Specificity of a cytochemical bioassay for arginine-vasopressin and its validation for plasma measurement

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The total Na+/K+ ATP-ase activity of the thick ascending limb of the loop of Henlé may be stimulated by arginine-vasopressin (AVP). Lysine-vasopressin (LVP), oxytocin (OT), and arginine-vasotocin (AVT) produce less than 5% of the enzyme activity induced by the same concentration of AVP. Physiological concentrations of a mixture of other hormones with known activity on the kidney (T₃, T₄, aldosterone, angiotensin II, and OT) did not significantly increase total Na+/K+ ATP-ase activity. Specific AVP antiserum consistently removed greater than 90% of the stimulatory effect of plasma. The concentration of AVP in plasmas from dehydrated subjects was greater than 10 times that of the same subjects hydrated. Intra-assay coefficient of variation was 35% and 52% from 200 μl and 20 μl of plasma respectively. The interassay coefficient of variation was 53% and 55% from plasma pools with high and low AVP content.

The cytochemical assay for arginine-vasopressin (AVP) was first reported by Baylis et al. (1980). Essentially, the bioassay relies upon an increase in the activity of either total or ouabain-sensitive Na+/K+-dependent ATP-ase in the thick ascending limb of the loop of Henlé, made in response to increasing concentrations of AVP. It is well known that various hormones such as tri-iodothyronine (T₃), thyroxine (T₄), aldosterone, and angiotensin II act through receptors at various sites along the the nephron (Katz & Lindheimer, 1977). We have examined the specificity of the total ATP-ase component of the AVP assay in the presence of physiological concentrations of oxytocin (OT), arginine-vasotocin (AVT), lysine-vasopressin (LVP), T₃, T₄, aldosterone, and angiotensin II.

Materials and Methods

Hormones

Oxytocin and AVP were obtained from the National Institute of Biological Standards and Control, London, as the 1st international standard issued. Lysine-vasopressin and AVT were obtained from

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Ferring AB, Malmo, Sweden. Aldosterone, T3, T4, and angiotensin II were from Sigma (Poole, Dorset).

To investigate whether other hormones known to act on the kidney would interfere with the assay, two mixtures of such hormones were produced. A low-concentration (LC) mixture of T3, T4, aldosterone, angiotensin II, and OT was used employing concentrations at the lower end of the normal physiological range and a high concentration (HC) of the same hormones at the upper end of the normal physiological range. A third pharmacological mixture was also tested with concentrations ten times higher than the high physiological levels (Table 1).

**Cytochemical assay**

A modification of the method of Baylis et al. (1980) was used in this study. The changes made to the reported method were as follows: (i) kidney segment revival and hormone dilution were carried out in 4.5 ml of T8 medium (Flow Lab, Sterling, Scotland) in 10-x-35-mm polystyrene cell-culture dishes (Sterilin); (ii) following incubation with the hormone (or just T8 medium for controls) kidney segments were chilled in isopentane (AnaJar-BDH) previously equilibrated with liquid nitrogen to -196°C.

Total Na+/K+ ATP-ase activity was demonstrated using the method of Chayen et al. (1981), with the following modifications: (i) 1 mM l-tetramisole (Sigma) was used in the reaction mixture to inhibit alkaline phosphatase activity; (ii) ATP-ase activity was visualized using a 1-in-300 dilution of saturated ammonium sulphide solution (BDH) in distilled water.

The reaction product was measured in duplicate sections in 20 cells from different thick ascending limbs at 550 nm on a scanning and light-integrating microdensitometer (Vickers, Model 85A).

**Results and Discussion**

The activity of total Na+/K+-dependent ATP-ase induced by the AVP analogues LVP, OT, and AVT at 2 and 10 pg/ml (equivalent to AVP in the physiological range) produced mean cross reactivities of 2% (range, 1.2-2.4), 0.3% (0.2-0.4), and 0.9% (0.7-1.0) respectively (Fig. 1). Pippard and Baylis (1983), using a modified cytochemical assay for AVP, found that the cross reactivity with LVP, OT, and AVT was 3%, 0.4%, and 10% respectively. Thus, with the exception of the AVT value, this is in close agreement with our own results.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Low concentration (LC)</th>
<th>High concentration (HC)</th>
<th>Pharmacological concentration (10 x HC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3</td>
<td>0.25 ng/ml</td>
<td>5 ng/ml</td>
<td>50 ng/ml</td>
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<tr>
<td>T4</td>
<td>10.00 ng/ml</td>
<td>200 ng/ml</td>
<td>2 μg/ml</td>
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<tr>
<td>Aldosterone</td>
<td>0.05 ng/ml</td>
<td>10 ng/ml</td>
<td>100 ng/ml</td>
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<td>Angiotensin II</td>
<td>20.00 pg/ml</td>
<td>200 pg/ml</td>
<td>2 ng/ml</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>1.00 pg/ml</td>
<td>20 pg/ml</td>
<td>200 pg/ml</td>
</tr>
</tbody>
</table>