Effects of D-mannoheptulose on blood glucose and alloxan sensitivity in mice

Lennart Boquist

Institute of Pathology, University of Umeå, S-901 87 Umeå, Sweden

Abstract. D-mannoheptulose (MH) administration induced a decreased serum insulin concentration in fed and starved mice, and a transient hyperglycaemia in fed mice, but not in starved ones. The liver glycogen concentration was decreased in starved controls and in fed mice treated with MH. Differences in the capacity for rapid hepatic glyconeogenesis may have contributed to the different blood glucose responses in fed and starved mice. The hyperglycaemia in fed mice was unaffected by pre-treatment with L-leucine, or p-hydroxymercuribenzoate (PMB), but was abolished by pre-treatment with tolbutamide, and by post-treatment with insulin. Treatment of fed mice with MH before alloxan caused a marked "initial" hyperglycaemia but no second hyperglycaemia, and thus no development of alloxan diabetes. In starved mice injected with MH before alloxan there was an inhibition of the initial hyperglycaemia, but occurrence of a "second" hyperglycaemia, suggesting an absence of protection against the development of alloxan diabetes. The data show that alloxan diabetes may develop in the absence of an "initial" hyperglycaemia and a triphase blood glucose response. The hyperglycaemic action of MH in fed mice is believed to underlie the protection against alloxan toxicity.

Mannoheptulose (MH) administration to rats (Simon & Kraicer 1957; Simon et al. 1962) and rabbits (Coore et al. 1963) produces transient hyperglycaemia, glucosuria and ketosis of short duration. The cause has not been clarified, but it has been established that MH induces a reversible block of glucose-induced insulin release and an acceleration of gluconeogenesis (Simon & Kraicer 1966). The block of glucose-induced insulin secretion has been suggested to be due to binding of MH to a glucose receptor at the B-cell membrane (Matschinsky et al. 1970) or to inhibited glucose phosphorylation (Coore & Randle 1964a; Malaisse et al. 1968; Ashcroft et al. 1970; Lernmark & Hellman 1970); MH inhibits rat liver glucokinase and hexokinases of rat liver, brain and adipose tissue (Coore & Randle 1964a).

MH has been used in alloxan-studies carried out in ob/ob-mice (Scheuvenius & Täljedal 1971) and rats (Zawalich & Beidler 1973; Rossini et al. 1975) in vivo, and in rat (Tomita et al. 1974) and mouse (Idahl et al. 1977) islets in vitro. These studies have shown that MH partly (Tomita et al. 1974) or completely (Scheuvenius & Täljedal 1971; Zawalich & Beidler 1973; Rossini et al. 1975) abolishes the protection against alloxan offered by glucose, whereas MH alone has been reported not to affect the diabetogenic action of alloxan.

Different factors altering the B-cell sensitivity to alloxan in vivo have been studied in this laboratory in C57BL mice (Boquist 1977, 1978, 1979b), e.g. L-leucine, tolbutamide, p-hydroxymercuribenzoate (PMB) and the state of feeding. Moreover, a transient hyperglycaemia has been observed in fed mice injected with PMB (Boquist 1979a). This hyperglycaemia is abolished by L-leucine, but not by MH (Boquist 1979b).

The present study was carried out with the aim to see whether MH affects the serum glucose, serum insulin and liver glycogen concentrations, and the B-cell sensitivity to alloxan in C57BL mice, and whether the blood glucose response to MH administration is affected by the state of feeding, and by pre-treatment with L-leucine, tolbutamide or PMB, or by post-treatment with insulin.
Table 1.
Details about the kind of treatment and the state of feeding in the experimental groups. The interval between treatment and post-treatment is 10 min.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Post-treatment</th>
<th>State of feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>saline</td>
<td>alloxan*</td>
<td>fed</td>
</tr>
<tr>
<td>II</td>
<td>saline</td>
<td>alloxan*</td>
<td>starved</td>
</tr>
<tr>
<td>III</td>
<td>MH</td>
<td>alloxan</td>
<td>fed</td>
</tr>
<tr>
<td>IV</td>
<td>MH</td>
<td>alloxan</td>
<td>starved</td>
</tr>
<tr>
<td>V</td>
<td>MH</td>
<td>alloxan</td>
<td>fed</td>
</tr>
<tr>
<td>VI</td>
<td>MH</td>
<td>alloxan</td>
<td>starved</td>
</tr>
<tr>
<td>VII</td>
<td>L-leucine</td>
<td>MH</td>
<td>fed</td>
</tr>
<tr>
<td>VIII</td>
<td>L-leucine</td>
<td>MH</td>
<td>starved</td>
</tr>
<tr>
<td>IX</td>
<td>tolbutamide</td>
<td>MH</td>
<td>fed</td>
</tr>
<tr>
<td>X</td>
<td>tolbutamide</td>
<td>MH</td>
<td>starved</td>
</tr>
<tr>
<td>XI</td>
<td>PMB</td>
<td>MH</td>
<td>fed</td>
</tr>
<tr>
<td>XII</td>
<td>PMB</td>
<td>MH</td>
<td>starved</td>
</tr>
<tr>
<td>XIII</td>
<td>MH</td>
<td>insulin</td>
<td>fed</td>
</tr>
</tbody>
</table>

* In some mice; those presented in Fig. 2.

Material and Methods

Animals and treatment
Non-diabetic adult C57BL-KsJ mice of both sexes were used. They were from a local stock kept under standard laboratory conditions at a constant temperature of 22°C. Before experimentation all animals had free access to water and a standard laboratory ration. Both fed and starved mice were used. Starved mice were deprived of food for 24 h before experimentation and were then allowed to eat again after blood sampling at the 4 h observation time in the alloxan experiments, and about 4 h after the injections in the other experiments. The experiments were started 8 a.m.

Table 1 gives details about the experimental groups. The following doses (aqueous solutions) were given: saline – 50 ml/kg b.w.; MH – 400/kg b.w.; L-leucine – 4 mmol/kg b.w.; tolbutamide – 200 mg/kg b.w.; tolbutamide – 5 x 10⁵ mol/kg b.w.; L-leucine – 200 mg/kg b.w.; and insulin – 0.4 IU/kg b.w. All injections were given ip under light ether anaesthesia. The 10 min interval used in most experiments corresponds to that commonly used in the preceding studies.

MH (D-mannoheptulose), L-leucine and PMB were from Sigma Chemical Co., St. Louis, Mo., USA; alloxan (Alloxan monohydrate) was from Eastman Kodak Co., Rochester, N.Y., USA; and tolbutamide (for injection) was from Svenska Hoechst AB, Stockholm, Sweden.

Serum glucose and insulin, and liver glycogen determinations
Blood was obtained by cutting the tip of the tail, and serum glucose was assayed by the glucose oxidase method at the following predetermined intervals: before (0 h) the first injection, and 1, 2, and 4 h and 1, 2, 3 and 4 days after the last injection.

Serum insulin was assayed radioimmunologically on blood obtained one h following the injections of saline in groups I and II, and MH in groups III an IV. The concentration of glycogen in the liver was determined according to Good et al. (1983) in the same groups and at the same observation time.

Student's t-test was used for statistical treatment of data.

Results

Serum glucose, serum insulin and liver glycogen concentrations
Fed mice injected with MH (group III) exhibited increased blood glucose concentration at 1 h (Fig. 1), whereas no significant blood glucose alteration was seen in starved mice given MH (group IV).

The serum insulin concentration was significantly decreased 1 h after MH injection both in fed and starved mice, and was significantly lower in starved than in the fed controls (Table 2). The liver glycogen concentration was significantly lower in starved than in fed controls, and lower in fed mice given MH than in fed controls, whereas no signifi-
Blood glucose response (mean ± SEM) to MH and saline in groups of differently treated mice: I. Saline – fed (n = 7); II. Saline – starved (n = 8); III. MH – fed (n = 18); IV. MH – starved (n = 19).

A significant difference was found between the two groups of MH-treated mice, or between the starved controls and the starved MH-treated mice.

**Alloxan sensitivity experiment**

Fed mice treated with MH before alloxan (group V) exhibited a potentiation of the initial hyperglycemia 1 h after alloxan (Fig. 2). Starved mice given MH before alloxan (group VI) showed no significant early blood glucose elevation, but a marked hyperglycaemia after 1 day. The starved controls (group II) exhibited a typical triphasic blood glucose response to alloxan, whereas a less marked triphasic blood glucose curve was observed in the fed controls (group I).

**Effect of pre-treatment with L-leucine, tolbutamide or PMB, or post-treatment with insulin on the blood glucose response to MH**

Pre-treatment with L-leucine did not alter the hyperglycaemic response to MH in fed mice (group VII) and had no influence upon the blood glucose curve of starved mice injected with MH (group VIII) (Fig. 3). Pre-treatment with tolbutamide on

**Table 2.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum insulin (mean ± SEM) (ng/ml)</th>
<th>Liver glycogen (mg/g wet wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.63 ± 0.09 (7)</td>
<td>28.9 ± 2.3 (8)</td>
</tr>
<tr>
<td>II</td>
<td>1.04 ± 0.08 (7)a</td>
<td>6.7 ± 2.1 (7)a</td>
</tr>
<tr>
<td>III</td>
<td>0.51 ± 0.15 (9)a</td>
<td>7.4 ± 2.5 (8)a</td>
</tr>
<tr>
<td>IV</td>
<td>0.30 ± 0.16 (9)b</td>
<td>3.1 ± 1.4 (8)</td>
</tr>
</tbody>
</table>

a Statistically different from group I at level of P < 0.001.
b Statistically different from group II at level of P < 0.001.

Effect of pre-treatment with MH or saline on the blood glucose response (mean ± SEM) to alloxan: I. Saline and alloxan – fed (n = 8); II. Saline and alloxan – starved (n = 7); V. MH and alloxan – fed (n = 17); VI. MH and alloxan – starved (n = 15). The interval between pre-treatment and alloxan is 10 min.
the other hand abolished the hyperglycaemic response to MH in fed mice (group IX).

Fed mice injected with PMB followed by MH (group XI) exhibited a hyperglycaemia similar to that observed in mice treated with MH alone, whereas no hyperglycaemic response was seen in starved mice injected with PMB followed by MH (group XII). These data indicate that MH-induced hyperglycaemia is not significantly affected by pre-treatment with PMB.

Injection of insulin in fed mice 10 min after MH administration caused decreased blood glucose concentration at the 1 (53.1 ± 4.8 mg/100 ml; mean ± SEM) and 2 (47.3 ± 5.0 mg/100 ml; mean ± SEM) h observation time, suggesting an abolishment of the hyperglycaemic response to MH.

Discussion

The hyperglycaemic action of MH has been ascribed to a reversible block of insulin secretion and an acceleration of gluconeogenesis (Simon & Kraicer 1966). The present study indicates that hepatic glycogenolysis contributes to the hyperglycaemia in fed mice treated with MH. Differences in the blood glucose response to MH between fed and starved mice have previously not been reported. They seem to be related to differences in the capacity for rapid hepatic glycogenolysis.

MH administration to starved mice did not inhibit the development of alloxan diabetes, but abolished the initial hyperglycaemia. In fed mice given MH on the other hand, the "initial" hyperglycaemia was preserved and seemingly potentiated, but the second hyperglycaemia was abolished, indicating a protection against alloxan. This influence of the state of feeding upon the alloxan sensitivity conforms to the observations made in mice injected with PMB (Boquist 1979b).

Since it is well-known that pre-treatment with glucose protects against alloxan toxicity, also in C57BL-mice in vivo (Boquist 1977), it is believed that the protection against alloxan in group V is due to the hyperglycaemic action of MH in fed mice. The seemingly potentiated initial hyperglycaemia in this group may represent a genuine MH-induced hyperglycaemia to which alloxan action has not contributed, or, less probably, be a result of a combined hyperglycaemic action of MH and alloxan.

In the absence of an "initial" hyperglycaemia in group VI there is no possibility for a protection against alloxan by increased serum glucose concentration, and alloxan diabetes may follow. One may then wonder why there is no alloxan-induced initial hyperglycaemia in group V. The reason of this is, so far, obscure. However, the findings show that alloxan diabetes may develop in the absence of an "initial" hyperglycaemia and a triphasic blood glucose response.

The present finding of a "second" hyperglycaemia in starved mice treated with MH conforms to the report (Scheynius & Täljedal 1971; Rossini et al. 1975) that MH does not protect against alloxan in starved mice. Under in vitro conditions MH does not protect against alloxan (Jain & Logothetopoulos 1976).

Pre-treatment with L-leucine protects against al-
loxan (Boquist 1978), and abolishes the hyperglycaemic response to PMB in fed mice (Boquist 1979b). In the present study no effect on the blood glucose response to MH was observed in mice pre-treated with L-leucine, whereas an abolishment of MH-induced hyperglycaemia was found in mice pre-treated with tolbutamide and in those post-treated with insulin. The abolishment of MH-induced hyperglycaemia by pre-treatment with tolbutamide is consistent with the report that MH not blocks, but rather stimulates insulin secretion induced by tolbutamide (Coore & Randle 1964b; Simon & Kraicer 1965; Renold 1970).

PMB-induced hyperglycaemia in fed mice is not affected by pre-treatment with MH (Boquist 1979b), and the data in the present study show that pre-treatment with PMB does not affect MH-induced hyperglycaemia.

Acknowledgments

Supported by grants from the Swedish Medical Research Council, Project No. B78-12X-00718-13.

References


Received on June 13th, 1979