enzymes, which may be membrane bound, in the receptor preparation, and in this way may be causing receptor binding rather than having intrinsic binding activity.

In several studies (1, 10), values for αANP in the circulation as measured by RRA and RIA have correlated well. We have not systematically studied the correlation between results obtained with our RRA and RIA. However, the observed correlation of the active forms detected by these assays leads us to expect that there would be a good relation. In some small-scale studies we have measured pooled plasma samples obtained from a normal person and a hypertensive individual after solid-phase extraction and found respective values of 7 ng/L (2.1 pmol/L) and 30 ng/L (10.4 pmol/L) by RRA and 6.5 ng/L (2.1 pmol/L) and 32 ng/L (10.4 pmol/L) for RIA.

In summary, we compared RRA and RIA for their specificity of measurement of αANP and found that the results by both methods correlated well. Our results confirm previous reports on the specificity of bovine adrenal receptor preparations and also show that our RIA is specific for the active form of αANP—a unique characteristic of our antiserum and an advantage over other RIAs. Both the RRA and the RIA can be applied to the accurate quantification of circulating αANP.

The RRA provides a rapid test that is by definition specific for active αANP. It is somewhat less sensitive than the RIA, but it may be particularly useful for rapid screening for high αANP values, because the whole assay can be done in 3 h. For example, above-normal values found in congestive heart failure can be measured quickly and accurately with this assay. The RIA is a more sensitive and precise assay, although it takes longer, and it may be particularly useful in measuring normal or near-normal values where slight differences are being looked for. It is of course at least as specific as the RIA for active αANP (1-28), and probably more so.

References

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Improved Method of Analysis for Aluminum in Brain Tissue

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To improve the accuracy and precision of the assay of aluminum in brain tissue, we modified for application to brain samples from rats and humans the wet-digestion method of Trapp et al. (Biol Psychiatry 1978;13:709–18), established the contribution of contamination, and examined the effect of precipitation of nonoxidizable fatty residues on the analysis. Specifications of the modified assay are a detection limit of 5 ng of aluminum per gram wet weight of brain tissue, a within-day CV of 4.8% (24.3 µg/L; n = 10), and a day-to-day CV of 5.5% (27.8 µg/L; n = 5). Contamination, a systematic error in the analysis of aluminum, was established to be 13 ng (SD = 7.9 ng; n = 8) per tube. The presence of indigestible fatty residues did not affect the accuracy of the method. Application of the method to brain hemispheres of nonexposed rats revealed an aluminum content of 0.041 mg/kg wet weight of tissue (SD = 0.032 mg/kg; n = 8). The aluminum content in human cortex samples, consisting of gray and white matter, ranged from 0.14 to 0.22 mg/kg. Modification of the wet-digestion method resulted in a reliable, simple sample pretreatment before analysis for aluminum in brain tissue. The extent of the aluminum contamination must be controlled by including appropriate blanks.

Additional Keyphrases: trace elements, sample handling, rats, tissue analysis

It is no longer disputed that aluminum is the cause of several dialysis-related diseases: microcytic anemia, vitamin-D resistant osteomalacia, and dialysis encephalopathy

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A role of aluminum in the pathogenesis of some neurological disorders—Alzheimer’s disease, amyotrophic lateral sclerosis, and Parkinsonian dementia—is suspected but not yet established (4–7).

Although some patients with Alzheimer’s disease show an increased aluminum content in the brain, it is not yet clear whether this increase is related to the neurological defect (8–12) or simply to the age of the patients (13, 14). Increased aluminum contents were also found in the brains of indigenous people of the Pacific island Guam and the Kii Peninsula (Japan) with amyotrophic lateral sclerosis and (or) Parkinsonian dementia (15–17). Chronic nutritional deficiencies of calcium and magnesium and relative excesses of aluminum may produce aberrations in mineral metabolism, resulting in abnormal deposition of aluminum in the central nervous system (15, 16).

There is, therefore, a need for measuring the accumulation of aluminum in brain. Even under pathological conditions, its concentration in tissue is low, so a precise, sensitive, and accurate analytical method is a requisite for clinical research in aluminum neurotoxicity.

An important step in the analytical procedure is the pretreatment of the sample before aluminum can be measured. Digestion of the tissue by heating with strongly oxidizing agents such as nitric acid is a simple and frequently used method of sample preparation. During the past 10 years several wet-digestion methods have been published as sample treatments before analysis for aluminum in brain tissue (8, 18–21). However, in most of them the analytical specifications were poorly defined: although they corrected for contamination by the inclusion of blanks, in none of these procedures were quantitative data given on the actual contribution of contamination to the analytical results.

In the present study, we improved the accuracy of aluminum measurement in brain tissue by modifying the method described by Trapp et al. (8), and evaluating various analytical variables. The contribution of the contamination was also examined and quantified. We then applied the method to samples of brain tissue from rats and humans.

Materials and Methods

Sample collection and storage. Samples were obtained from female Wistar rats, body weight 200–230 g. The animals were killed, and the total brain was removed and stored in screw-capped, disposable polypropylene tubes (13 mL, 101 × 16 mm, no. 60.541; Sarstedt, Nümbrecht, F.R.G.) at −20 °C to be analyzed later. Separate hemispheres were used for methodological development and application of the method.

Samples of brain cortex from six patients who had died from various causes were provided by the Department of Pathology of this hospital. The samples, consisting of grey and white matter, were stored in 100 mL/L formaldehyde solution (aluminum concentration 7 μg/L) before being analyzed.

Wet-digestion method. A 0.6- to 0.7-g sample of brain tissue (i.e., one hemisphere of a rat brain) was allowed to stand overnight at 40 °C in a screw-capped, disposable polypropylene tube with 0.8 mL of concentrated nitric acid (Suprapur no. 441; Merck, Darmstadt, F.R.G.) and 0.2 mL of concentrated sulfuric acid (Suprapur no. 741; Merck). The caps were pierced with a needle to allow nitric vapors to escape. The next morning the tissue had partly disintegrated, and the temperature was increased to 70 °C. Within 2 h the digestion of the tissue was essentially completed except for some fatty residues floating on top of the digest. Increasing the temperature again, to 105 °C, resulted in a clear yellow solution within 1 h. Finally, the digest was diluted to 3.0 mL with de-ionized water that had been further purified by reverse osmosis (Millipore Corp., Bedford, MA); water thus treated was used throughout. By diluting, the digestion mixture was abruptly cooled, and small residues of fat precipitated onto the inner wall of the tube.

Instrument settings. Aluminum in the digest was measured by electrothermal atomic absorption spectrophotometry (Model 3030; Perkin-Elmer Corp., Norwalk, CT) with Zeeman background correction. Pyrolytically coated graphite tubes were used. A hollow-cathode lamp was operated at 15 mA. Atomic absorption was measured at 309.3 nm, with a spectral band width of 0.7 nm. We used a Perkin-Elmer AS-60 autosampler to inject 20-μL samples. A previously described instrument program (22) was modified for these analyses (Table 1).

Standard addition. Aluminum concentration in the digest was determined by the standard-addition method. We prepared an aqueous intermediate solution containing 10 mg of aluminum per liter by diluting with water a stock standard containing 1 g of aluminum (9.13 g of AlCl3 · 6H2O) per liter. Aqueous standard solutions containing 0, 10, 20, and 50 μM/L were prepared from this intermediate solution. We mixed a 250-μL sample of digest with an equal volume of the standard solutions before analysis in duplicate. The detection limit of this assay, determined according to Price (23), was 5.0 ng of aluminum per gram wet weight of brain tissue. The within-day CV was 4.8% (24.3 μg/L; n = 10), the day-to-day CV 5.5% (27.8 μg/L; n = 5).

Results

Contamination by aluminum. To study the effect of dilution of the digest on the extent of the aluminum contamination, we diluted to different volumes the nitric/sulfuric acid mixture without brain tissue, after having subjected this mixture to the digestion procedure.

We diluted the acid mixture to 10 mL in 10 tubes and then, and on days 1 and 3, measured the aluminum content of each. The course of the aluminum content in these 10 tubes is shown by the upper curve in Figure 1. A repeated-measurement MANOVA model, used to analyze the difference between the aluminum content in different dilutions and on different days, showed that the mean aluminum content increased significantly between day 0 and day 1 (P = 0.002), and also between day 1 and day 3 (P = 0.014).

In eight other tubes we diluted the acid mixture to a total volume of 3.0 mL, and measured the aluminum content

| Table 1. Program for Determination of Aluminum in Digest |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|
| Step no. | Temperature, °C | Ramp time, s | Hold time, s | Internal N2 flow, mL/min |
| 1 | 90 | 1 | 20 | 300 |
| 2 | 150 | 10 | 20 | 50 |
| 3 | 500 | 10 | 20 | 50 |
| 4 | 1400 | 1 | 30 | 50 |
| 5 | 2600 | 0 | 6 | 10 |

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then and on days 1, 3, 5, and 7. As shown by the lower curve in Figure 1, the mean aluminum content in these tubes did not change during the first five days after the digestion. However, between day 5 and day 7, the mean aluminum content in these tubes increased significantly (P = 0.001).

The mean aluminum content—i.e., the contribution of contamination to the analytical result—in the tubes with the 3.0-mL dilution was not only significantly lower than the mean content in 10-mL dilution (P < 0.001), but was also constant during the first five days after the digestion. We therefore decided to dilute the tissue digest to a total volume of no more than 3.0 mL: the absolute amount of contamination was established to be 13 ng of aluminum per tube (SD = 7.9 ng; n = 8) in the 3-mL samples during the first five days after the digestion.

Analytical recovery. To determine the recovery of aluminum in this assay, we used six rat brains. One hemisphere was digested to determine the aluminum content of the rat brain, whereas we added to the tube with the other hemisphere 250 ng of aluminum (AlCl₃ · 6H₂O) in 50 μL of water before the digestion. After measuring the aluminum concentration in both digests, we calculated the recovery to be 106% (SD = 9.5%; n = 6).

Application of the method. Blanks were included in all analyses, so that we could correct for any contribution from contamination.

A mean value of 0.041 mg of aluminum per kilogram wet weight of brain tissue (SD = 0.032 mg/kg; n = 8) was obtained in samples from rats that had not been deliberately exposed to aluminum.

Applying the modified wet-digestion method to human brain cortex samples—five men and one woman, ages 37 to 82 years, who had died from various causes—gave the results listed in Table 2. The analysis was carried out in triplicate.

Fatty residues. Dilution of the tissue digest resulted in the precipitation of fatty residues. To examine whether this affected the accuracy of the method, we also measured aluminum in the insoluble fatty residues remaining in the tubes used to digest tissues from the eight non-exposed rats and the six humans.

Before solubilizing the fatty residues in absolute ethanol (no. 983; Merck; aluminum content <1 μg/L, according to the manufacturer’s specifications), we rinsed the tubes three times with water and dried them.

The absolute amount of aluminum in the fatty residues from rat origin was 6.17 ng per tube (SD = 3.71; n = 8); the residues from human origin contained 5.93 ng per tube (SD = 5.90; n = 18).

Apparent ly the amount of aluminum in the fatty residue is independent of the total amount of aluminum in the tube and has approximately the same order of magnitude as the contamination. We therefore conclude that the aluminum present in the fatty residues is due to contamination and does not further affect the accuracy of the method.

Discussion

Determination of aluminum in brain tissue requires an accurate and reliable analytical procedure.

Digestion of tissue with strong oxidizing agents in combination with heating before measuring the aluminum by electrothermal atomic absorption spectrophotometry is a simple sample-treatment method. Although this method has often been used in the analysis for aluminum in brain tissue (8, 18–21), analytical specifications were poorly defined. Moreover, in none of these studies was much attention given to contamination, a widespread and well-known problem in the determination of this ubiquitous element.

In the present study, we modified the digestion method introduced by Trapp et al. (8), then evaluated the analytical parameters and applied the method to samples of brain tissue from rats and humans. The most significant modifications were the dilution of the digest to a total volume of 3.0 mL (instead of 25 mL) and the use of disposable polypropylene tubes (instead of nondisposable quartz vessels).

By diluting the digest to a smaller volume, contamination was reduced significantly (P < 0.001; Figure 1). Even more important, the extent of the contamination remained constant during the first five days after the digestion, a finding that contributes to the usefulness of the method. By including blanks, the analytical results can be corrected for the contribution of the contamination.

The analysis for aluminum in rat brain is more affected by contamination (up to 20%) than the analysis in human cortical samples (<5%), owing to the lower absolute aluminum content in rat brain.

Although the wet-digestion methods described in the literature are largely comparable (8, 18–21), only Slainina et al. (19) reported the precipitation of insoluble fat particles. The present study confirms this precipitation, and shows that the accuracy of the procedure is not affected by it. Aluminum in the fatty residues probably originates from contamination and may therefore be neglected.
Table 3. Reported Values for Normal Aluminum Content of Rat Brain

<table>
<thead>
<tr>
<th>Al content, mg/kg wet wt.</th>
<th>Ref. no.</th>
<th>This study</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>0.28 ± 0.1</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>0.77 ± 0.19</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>&lt;0.5</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>0.28</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>0.013*</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>0.016*</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>0.041 ± 0.032</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* In cortex.

Application of the digestion method to brain hemispheres of nonexposed rats demonstrated a normal aluminum content of 0.041 mg/kg wet weight of tissue (SD = 0.032 mg/kg; n = 8). Except for Slanina et al. (19, 24), who reported values in the same order of magnitude, all other groups reported a higher value (Table 3). We suspect that the analytical results of these latter groups were affected by contamination, causing a systematic error in the analysis of aluminum.

Results of this method for brain cortex samples from six patients, an aluminum content ranging between 0.14 and 0.22 mg/kg wet weight of tissue (Table 2), are also lower than others have reported. According to Ganrot (7), the value for normal aluminum content of human brain cortex varies between 0.25 and 0.75 mg/kg. The discrepancy between our findings and those reviewed by Ganrot (7) can, again, most probably be ascribed to contamination.

To summarize: the modification of the wet-digestion method is a reliable, simple sample pretreatment of brain tissue for aluminum analysis. The contribution of aluminum contamination, a systematic error in the analysis of aluminum, must be controlled by including appropriate blanks.

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