Direct Non-Chromatographic Radioimmunoassay of Aldosterone: Validation of a Commercially Available Kit and Observations on Age-Related Changes in Concentrations in Plasma

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Human plasma aldosterone can be rapidly, directly, and reliably measured by radioimmunoassay with use of a commercially available anti-aldosterone antibody. With this method, we show that values tend to decrease with advancing age, especially after the sixth decade.

Currently used antibodies for radioimmunoassay (RIA) of aldosterone lack the necessary high specificity for its direct RIA in plasma (1-6), and tedious separation procedures commonly are needed to eliminate interfering steroids. We have recently tested a highly specific and commercially available anti-aldosterone antibody recommended for assay of aldosterone in human plasma after a simple extraction step, without need for prior chromatography. This communication documents the reliability of the method and reports our data on changes with age in aldosterone concentrations in human plasma.

Materials and Methods

The anti-aldosterone antibody, aldosterone standard, and 1,2,6,7-[3H]aldosterone were provided as part of an RIA kit from Diagnostic Products Corp., Los Angeles, Calif. 90064. The antibody was obtained from rabbits immunized against the 6-carboxy-methoxy-oxime derivative of aldosterone conjugated to thyroglobulin. The nonchromatographic extraction procedure consists of a protein precipitation step followed by extraction with methylene chloride and an NaOH wash (Figure 1). The RIA step calls for incubation in borate buffer at 0-4 °C for 2 h in a total volume of 0.8 ml. The standard assay curve extends from 0 to 320 pg. Dextran-coated charcoal is used to separate free from bound steroid; the radioactivity of the supernate is counted in Aquasol, and counts are accumulated to give a counting error not exceeding ±3%.

To study the effect of age on plasma aldosterone levels, we recruited 91 apparently healthy human volunteers spanning five decades of life. Informed consent was obtained, and a standardized furosemide stimulation test as described previously (7) for renin profiling was used. This test consisted of two oral 40-mg doses of furosemide (one the evening before and one on the morning of the test), followed by 3 to 4 h of ambulation. Blood was sampled at noon and the plasma separated by centrifugation at 4 °C and kept frozen until analyzed. There were no dietary restrictions except for a standardized breakfast consisting of coffee, juice, and toast.

Results

The mean binding of [3H]aldosterone to the antibody (B0) was 38 ± 0.5 (SE)% (n = 100). The mean recovery of [3H]aldosterone added to plasma and carried through the entire procedure was 55.3 ± 0.7% (n = 100).

Analytical Variables

Sensitivity: The assay is highly sensitive as judged by the standard assay curve depicted in Figure 2. The percentage binding in the presence of 5 pg of added aldosterone was 93.1 ± 1% that at B0 (n = 18). In repeated assays, the binding at 5 pg was always lower than that at B0 (n = 18, P < 0.005), so that 5 pg was considered to be the lower limit of sensitivity. There was a 50% change in binding at a mean aldosterone dose of 71.9 ± 2.2 pg (n = 18). With the usual aliquot (20% of 2 ml) and with a correction for recovery, the lowest detectable aldosterone value thus is about 20 ng/liter of plasma.

Specificity. The most outstanding characteristic of this RIA method is the extreme specificity of the antibody. Many different steroids, tested in the system at extraordinarily high concentrations, were without significant effect on the binding of [3H]aldosterone to the antibody (Table 1). All 13 steroids, tested at relatively high doses, were noncompetitive in the assay. Furthermore, both spironolactone and canrenone at doses above therapeutic blood concentrations were nonreactive. For a plasma pool with an aldosterone concentration of 57 ng/liter, addition of cortisone and cortisol in concentrations 10-fold those normally found in circulating plasma resulted in aldosterone readings of 56 and 55 ng/liter, respectively. Further to prove the specificity of the method, we assayed two separate plasma pools in triplicate before and after column chromatography on Sephadex LH-20 (23 × 1 cm; methylene chloride as eluting solvent). Aldosterone values were comparable before and after chromatography: 90 ± 3 vs. 92 ± 5 ng/liter, and 326 ± 5 vs. 349 ± 23 ng/liter.

Accuracy. This was assessed by adding 120 and 240 ng of aldosterone per liter to a specimen of pooled plasma. After correcting for endogenous aldosterone values, 127 ± 8 pg (n = 10) and 289 ± 12 pg (n = 9), respectively, were accounted for.

Reproducibility. The inter-assay coefficients of variation for two separate plasma pools with mean values of 90 and 289 ng/liter were 11 and 9%, respectively (n = 12).

Blanks. Water blanks were indistinguishable from zero. In plasma obtained from an adrenalectomized patient receiving cortisone acetate, an aldosterone reading of 5 pg per sample was obtained. In plasma from an adrenalectomized rat, al-

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Table 1. Specificity of Aldosterone Antibody

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Aldosterone</th>
<th>Dose tested, pg</th>
<th>Affinity, %</th>
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<tr>
<td></td>
<td>equivalent (pg)</td>
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<td>1000</td>
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<tr>
<td>Pregnenolone</td>
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<tr>
<td>Progesterone</td>
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<tr>
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<tr>
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<td>0</td>
</tr>
<tr>
<td>11-Deoxycorticosterone</td>
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</tr>
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<td>Canrenone</td>
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</table>

* Binding equivalent of 5 pg aldosterone was also observed at a dose of 100 ng (100 000 pg).

**No binding was demonstrable at 100 and 500 ng.

Aldosterone readings of 2–3 pg per assay tube were obtained. Such an insignificant blank was negligible in the sample calculation.

Age and Plasma Aldosterone

Figure 3 shows plasma aldosterone values obtained for our 91 human subjects between the ages of 20 and 66. Values averaged 481 ± 58 ng/liter (n = 16) for subjects in their twenties and were significantly lower (P < 0.005) by age 60 (169 ± 29 ng/liter). Twenty-six men of age 20–40 did not have significantly different values from 12 age-matched women (435 ± 63 vs. 419 ± 39 ng/liter, P = 0.9). Although aldosterone values tended to be lower in subjects older than 30, the most dramatic decrease appeared to take place in the sixth decade (Figure 4). Linear regression analysis gave a correlation coefficient of 0.29 (P < 0.05).

Discussion

The above method offers two advantages, simplicity and availability. About 20 plasma samples can be assayed by a trained technician during a working day. Accuracy and reproducibility are good. The remarkable specificity of the antibody is an essential feature of the method. None of the steroids tested in concentrations 2000 to 100 000 times the lower detection limit for aldosterone displayed significant affinity in excess of 0.1%. The steroids tested included cortisol and cortisone, two corticoids present in relative abundance in human plasma. The affinity of these steroids for the antibody was less than 0.005%; at 100 ng, both cortisol and cortisone exhibited the binding equivalent of only 5 pg aldosterone. Addition of a 10-fold excess of both steroids to the control plasma pool did not result in a higher reading for aldosterone, nor did chromatographic purification affect aldosterone values. Finally, it should be noted that both spironolactone and its chief plasma metabolite, canrenone, did not interfere in the assay at concentrations up to 500 ng; the interference of these drugs or their metabolites, or both, has been noted in at least one aldosterone RIA system (9).

The plasma aldosterone response to furosemide declined with age, with the most impressive drop after age 60, con-
firming previous observations on fewer subjects (8). This decline is probably secondary to the lower plasma renin activity seen in older subjects (7, 10), although an enhanced aldosterone metabolic clearance rate cannot be ruled out, especially in view of recent evidence indicating that aldosterone-binding globulin concentrations decrease with age in normal subjects (11). Decreases in aldosterone secretion rate (12) and in its urinary excretion (10) by elderly subjects have been reported previously. Plasma aldosterone after 2 h of ambulation has been reported to be lower in older subjects whose sodium intake is normal (13). More recently, Wiedmann et al. (14) also reported an age-related decline in plasma aldosterone response to Na+ restriction and furosemide stimulation, although the response to corticotropin (ACTH) remained unaltered in the elderly.

The availability of this rapid, direct RIA method should make possible the routine measurement of plasma aldosterone in most clinical laboratories. Our data indicate that a factor in clinical interpretation is the age of the subject. Our initial experience suggests that this method is also suitable for the measurement of the acid-labile glucuronide conjugate of aldosterone in human urine. A recent report (15) has favorably compared this RIA method with another commercially available kit, although it did not evaluate blanks, procedural recoveries, sensitivity and detailed specificity.

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References