Studies on Ethionine

V. SEX DIFFERENCE IN INCORPORATION IN VIVO OF ETHIONINE INTO RAT PROTEINS*

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Among the various effects of administering ethionine to rats, the induction of a fatty liver was shown to occur in female but not in male rats (2, 3). A similar sex difference was observed in the ethionine inhibition of hepatic protein synthesis (4). The sex difference in the ethionine-induced fatty liver has been attributed to the presence of androgens in the male, since orchidectomy makes the male susceptible, and the administration of androgens protects intact or ovariectomized females (3, 5).

The correlation between the occurrence of the fatty liver and inhibition of hepatic protein synthesis led to the suggestion that the sex difference in the effects of ethionine upon hepatic protein metabolism was also mediated by the presence of androgens in the male (4). Ranney and Drill (6) reported that 17-ethyl 19-nortestosterone, a synthetic testosterone derivative, was especially effective in preventing the induction of fatty livers by ethionine. The present experiments were designed to study the sex difference in the incorporation in vivo of radioactive ethionine into various proteins of rats, and to test the effects of castration and 17-ethyl-19-nortestosterone administration.

EXPERIMENTAL PROCEDURE

1-Ethionine-ethyl-C14 (0.98 mc per mmole) was synthesized in this laboratory by Dr. D. Gross. dl-Leucine-1-C14 (2.5 mc per mmole) was purchased from Research Specialties Company. 17-Ethyl-19-nortestosterone (Nilevar) is a product of G. D. Searle and Company. Rats of the Long-Evans strain, maintained on Wayne Lab Blox rat food and weighing from 200 to 250 g, were used in all experiments. Castrated male rats were purchased from Diablo Animal Laboratories.

Administration of Hormone—Rats weighing within 20 g of each other were divided into two groups of five animals. The hormone was administered to one group; the other group served as controls. The hormone group was treated by intramuscular injection daily for 8 days with a solution (25 mg per 100 ml) of 17-ethyl-19-nortestosterone in a sesame oil-benzyl alcohol mixture (9:1). The total dose was 1.0 mg of the hormone per kg of body weight. The control group was injected with the same volume of sesame oil-benzyl alcohol mixture. On the 8th day, food was withdrawn and all animals were fasted overnight. On the 9th day, an aqueous solution of the radioactive amino acid was administered intraperitoneally and the animals were killed 24 hours afterward by ether anesthesia. The castrated rats began to receive the hormone on the 8th day after castration. On the 10th day, radioactive ethionine was injected, and the animals were killed on the 17th day after castration.

Preparation of Protein Samples—Liver microsomes were prepared by the method of Keller and Zamecnik (7), with minor modifications. The liver was rapidly removed from the animal and 3 g of the tissue were homogenized for 30 seconds in 7 ml of cold Medium A of Keller and Zamecnik in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 15,000 × g for 10 minutes in a Spinco model L preparative ultracentrifuge with a No. 40 head. The supernatant, excluding the fluffy coat, was carefully withdrawn with a needle and syringe, and then was centrifuged at 105,000 × g for 1 hour. The supernatant from this centrifugation was discarded and the sedimented microsomal pellet was rinsed three times with 1-ml portions of Medium A.

The microsomal pellet was treated with 10% trichloroacetic acid and the precipitate was washed out each with cold and hot 5% trichloroacetic acid. The precipitate was then dissolved in 1 ml of 98 to 100% formic acid and was oxidized for 30 minutes at room temperature, with the addition of 0.2 ml of 30% hydrogen peroxide. This oxidation step served to minimize the nonspecific adsorption of radioactive compounds onto the protein sample. The protein was reprecipitated from the oxidizing solution with 10% trichloroacetic acid. The precipitate was then washed once with warm alcohol, twice with a warm alcohol-ether mixture (3:1), and finally once with ether.

Total liver and pancreatic proteins were prepared by homogenizing the tissue with acidic acetone (1 ml of concentrated hydrochloric acid in 1 liter of acetone). The subsequent washing procedure was the same as that used for liver microsomal protein.

Radioactivity determination of the protein samples was carried out with a Packard Tri-Carb liquid scintillation counter. Protein samples were dissolved in scintillation solution with the use of Hyamine hydroxide. The amount of protein was estimated by directly weighing the samples in counting vials.

RESULTS

Sex Difference in Ethionine Incorporation in Vivo—In a 24-hour period, incorporation of radioactive ethionine into all of the protein fractions investigated was definitely greater in female rats than in male rats, whereas incorporation of radioactive leu-
Sex difference in incorporation in vivo of radioactive L-ethionine and DL-leucine into various proteins

<table>
<thead>
<tr>
<th>Protein source</th>
<th>Sex</th>
<th>Protein source</th>
<th>Sex</th>
<th>Specific activity</th>
<th>Difference</th>
<th>Specific activity</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L-Ethionine-ethyl-C&lt;sup&gt;14&lt;/sup&gt;</td>
<td></td>
<td>d.p.m. / mg</td>
<td>%</td>
<td>d.p.m. / mg</td>
<td>%</td>
</tr>
<tr>
<td>Liver microsome</td>
<td>F</td>
<td>115 ± 3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>524 ± 42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-37</td>
<td>549 ± 44</td>
<td>+5</td>
</tr>
<tr>
<td>M</td>
<td>72 ± 3.8</td>
<td>308 ± 23</td>
<td>41</td>
<td>270 ± 18</td>
<td>-12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total liver</td>
<td>F</td>
<td>64 ± 1.8</td>
<td></td>
<td>242</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>38 ± 2.0</td>
<td>238</td>
<td>-18</td>
<td></td>
<td>-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas&lt;sup&gt;d&lt;/sup&gt;</td>
<td>F</td>
<td>95</td>
<td></td>
<td>242</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>98</td>
<td>238</td>
<td>-18</td>
<td></td>
<td>-2</td>
<td></td>
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</tr>
</tbody>
</table>

* 3.5 μmoles, containing 3.4 μc.
<sup>a</sup> 1.5 μmoles, containing 3.8 μc.
<sup>c</sup> Specific activity is corrected to a 200-g rat weight. It is equal to observed specific activity X weight of rat in grams / 200.
<sup>d</sup> (Specific activity of male - specific activity of female) / specific activity of female X 100.
<sup>e</sup> Mean of the values from five animals ± standard error of the mean.
<sup>f</sup> Pancreas of five animals pooled.

Comparison of the results in Table II with those of Table I indicates that, upon castration of male rats, the ethionine incorporation increases to the level of female rats, and administration of 17-ethyl-19-nortestosterone to the castrated males brings the extent of incorporation down to the normal level of intact males.

**DISCUSSION**

The results of this investigation clearly demonstrate that a definite sex difference exists in the extent of ethionine incorporation in vivo into the total liver and hepatic microsomal proteins. It should be emphasized that this effect is observed with doses of ethionine much smaller than those necessary to produce fatty livers. This sex difference corresponds to that observed in the fatty liver induction (2, 3), as well as in the inhibition of hepatic protein synthesis by ethionine (4), and suggests that these biochemical and morphological effects of ethionine are somehow interrelated.

Although the mechanism of action of ethionine upon protein and lipid metabolism is not understood, the observed effect of ethionine toxicity may be due, at least in part, to the formation of abnormal enzyme proteins in the hepatic microsomes. The partial replacement of methionine by ethionine could lead to the formation of functionally altered enzyme systems which are responsible for some phases of protein biosynthesis. This disturbance of protein synthesis would, then, lead to disturbances in lipid metabolism. That abnormal proteins are formed in the presence of ethionine was previously shown in this laboratory (8). However, this cannot be the mechanism of action of ethionine in the present experiments because the dose of ethionine was so small that the amount of modified protein formed must likewise have been very small.

The present demonstration of the effects of castration of male rats and subsequent administration of 17-ethyl-19-nortestosterone one upon ethionine incorporation in vivo coincides with the previous observation of the effects of those treatments upon ethionine-induced fatty liver (3, 5, 6). This finding renders further support to the hypothesis that androgens play an important role in the sex-dependent action of ethionine (4). In view of the observation that incorporation of trace doses of methionine and leucine is the same in both sexes, it seems rather improbable that androgens directly participate in some phase of protein biosynthesis in such a manner as to influence the extent of the incorporation of trace doses of ethionine. The possibility that androgens influence the rate of catabolic degradation of ethionine in the body is now under investigation.

In the present study, sex difference in ethionine incorporation was observed in the pancreas, corresponding to that of liver. Of interest is the fact that no sex difference has previously been reported in ethionine-induced pancreatic malfunctions such as increase in protein synthesis (9) and production of acinar cell degeneration (10, 11). Whether this difference in the effect of ethionine indicates a different mode of action for various bio-

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chemical processes within the pancreas, or whether it is a reflection of the difference in ethionine action between the two different organs, remains to be determined.

SUMMARY

1. The incorporation in vivo of radioactive ethionine into proteins of liver microsomes, total liver, and pancreas was found to be greater in female rats than in males, whereas the incorporation of leucine is the same in both sexes. This sex difference corresponds to that previously described for the induction of fatty liver and the inhibition of hepatic protein synthesis by ethionine.

2. Castration of male rats resulted in greater incorporation of radioactive ethionine, comparable to the level of the intact female, and subsequent administration of 17-ethyl-19-nortestosterone to castrated males restored the extent of ethionine incorporation to the level of the intact male.

REFERENCES

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