COMPARATIVE TOLERANCE TO MIXTURES OF NATURAL AND RACEMIC AMINO ACIDS ON INTRAVENOUS INFUSION IN THE DOG

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Madden and his collaborators (1-3) have reported that certain mixtures of crystalline amino acids are well tolerated when infused intravenously at high rates into dogs, whereas protein hydrolysates must be given fairly slowly to be tolerated. They have also found that glycine added to mixtures of pure amino acids improves their tolerance. They further report that glutamic acid is particularly likely to produce vomiting and suggest that the lower tolerance to the hydrolysates may be due, in part at least, to their content of this amino acid. Unna and Howe (4) have corroborated this observation concerning glutamic acid and have also demonstrated that aspartic acid has an equal emetic effect. Madden et al. (5) more recently have also reported that dl-aspartic acid in mixtures of crystalline amino acid induces vomiting.

In this communication we wish to record striking differences in the tolerance of mixtures of racemic and natural amino acids, to elaborate on some of Madden's findings, and to present a preliminary study of the tolerance to single amino acids.

EXPERIMENTAL

Healthy adult mongrel dogs maintained on a diet of Purina dog chow were given single intravenous infusions of solutions of the mixtures listed in Table I, of variations of these mixtures, and of single amino acids. Food was withdrawn from the cages in the afternoon of the day preceding the experiment. The rates of infusion were carefully controlled by use of a variable speed infusion pump designed by Mr. Michael Kniazuk of the Merck Institute. With this instrument an 8 per cent solution of amino acids may be given to a 10 kilo dog at a maximum rate of 35 mg. of nitrogen per kilo per minute. Pulse and temperature were recorded at intervals during the infusion and for 2 hours thereafter. No dog was used more often than twice weekly.

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Vomiting was used as the criterion of non-tolerance. The vomiting was preceded by a short period of nausea, retching, and a slight fall in rectal temperature. On continuation of the infusion the vomiting often reoccurred in short intervals. However, after the termination of the infusion, vomiting ceased and the animals recovered quickly within 2 hours. No elevation in temperature was observed during the infusion. Only occasionally a rise of 1° or 2° was encountered 1 to 2 hours after the infusion. Such temperature reactions were presumably due to contamination with pyrogens, and not to the amino acids per se.

Table I

Per Cent Composition of Mixtures of Amino Acids

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Mixture I</th>
<th>Mixture II</th>
<th>Mixture III</th>
<th>Mixture IV</th>
<th>Vuj mixture</th>
<th>Vuj-N mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>l(+)-Arginine HCl</td>
<td>14.2</td>
<td>16.6</td>
<td>16.5</td>
<td>10.8</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>l(+)-Histidine HCl-H₂O</td>
<td>32.0</td>
<td>16.1</td>
<td>23.5</td>
<td>21.7</td>
<td>15.4</td>
<td>17.5</td>
</tr>
<tr>
<td>dl-Isoleucine</td>
<td>16.7</td>
<td>9.2</td>
<td>10.7</td>
<td>6.2</td>
<td>6.1</td>
<td>1.2</td>
</tr>
<tr>
<td>l(+)-Leucine</td>
<td>18.7</td>
<td>10.7</td>
<td>9.2</td>
<td>10.8</td>
<td>6.9</td>
<td>8.7</td>
</tr>
<tr>
<td>dl-Leucine</td>
<td>9.2</td>
<td>18.7</td>
<td>10.6</td>
<td>2.2</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>l(-)-Lysine HCl</td>
<td>6.2</td>
<td>3.7</td>
<td>16.5</td>
<td>10.8</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>l(-)-Methionine</td>
<td>2.7</td>
<td>11.1</td>
<td>2.7</td>
<td>7.6</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>dl-Methionine</td>
<td>12.9</td>
<td>11.1</td>
<td>21.0</td>
<td>13.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l(-)-Phenylalanine</td>
<td>3.2</td>
<td>3.7</td>
<td>16.5</td>
<td>7.6</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>dl-Phenylalanine</td>
<td>12.9</td>
<td>3.7</td>
<td>16.5</td>
<td>13.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l(-)-Threonine</td>
<td>12.9</td>
<td>12.9</td>
<td>21.0</td>
<td>10.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dl-Threonine</td>
<td>11.1</td>
<td>12.9</td>
<td>21.0</td>
<td>10.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l(+)-Valine</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dl-Valine</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The amino acids were dissolved in distilled water by heating on a steam bath to 70-80°. The solutions in all cases with the exception of dl-phenylalanine (to be discussed later) were adjusted to pH 6 to 7 with strong sodium hydroxide solution. The nitrogen content of the complete amino acid mixtures (those containing all of the essential amino acids) and of the hydrolysates tested varied from 1.0 to 1.1 gm. per 100 cc. The total amount of nitrogen administered in these preparations was usually approximately 220 mg. per kilo of body weight. Correspondingly smaller amounts of the incomplete mixtures were given.

In Table I the amino acid composition of some of the infusion mixtures
is shown. Mixture I is the monoaminomonocarboxylic fraction of a protein hydrolysate obtained from a casein hydrolysate by Dakin's butanol extraction procedure. Its amino acid composition was determined by microbiological assay. The unidentified portion of this mixture containing 15 per cent of the total nitrogen is possibly to a large extent natural non-essential amino acids. Mixture II is a mixture of the essential racemic acids in the same proportion in which the natural acids are found in the butanol extract, whereas Mixture III is a mixture of the monoaminomonocarboxylic acids in the proportions in which they are found in Madden's Vuj mixture (6). No. IV is a mixture of natural amino acids made by adding the pure basic amino acids to Mixture I. The fifth mixture is Madden's Vuj mixture which has, to some extent, been used clinically. The final mixture is obtained by addition of certain racemic amino acids and glycine to Mixture IV to make it resemble the Vuj mixture with respect to natural amino acid content. Because of this close resemblance and because it consists predominantly of natural amino acids, it has been given the designation Vuj-N mixture.

RESULTS AND DISCUSSION

The monoaminomonocarboxylic fraction (Mixture I) was infused at rates varying from 5 to 22 mg. of N per kilo per minute in a series of seventeen experiments with eleven dogs without causing vomiting or any apparent nausea. However, when a mixture of the same essential amino acids in the same proportions but in the racemic form (Mixture II) was given to three dogs at rates between 1 and 2 mg. of N per kilo per minute, all of these animals vomited repeatedly. In a like manner, Mixture III containing racemic monoaminomonocarboxylic acids and l(+)-leucine in the proportions found in the Vuj mixture produced violent retching for twelve of fourteen infusions when it was administered at rates of 2 to 4 mg. of N per kilo per minute. Again, when the l(+)-leucine of Mixture III was replaced by dl-leucine (Mixture IIIa) two of four dogs vomited at the rate of 2 mg. of N per kilo per minute.

The mixture of nine natural essential amino acids (Mixture IV) was used in nine infusions at rates of 20 to 35 mg. of N per kilo per minute. Only two of the animals vomited and in these experiments the rates of infusion were 30 and 31 mg. of N per kilo per minute. Similarly, when this mixture was fortified with about 10 per cent racemic amino acids and 10 per cent glycine (Vuj-N mixture) it was extremely well tolerated, producing no vomiting for eighteen infusions at rates of 6 to 30 mg. of N per kilo per minute. In contrast, the Vuj mixture produced vomiting in eight of a series of sixteen infusions at rates between 5 and 6.5 mg. of N per kilo per minute. Table II summarizes the results of the experiments.
which show the striking differences in the tolerance to natural and racemic amino acids.

Although these great differences in tolerance may not be considered evidence of non-utilization of the d forms of the amino acids, they become of prime importance in parenteral amino acid alimentation, when speed of infusion may be advantageous.

The effect of glycine in increasing the tolerance of the dog to mixtures consisting predominantly or entirely of racemic amino acids is not great. Three dogs vomited when they received Mixture III plus 10 per cent glycine (Mixture VII) at rates of 2.5 to 3.5 mg. of N per kilo per minute.

Addition of 10 per cent glycine to Mixture IIIa (Mixture VIII) again showed little or no increase in tolerance, since three of four dogs vomited at rates of 2.5 to 3.5 mg. of N per kilo per minute. When 25 per cent of glycine was added (Mixture IX) five of six dogs vomited at rates of 3 to 4 mg. of N per kilo per minute.

That glycine does, however, greatly increase the tolerance for racemic amino acids in some mixtures of racemic and natural acids is demonstrated by a series of experiments in which the Vuj-N mixture minus glycine (Mixture X) was used. As has been previously recorded, a mixture of natural amino acids is tolerated at rates up to 30 mg. of N per kilo per minute. Similarly, when 10 per cent racemic acids and 10 per cent glycine are added to this mixture it may again be given at a rate of 30 mg. of N per kilo per minute without nauseating effects. However, when the
glycine is omitted vomiting occurs quite frequently at rates as low as 9 mg. of N per kilo per minute. Forty infusions were carried out at rates between 6 and 30 mg. of N per kilo per minute. In twenty-one of the forty vomiting occurred. Closer examination of the data indicates that this mixture behaves somewhat anomalously, i.e., the incidence of vomiting was greater upon infusion at rates of 9 to 11 mg. when seven of ten dogs vomited than at 18 to 22 mg., when vomiting occurred in only seven of fourteen. As an example of individual performance Dog 571 vomited when the infusion rate was 11 mg. of N per kilo per minute but in two infusions at 21.5 mg. vomited only once. This great individual variation was not encountered with the other mixtures used.

Table III
Effect of Glycine on Tolerance to Amino Acid Mixtures

<table>
<thead>
<tr>
<th>No. of dogs</th>
<th>Mixture No.</th>
<th>Description of mixture</th>
<th>Nitrogen, total mg. per kg</th>
<th>Nitrogen rate ms. per kg. per min.</th>
<th>No. of infusions</th>
<th>No. of vomitings</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>III</td>
<td>Monoaminomonocarboxylic acids as found in Vuj mixture</td>
<td>120</td>
<td>2 - 4</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>VII</td>
<td>Mixture III + 10% glycine with dl-leucine replacing l(+)-leucine</td>
<td>120</td>
<td>2.5 - 3.5*</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>IIIa</td>
<td>&quot;</td>
<td>120</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>VIIII</td>
<td>Mixture IIIa + 10% glycine</td>
<td>120</td>
<td>2.5 - 3.5*</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>IX</td>
<td>&quot;                  + 25% &quot;</td>
<td>120</td>
<td>3 - 4*</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>IV</td>
<td>Natural amino acids</td>
<td>216</td>
<td>20 -35</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>VI</td>
<td>Mixture IV + 10% racemic amino acids + 10% glycine</td>
<td>220</td>
<td>6 -30</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>X</td>
<td>Vuj-N mixture minus glycine</td>
<td>214</td>
<td>6 -30</td>
<td>40</td>
<td>21</td>
</tr>
</tbody>
</table>

* These figures do not include the glycine nitrogen.

The results of the experiments showing the effect of glycine upon the tolerance to amino acid mixtures are summarized in Table III.

The poor tolerance to the racemic amino acid mixtures (Mixtures II and III) might be a cumulative effect of all or, on the other hand, might be due to a comparatively great emetic effect of one or a limited number of them. Accordingly, each of these racemic acids was infused singly at several times the rates and in at least twice the total amounts that they were given in the mixtures. The results, found in Table IV, show that each amino acid is remarkably well tolerated. Even dl-methionine, by far the worst offender, could be injected at 4 times the rate and in twice the total quantity that it is given in the Vuj solution when infused at the maximum tolerated speed of 6 mg. of N per kilo per minute.
Owing to its low solubility, \textit{dl}-phenylalanine was given in solution at pH 8.5. This amino acid produced vomiting in one of three experiments when a total of 1 gm. per kilo of body weight was administered at 2.8 mg. of N per kilo per minute. This positive result is probably without significance, however, since glycine which is normally innocuous produced vomiting in one of two experiments at pH 8.5.

\textbf{Table IV}

\textit{Tolerance to Single Amino Acid}

\begin{tabular}{|c|c|c|c|c|c|}
\hline
No. of & Description of material & Amino acid & Nitrogen & No. of & No. of \vomitings \\
& & & gm. per & mg. & per kg. per min. & per kg. per min. & infusions & \\
& & & kg. & mg. & & & & \\
\hline
2 & \textit{dl}-Isoleucine & 1.0 & 33 & 105 & 3.5 & 2 & 0 \\
2 & \textit{dl}-Leucine & 1.0 & 33 & 105 & 3.5 & 2 & 1* \\
2 & \textit{dl}-Methionine & 0.2 & 10.6 & 18 & 1.0 & 2 & 0 \\
2 & " & 0.6 & 16-22 & 5.6 & 1.6-2.1 & 2 & 2 \\
3 & \textit{dl}-Phenylalanine & 1.0 & 33 & 8.4 & 2.8 & 3 & 1† \\
4 & \textit{dl}-Threonine & 1.0 & 33 & 116 & 3.9 & 4 & 2 \\
2 & \textit{dl}-Tryptophane & 0.06 & 3.2 & 8 & 0.4 & 2 & 0 \\
2 & \textit{dl}-Valine & 1.0 & 33 & 119 & 4.0 & 2 & 0 \\
9 & Mixture III, monoamino- & 120 & 2-4 & & & 14 & 12 \\
& monocarboxylic acid as & & & & & & \\
& found in Vuj & & & & & & \\
5 & Mixture III minus \textit{dl}-methi- & 70-100 & 2.5-5 & & & 5 & 2 \\
& tonine & & & & & & \\
2 & Mixture III minus \textit{dl}-threo- & 98 & 2-3 & 2 & 2 & & \\
& nine & & & & & & \\
4 & Mixture III minus \textit{dl}-methi- & 102 & 4-5 & 4 & 1 & & \\
& tonine and \textit{dl}-threonine & & & & & & \\
2 & " & 102 & 7 & 2 & 2 & & \\
11 & Mixture I, natural mono- & 110-120 & 5-22 & & & 17 & 0 \\
& aminomonocarboxylic acids & & & & & & \\
\hline
\end{tabular}

* Vomiting possibly due to the large volume of solution given.
† Vomiting probably due to the pH of the solution (8.5).

Since vomiting was encountered with alkaline solutions, no attempt was made to increase the solubility of \textit{dl}-leucine by forming the sodium salt. Accordingly, this amino acid was infused in a 1.2 per cent solution. In this connection it is of interest to note the volume of solution which may be infused without apparent ill effects. Two dogs each received 80 cc. of solution per kilo over a period of 30 minutes. One vomited a few minutes after the infusion; in the second there was no reaction. Because of the large volume of solution infused the positive reaction in the first dog cannot be definitely ascribed as an effect of the \textit{dl}-leucine.
To eliminate the possibility that the poor tolerance of the racemic amino acids might be due to either or both of the two least well tolerated of the amino acids, methionine and threonine, these acids have been omitted from the infusion mixture. The results of these experiments are shown in Table IV. When methionine is eliminated from Mixture III there is a possibility of a slight increase in tolerance to the mixture. When threonine is left out, there is apparently no appreciable change. However, when both are removed, the rate at which the mixture may be given without inducing vomiting seems to be appreciably increased although the ceiling of tolerance is still quite low in comparison with that of the natural amino acids.

SUMMARY

1. Mixtures of natural amino acids can be given to dogs intravenously at much higher rates without inducing vomiting than can similar mixtures containing racemic amino acids.

2. Glycine causes a definite increase in the tolerance of the dog to infusion of certain mixtures containing racemic amino acids.

3. Of the single racemic amino acids tested, dl-methionine is the most poorly tolerated. The poor tolerance of the mixtures of amino acids is, however, undoubtedly due to cumulative effect of the racemic acids present.

The authors wish to express indebtedness to Dr. Max Tishler for valuable suggestions and guidance in this work, and to Dr. J. L. Stokes for the amino acid assays.

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