SHORT COMMUNICATION

THE EFFECT OF VARYING THE AMOUNT OF UNLABELLED 5,5-DIMETHYLOXAZOLIDINE-2,4-DIONE (DMO) IN THE MEASUREMENT OF RAT HEPATIC INTRACELLULAR pH USING [14C]DMO

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SUMMARY

1. Hepatic intracellular pH (pHi) was measured in intact rats by distribution of 5,5-dimethyloxazolidine-2,4-dione (DMO), using [14C]DMO and a range of doses of unlabelled DMO, previously neutralized with sodium hydroxide.

2. Mean calculated pHi fell from 7.27 to 7.17 as the dose of added unlabelled DMO was increased from zero to 0.19 mmol/kg (25 mg/kg) and thereafter remained constant up to and including the highest dose group (1.55 mmol/kg; 200 mg/kg).

3. Control observations showed that the change in calculated pHi with DMO dose could have been related to the consequential sodium load, rather than to the presence of a saturable process determining the distribution of DMO.

4. Reasons are given why it is nevertheless desirable to add carrier DMO when employing [14C]DMO for the measurement of hepatic pHi in the rat.

Key words: hepatic intracellular pH, 5,5-dimethyloxazolidine-2,4-dione (DMO).

The distribution by non-ionic diffusion of the weak acid 5,5-dimethyloxazolidine-2,4-dione (DMO) between intra- and extra-cellular compartments has been widely used for the measurement of mean intracellular pH since its introduction by Waddell & Butler (1959). The validity of the calculations involved depends on the assumption that the concentrations of the unionized fraction of DMO are equal in extra- and intra-cellular fluids when equilibrium has been reached after addition of DMO to the system. If protein binding of DMO within either compartment occurred, or active transport of DMO across the cell membrane, then these would be ways by which the assumption would be invalidated. This possibility has been tested in rat skeletal muscle (Miller, Tyson & Relman, 1963), Ehrlich ascites tumour cells (Poole, Butler & Waddell, 1964), human platelets (Zieve & Solomon, 1966) and ox-heart mitochondria.

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(Addanki, Cahill & Sotos, 1967), by measuring intracellular or intra-organelle pH (pH_i) and using a large range of concentrations of DMO. If protein binding or active transport occurred, then these mechanisms would be expected to be saturated at the higher concentrations of DMO; only well above the saturating concentration would the calculated pH_i be independent of DMO concentration and negligibly affected by such mechanisms. In fact, calculated pH_i in all these systems was independent of the DMO concentration used and it was concluded that there was no evidence for the presence of saturable mechanisms which affected the measurement of pH_i.

However, in studying the distribution of DMO across epithelial surfaces Dietschy & Carter (1965) found evidence compatible with active transport of DMO across rat intestinal mucosa, and Rollins & Reed (1970) found evidence of a saturable process partly responsible for the distribution of DMO between cerebrospinal fluid and the blood. Makoff, Reid, Bar Khayim & Kuyt (1971) found a substantial rise of measured mean whole-body pH_i in the dog as the dose of unlabelled DMO used was increased up to 15.5 mmol/kg, a major part of this rise occurring as the dose increased from tracer amounts to 1.55 mmol/kg.

The DMO method has been used for measuring hepatic pH_i by a number of authors (e.g. Longmore, Niethe & McDaniel, 1969; Walker, Goodwin & Cohen, 1969; Cohen, Iles, Barnett, Howell & Strunin, 1971; Williams & Woodbury, 1971). In view of the epithelial nature of the hepatocyte in respect of biliary secretion, and the embryological relationship to intestinal epithelium, it was thought important to search for evidence of a saturable process which might have affected the calculation of pH_i.

METHODS

Hepatic pH_i was measured in resting Glaxo Wistar rats (weight 250–350 g) 3 h after nephrectomy and intravenous injection of [2-14C]DMO, using the techniques of Walker et al. (1969) and Cohen et al. (1971) with the following modifications.

(a) The 1 h hydroxy[14C]methylinulin space was used to measure the extracellular compartment of the liver; Williams & Woodbury (1971) have shown that this marker gives the best estimate of hepatic extracellular space. This change necessitated a brief second ether anaesthetic for the injection of the labelled inulin 1 h before the animal was killed.

(b) [14C]DMO was injected either without added unlabelled DMO (specific radioactivity 11 mCi/mmol) or mixed with unlabelled DMO to give doses of 0.001, 0.03, 0.19, 0.39, 0.78 and 1.55 mmol/kg rat body weight (equivalent to 0.14, 4, 25, 50, 100 and 200 mg/kg); a dose of 1.55 mmol/kg gives a plasma level of approximately 3.5 mmol/l, as measured by the method of Waddell & Butler (1959). The DMO, previously neutralized to pH 7.4 with sodium hydroxide, was injected in a total volume of 0.4–0.5 ml. There were eight to eleven rats in each dose group.

Since the higher-dose groups received increasing amounts of sodium in the neutralized DMO injected, a further group was studied in which [14C]DMO was injected with sodium chloride (rather than neutralized DMO) in a dose containing an amount of sodium similar to that given to the group receiving neutralized DMO (1.55 mmol/kg).

RESULTS

The mean values for right atrial venous blood pH (pH_v) and calculated pH_i in the different
dose groups are shown in Table 1. The group without added carrier DMO has either a significantly higher mean pH, or a mean pH which is close to being significantly higher, than all other groups except the adjacent dose group (0.031 mmol/kg). No further decrease in pH is observed in groups with doses of 0.194 mmol/kg or greater. However, the mean pH in the ‘no added carrier’ group with added sodium chloride is significantly less than that in the ‘no added carrier’ group without sodium chloride, but not different from any of the other groups.

Right atrial blood mean PCO2, which was measured in five to seven animals in each group, did not differ significantly between any of the dosage groups; the overall mean value was 37.1 ± SEM 0.60 mmHg (n = 35).

<table>
<thead>
<tr>
<th>Amount of added carrier DMO (mmol/kg rat body wt.)</th>
<th>No. of rats</th>
<th>Mean pH, ± SEM</th>
<th>Mean pH, ± SEM</th>
<th>P(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9</td>
<td>7.400 ± 0.010</td>
<td>7.27 ± 0.032</td>
<td></td>
</tr>
<tr>
<td>0.03</td>
<td>10</td>
<td>7.400 ± 0.010</td>
<td>7.23 ± 0.040</td>
<td>&gt;0.4</td>
</tr>
<tr>
<td>0.19</td>
<td>11</td>
<td>7.390 ± 0.008</td>
<td>7.17 ± 0.023</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>0.39</td>
<td>8</td>
<td>7.395 ± 0.009</td>
<td>7.17 ± 0.038</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>0.78</td>
<td>9</td>
<td>7.382 ± 0.009</td>
<td>7.20 ± 0.032</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>1.55</td>
<td>10</td>
<td>7.371 ± 0.007</td>
<td>7.17 ± 0.023</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>0 plus NaCl</td>
<td>9</td>
<td>7.380 ± 0.011</td>
<td>7.17 ± 0.017</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

(1) Significance of difference from mean pH, of ‘no added carrier’ group (t-test).

**DISCUSSION**

The fall in calculated mean hepatic pH, observed as the dose of unlabelled DMO increased contrasts with the rise in mean whole body pH, observed by Makoff *et al.* (1971). The findings are compatible with the presence of a saturable process whereby DMO is either bound in the hepatocyte or actively transported into it. However, in order to avoid a hydrogen ion load, the weak acid DMO was neutralized with sodium hydroxide before injection. When sodium chloride instead of sodium-DMO was added to [14C]DMO before injection, the calculated mean pH, was now not significantly different from that of any of the other dose groups. It may therefore be that the change of pH, with dose is not due to saturation of a binding or transport process by excess of DMO, but to an effect of altering the consequential sodium load.

Nevertheless it remains possible that both the consequential sodium load and excess of DMO are capable of making a hypothetical saturable process appear saturated, and for practical purposes we have chosen to use ‘carrier’ DMO in experiments from this laboratory in which hepatic pH, has been measured in vivo or in the isolated perfused rat liver (Walker *et al.*, 1969; Cohen *et al.*, 1971; Lloyd, Iles, Simpson, Strunin, Layton & Cohen, 1973), approximately 0.2 mmol/kg being used for injection into intact rats or approximately 0.4 mmol/l or...
greater in the perfusate of isolated liver preparations; these amounts are in the range where calculated pH would be independent of any true effect of DMO dose.

REFERENCES


