Almost 50 years ago, Davies (1) determined equilibrium constants for the association between calcium and some organic anions. He measured the solubility of calcium iodate in the presence of the sodium salt of the organic acid. We have re-determined three of these association constants. By using a reference calcium electrode (2) with saturated potassium chloride for salt bridge, and by substituting the organic anion (A⁻) for chloride in aqueous solutions with pH 7.4,10.16

<table>
<thead>
<tr>
<th>Table 1. Apparent Association Constants between Calcium and Organic Anions</th>
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<tr>
<td>Device 1983 (1)</td>
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<tr>
<td>Lactate</td>
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<tr>
<td>Pyruvate</td>
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<td>β-OH-butyrate</td>
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</table>

The values are for aqueous solutions with \( I = 0.16 \text{ mol/kg}, T = 37 \, ^\circ \text{C}, \text{and pH} = 7.4 \). We used \( y_{\text{Ca}^2+} = 0.34 \) (2) to re-calculate Davies' association constants to a physiological ionic strength.

7.4, \( I = 0.16 \text{ mol/kg}, \) and \( T = 37 \, ^\circ \text{C} \) and observing the decrease in ionized calcium (Ca²⁺), we determined the association constants \( K \) (Table 1) from

\[
K = \frac{\text{CaA}^+}{(\text{Ca}^2+ \cdot \text{A}^-)}
\]

(1)

The differences between Davies' and our results are small in view of the different methods. This may indicate that our reference method is unbiased by the organic anions.

If the organic anion in plasma binds only to calcium, the concentration of a calcium complex can be calculated from the Ca²⁺ and total anion concentration (A⁻) in mol/L by

\[
\text{CaA}^+ = \text{A} \cdot \text{Ca}^2+ \cdot K / (1 + \text{Ca}^2+ \cdot K)
\]

(2)

The highest concentrations occur in untreated diabetes mellitus and lactic acidosis, and the concentration of the calcium lactate complex can exceed 0.50 mmol/L.

Some calcium complexes may have a calcium-like effect on cells (3) or may increase the excretion of calcium by being less readily reabsorbable in the kidney tubules than ionized calcium and the calcium bicarbonate complex (4).

References

Determination of plasma concentrations of imipramine and its major metabolite, desmethylimipramine, are useful to guide the effective treatment of depression and cardiac arrhythmias (1). Recently, we encountered an interfering compound in the "blank" plasma of a patient about to take imipramine, a tricyclic antidepressant, for suppression of ventricular arrhythmias. The interfering compound was subsequently identified as flexeril (Benзaprine HCl; Merck, Sharp & Dohme), a tricyclic muscle relaxant. Flexeril, 3-(5H-dibenzo[a,d]cylohepten-5-ylidenene)-N,N-dimethyl-1-propanamine hydrochloride, is not considered an antidepressant, but it is a tricyclic with a chemical configuration similar to imipramine's. Moreover, flexeril is extracted with imipramine, desmethylimipramine, and the internal standard (8-hydroxychlorimipramine) in the following method:

To extract the drugs from plasma, combine 1.0 mL of sample with 50 \( \mu \text{L} \) of internal standard solution (0.4 mg/L), 1.0 mL of carbonate buffer (0.6 mol/L, pH 9.7), and 9.0 mL of methyl-t-butyl ether in a 15-\( \text{mL} \) test tube. Mix and centrifuge for 15 min. Transfer the organic layer to a 15-\( \text{mL} \) test tube containing 250 \( \mu \text{L} \) of 0.1 mol/L HCl, shake, then centrifuge for 10 min. Discard the organic layer and inject 50 \( \mu \text{L} \) of the aqueous layer into the liquid-chromatographic solvent delivery system attached to an amperometric detector (Bioanalytical Systems). Elute with a filter and degassed mobile phase of 0.1 mol/L acetate buffer and acetonitrile (65/35, by vol) containing 5 \( \text{mmol} \) of 1-heptanesulfonic acid per liter. The flow rate is 1.4 mL/min, and the pressure <14 kPa.

The retention time of flexeril and imipramine differs by only 20 s and could be measured as spuriously high concentrations of either analyte when subjects receive both agents. This method has been used to determine imipramine and desmethylimipramine concentrations in thousands of samples in multi-center trials sponsored by the National Institute of Mental Health (2). Other compounds such as chlorpromazine, thiadiazine, and loxapine reportedly have retention times that interfere with imipramine determination (2), but the observation that flexeril also interferes is new. Other laboratories measuring tricyclic concentrations in plasma should be made aware of this interference. Whether flexeril and imipramine also interact pharmacokinetically or pharmacodynamically is unknown at this time.

References