accepted is that one has not been found that meets all of these criteria.

However, if we identify which of the characteristics are the most important, we should then, making minor compromises, be able to select the best acceptor. Some of the compounds that have been evaluated by investigators include the following: Tris, AMP, 2-amino-2-methyl-1,3-propanediol, diethanolamine (DEA), 2-(ethylamino)ethanol (EAE), ethanolamine, and N-methyl-D-glucamine. Elsewhere my coworkers and I published a thorough kinetic assessment of these compounds as phosphoacceptors for the human ALP isoenzymes from placenta, adult intestine, and liver, and made some conclusions about the preferred acceptor for routine assay of ALP in sera (3). Under saturating conditions, DEA was the best acceptor of phosphate. However, the $K_m$ of this compound was 9.8 times higher for the placental isoenzyme and 2.5 times higher for the liver enzyme than the $K_m$ for EAE. Therefore, to saturate the placental enzyme would require a DEA concentration of at least five times its $K_m$ or about 25 mol/L. Stated another way, the placental isoenzyme would exhibit only 11% of its $V_{max}$ in DEA (1 mol/L) but 66% of its $V_{max}$ in an equal concentration of EAE. The equivalent value in N-methyl-D-glucamine is 53%, but this compound did not increase $V_{max}$ to the extent that DEA and EAE did. Thus, the rate of conversion of p-nitrophenol from p-nitrophenyl phosphate in the presence of ALP is greatest in EAE. Another consideration, sometimes part of a thorough evaluation, although not addressed here, is how these phosphoacceptors affect the $K_m$ for p-nitrophenyl phosphate.

Considering the phosphoacceptors as activators allows us to evaluate their activation effects on the phosphohydrolytic activity of ALP and the effects of inhibitors as impurities in these chemicals in the presence of the enhanced phosphotransferase activity. This measure of efficiency of the phosphoacceptors is most important in routine clinical assays and is reflected by a ratio of $V_{max}/K_m$ (U/mol). This ratio (averaged for the isoenzymes from placenta, intestine, and liver) was 19 for EAE, 8.6 for DEA, and only 5.3 for N-methyl-D-glucamine (3). Clearly, under the conditions we used, EAE is the compound of choice. Of great importance in an evaluation of this type is the purity of the phosphoacceptors. We used in each case what we believed to be the highest quality available, including “Gold Label” EAE from Aldrich Chemical Co.

I urge investigators who are evaluating compounds to serve as phosphoacceptors and buffers in ALP assays to keep these basic kinetic considerations in mind.

References


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Digoxin-like immunoreactivity in Saliva and Plasma of Pregnant Women

To the Editor:

Endogenous digoxin-like immunoreactivity (EDLI) has been detected in different clinical conditions, especially associated with volume expansion (1–3), including pregnancy (5). Drug assays in saliva samples are particularly attractive as an alternative to blood sampling for therapeutic drug monitoring in patients with inaccessible peripheral veins and for pharmacokinetic studies, where repeated sampling is required. Saliva has been used to assay steroid hormones (6) and several drugs such as digoxin (7) and theophylline (8). Although EDLI has been detected in several biological fluids (3, 4), so far no study on EDLI in saliva has been published. In view of our previous finding (7) of significant correlation between salivary and serum concentrations of digoxin, we undertook the present study to examine the possibility of finding EDLI in saliva. Furthermore we found a significant correlation between salivary magnesium and serum digoxin concentrations in patients treated with digoxin (9).

We measured EDLI expressed as nanograms of digoxin equivalent per liter (ng/L) in stimulated and unstimulated saliva and plasma of 20 term pregnant women, known to have increased EDLI in plasma (5) and in 10 healthy nonpregnant women. All the patients and controls were non-digoxin, and none of them received digoxin, contraceptives, or other medication known to cross-react with the digoxin radioimmunoassay.

EDLI was measured, in duplicate on the same day, by radioimmunoassay (RIANEN; New England Nuclear, Billerica, MA) as described by us elsewhere (3), with an intra-assay CV of 6.8%. Saliva and blood specimens were collected concurrently into chilled heparinized glass tubes. Blood was centrifuged for 10 min (1000 × g) and plasma was stored at −30 °C until assay. For the collection of unstimulated saliva, subjects were instructed to let saliva accumulate in their mouth, then expectorate it into a test tube for a 10-min period (9). For stimulated saliva, subject’s tongues were swabbed every 30 s with a cotton applicator containing citric acid solution before expectoration into the test tube for a 10-min period (8). All saliva samples were centrifuged for 20 min (1000 × g) and the supernates stored at −30 °C until assay, as described for plasma. We also stored 1 mL of unstimulated saliva at 4 °C after centrifugation until assayed for magnesium. The lower limit of sensitivity for EDLI was 50 ng/L (3). For statistical analysis, all EDLI values below the detection limit were considered as having concentrations at the lower limit of detection. Saliva was assayed for magnesium by atomic absorption spectrophotometry (9).

Table 1 summarizes the mean EDLI in saliva and plasma and the salivary magnesium concentration in the studied population.

We found that EDLI is present in saliva in both groups. However, although EDLI in plasma of pregnant women is significantly higher than in the controls, pregnancy does not have a similar effect on salivary EDLI. No correlation between plasma and salivary EDLI was found in either patients or controls; also, no correlation was found between plasma EDLI and salivary magnesium in both groups. Despite the twofold increase in plasma EDLI, salivary magnesium concentrations remained unchanged.

Stimulation did not significantly affect salivary EDLI in controls or patients, indicating that salivary flow
Table 1. Salivary and Plasma Endogenous Digoxin-like Immunoreactivity (EDLI) and Salivary Magnesium Concentrations (±SD)

<table>
<thead>
<tr>
<th></th>
<th>EDLI, ng/L</th>
<th>Saliva Mg, mmol/L</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Unstimulated</td>
<td>Stimulated</td>
</tr>
<tr>
<td>Controls</td>
<td>174.5 (49)</td>
<td>140 (129)</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>167 (65)</td>
<td>132 (121)</td>
</tr>
</tbody>
</table>

* Significantly different from controls: P <0.001.

Rate does not change EDLI in saliva. Some substances that passively diffuse into the saliva are independent of flow rate; thus it can be reasoned that salivary measured EDLI, in this respect, follows the same diffusion principles as digoxin (9) and other lipid-soluble unconjugated steroids (6).

As the above results indicate, EDLI as measured in saliva by this RIA may have immunological similarity to digoxin in vitro but may have different biological behavior.

References