, the correlation coefficients (linearity) for the tertiary amines are much improved when TR is used as the internal standard, and conversely, correlation values for the secondary amines are better when PT is used for quantitation.

Drug losses during evaporation may be caused by adsorption onto glass surfaces, volatilization, or both (5–7). Regardless of the cause, however, these losses are minimized by using silanized glassware for evaporation, evaporating the solvent at 40°C, removing the drug residues promptly after the solvent has evaporated, and using two internal standards, a tertiary and a secondary amine, to compensate appropriately for the losses that do occur.

We thank the Hewlett-Packard Foundation for the liquid chromatograph used in these studies.

References

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Effect of Contraceptive Steroids on Creatine Kinase Activity in Serum

To the Editor:

Administration of steroids has been reported to decrease creatine kinase (CK; EC 2.7.3.2) activity in sera of patients suffering from various diseases (1). Recently, one of us reported a decrease in serum CK activity in healthy women of reproductive age taking a contraceptive steroid (Primoviar, Schering) for nine months (2). We continued the study, to investigate the effect of another contraceptive steroid preparation (Ovuilen, Searle) on serum CK.

CK activity was measured in sera of 10 healthy women of reproductive age three, six, and nine months after they started taking Ovuilen and in 20 healthy women of comparable age (control group), as described earlier (2). The data were analyzed statistically by Student's t-test.

There was no significant difference (p

Table 1. Serum CK Activity In Control Subjects and Women Taking Ovuilen

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Women taking Ovuilen</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK acty, kU/L</td>
<td>Range  Mean ± SD</td>
<td>Range  Mean ± SD</td>
</tr>
<tr>
<td>Control group</td>
<td>2.0–18.0</td>
<td>7.65 ± 3.17</td>
</tr>
<tr>
<td>Ovuilen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 mo</td>
<td>2.0–10.0</td>
<td>5.50 ± 2.38</td>
</tr>
<tr>
<td>6 mo</td>
<td>2.0–10.0</td>
<td>6.00 ± 2.36</td>
</tr>
<tr>
<td>9 mo</td>
<td>2.0–10.0</td>
<td>5.60 ± 2.56</td>
</tr>
</tbody>
</table>
>0.05) in the serum CK activity of control subjects and women taking Ovulen for three, six, and nine months (Table 1). All the individual values were within the normal range. We conclude that the serum CK-lowering effect of contraceptive steroids depends on the specific steroids involved. Both Primovlar and Ovulen are estrogen/progestogen combinations, but Primovlar contains 0.05 mg of ethinyl estradiol and 0.5 mg of norgestrel, and Ovulen contains 0.1 mg of mestranol and 1.0 mg of ethynodiol diacetate.

References

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Dopamine β-Hydroxylase Activity in Human Cerebrospinal Fluid from Various Age Groups

To the Editor:

Dopamine β-hydroxylase (DBH, EC 1.14.17.1) is present not only in serum but also in cerebrospinal fluid (CSF) of humans (1–3) and rabbits (4). DBH in serum originates from the sympathetic nerves, whereas that in CSF may be derived from the noradrenergic neurons in the brain (5). Therefore, DBH activity in CSF may be an index of central noradrenergic activity and may be useful for diagnosis of central nervous system diseases—such as Parkinson’s disease, in which DBH activity in the brain is decreased (6).

We reported the DBH activity in human CSF of adult normal subjects (3) but no study has been made on normal values for DBH in CSF from subjects of various age groups. We have applied the statistical method of Hoffmann (7) to the computation of normal values for DBH activity in CSF of various age groups. The principle of the method (7) is computer generation of a normal value, by repeated omitting of measured values outside the mean ± 2.2 SD range until a constant mean and SD values are obtained. We had already used the method of Hoffmann to report normal values for human serum DBH (8).

CSF was obtained from patients by lumbar puncture at Fujita-Gakuen University School of Medicine Hospitals. The patients were undergoing surgery under lumbar anesthesia. No patient suffering from central or peripheral neurological disease was included, and the general physical and nutritional states of the patients were normal. The first 5 mL of CSF was removed for chemical and cytological examination, and the next 5 mL was used for the assay of DBH activity. The samples of CSF were all clear, and no erythrocytes were detected.

DBH activity was determined according to the method of Fujita et al. (8) by "high-performance" liquid chromatography with fluorescence detection and o-phthalaldehyde as reagent.

Under our assay conditions the effect of endogenous inhibitors in CSF could be completely removed by the presence of both N-ethylmaleimide and Cu²⁺, as judged by nearly 100% recovery of the activity of pure DBH from bovine adrenal medulla added to CSF samples.

DBH activity in CSF was less stable than that in serum. Unless the assay was done within 2 h after the CSF samples were collected, the CSF was frozen at 80 °C and measured within a week. DBH activity in CSF was stable for at least three months at 80 °C.

The rate of octopamine formation from tyramine, with human CSF as the enzyme source, proceeded linearly for 60 min; complete linearity was observed between the volumes of human CSF used (100 to 500 μL) and the quantities of octopamine produced.

The mean DBH activity of human CSF samples before and after the Hoffmann computation (7), from subjects of ages from 6 to 77 years, agrees well with the previously reported DBH activity (3). The mean (±SD) DBH activity after the Hoffmann computation was 0.146 ± 0.094 μmol/min per liter of CSF (range 0.098–0.379; n = 114). The activity was similar between males and females.

As shown in Table 1, the DBH activity was nearly constant in human CSF from 10 to 50 years of age. After age 50, the activity started to decrease, especially after age 70. Although only one case was available, the activity in CSF from a boy six years old showed a lower activity than the adult mean activity.

This is the first report on the normal value for DBH activity of CSF for various age groups. DBH activity in CSF appears to be constant during age 10–50 years, but it may decrease in older ages, especially above 70 years. The computer-generated normal value for DBH activity in human CSF may be a suitable

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Table 1. Dopamine β-Hydroxylase (DBH) Activity in Normal Subjects of Various Age Groups without or with the Statistical Computation of Hoffmann (7)

<table>
<thead>
<tr>
<th>Age, years</th>
<th>No. subjects</th>
<th>DBH acyt, μmol/min per liter of CSF</th>
<th>Mean ± SD</th>
<th>Age, years</th>
<th>No. subjects</th>
<th>DBH acyt, μmol/min per liter of CSF</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>1</td>
<td>—</td>
<td>0.075</td>
<td>6</td>
<td>1</td>
<td>—</td>
<td>0.075</td>
</tr>
<tr>
<td>10–19</td>
<td>7</td>
<td>0.051–0.913</td>
<td>0.270 ± 0.293</td>
<td>6</td>
<td>13</td>
<td>0.051–0.241</td>
<td>0.163 ± 0.082</td>
</tr>
<tr>
<td>20–29</td>
<td>32</td>
<td>0.009–0.349</td>
<td>0.147 ± 0.099</td>
<td>20–29</td>
<td>32</td>
<td>0.009–0.349</td>
<td>0.147 ± 0.099</td>
</tr>
<tr>
<td>30–39</td>
<td>23</td>
<td>0.018–0.525</td>
<td>0.163 ± 0.106</td>
<td>30–39</td>
<td>23</td>
<td>0.018–0.525</td>
<td>0.163 ± 0.106</td>
</tr>
<tr>
<td>40–49</td>
<td>23</td>
<td>0.018–0.947</td>
<td>0.213 ± 0.204</td>
<td>40–49</td>
<td>21</td>
<td>0.018–0.327</td>
<td>0.159 ± 0.088</td>
</tr>
<tr>
<td>50–59</td>
<td>12</td>
<td>0.048–0.758</td>
<td>0.270 ± 0.273</td>
<td>50–59</td>
<td>9</td>
<td>0.048–0.379</td>
<td>0.129 ± 0.102*</td>
</tr>
<tr>
<td>60–69</td>
<td>15</td>
<td>0.048–0.267</td>
<td>0.136 ± 0.072*</td>
<td>60–69</td>
<td>15</td>
<td>0.048–0.267</td>
<td>0.136 ± 0.072*</td>
</tr>
<tr>
<td>70–77</td>
<td>7</td>
<td>0.009–0.285</td>
<td>0.101 ± 0.099*</td>
<td>70–77</td>
<td>7</td>
<td>0.009–0.285</td>
<td>0.101 ± 0.099*</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>0.009–0.947</td>
<td>0.176 ± 0.165</td>
<td>120</td>
<td>114</td>
<td>0.009–0.379</td>
<td>0.146 ± 0.094</td>
</tr>
</tbody>
</table>

* Differs significantly from the value during 40–49 years, p < 0.01.

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