Estrogen and prothrombin synthesis: effect of estrogen on absorption of vitamin K$_1$

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JOLLY, DENNIS W., CAROLYN CRAIG, AND THOMAS E. NELSON, Jr. Estrogen and prothrombin synthesis: effect of estrogen on absorption of vitamin K$_1$, Am. J. Physiol. 232(1): H12-H17, 1977 or Am. J. Physiol: Heart Circ. Physiol. 1(1): H12-H17, 1977 - Intact male and female albino rats fed a vitamin K-deficient diet develop a plasma prothrombin-proconvertin deficiency. Male rats respond with a precipitous drop to approximately 20-30% of normal plasma levels within 2-5 days, whereas female rats respond at a slower rate. Ethynylestradiol, 5-10 µg/day, or castration, reduces the progressive decline of plasma prothrombin-proconvertin seen in nonsupplemented intact male rats. The response of castrate females differs little from the response of intact females. Ethynylestradiol, 5-10 µg/day, affects both castrate males and females similarly, limiting the prothrombin-proconvertin decrease to about 13% below control value after 14 days. Intestinal absorption of vitamin K$_1$, measured in the thoracic duct lymph of pentobarbital-anesthetized castrate male and female rats was shown to increase significantly after estrogen treatment. Estrogen-treated castrate male and female rats absorbed 25.8 µg and 1.2 µg, respectively, during a 240-min collection period. Use of radioactive vitamin K$_1$ in similar experiments confirmed these results. Estrogen-treated castrate males absorbed vitamin K$_1$ at the rate of 30-40 µg/g lymph whereas nontreated control males absorbed only about 6 µg/g lymph.

In this paper, we report experiments that undertake to a) demonstrate the effects of estrogen on the development of hypoprothrombinaemia in intact and castrate animals, and b) determine whether estrogen affects the ability of the intestine to absorb available vitamin K$_1$ into the thoracic duct lymph.

MATERIALS AND METHODS

Animals and Diet

Simonsen albino rats (Simonsen Laboratories, Inc., Gilroy, Calif.) weighing between 160 and 200 g were used in the hypoprothrombinaemia experiments. The animals were maintained on Purina rat chow (Ralston Purina Co., St. Louis) for approximately 1 wk prior to the beginning of each experiment. In experiments involving castrate rats the animals were castrated approximately 1 wk before being placed on the vitamin K$_1$-free diet. During the course of each experiment, the animals were housed separately in suspended, wire-bottom cages with individual food and water supplies.

Food and water were supplied ad libitum, and food consumption and weight gain were recorded to assess acceptance of the diet. These data indicated that growth rate and dietary intake, although reduced, were acceptable after an initial adjustment to the synthetic diet, even when the diet contained ethynylestradiol (Fig. 1, B and D).

The vitamin K$_1$-free diet for all experiments was prepared in our laboratory according to Mameesh and Johnson (2). The estrogen-supplemented diet was prepared by first dissolving the ethynylestradiol in 5 ml of 100% ethyl alcohol and this solution was then dispersed in, and added with, the wheat germ-oil component. The diet was then completely mixed and reground 2 or 3 times to ensure a homogeneous preparation.

In the cannulation experiments larger Simonsen albino rats of both sexes were used, weighing between 325 and 450 g. These animals were maintained on Purina rat chow during the period between castration and lymph duct cannulation. The day of castration is referred to as day 0. Injections of 17β-estradiol, 100 µg in sesame oil, or sesame oil alone were given intraperitoneally on day 0 and once more on day 5. The animals were then fasted for 24 h, at which time they were force-fed 3 ml of whipping cream by stomach tube to visualize the ducts for cannulation. One hour later the thoracic
Chemicals and Reagents

Dietary components, with the exception of sucrose (C&H granulated sugar), were obtained from Nutritional Biochemical Corp., Cleveland, and ethynylestradiol and 17β-estradiol were purchased from Sigma Chemical Co., St. Louis. The radioactive 14C-labeled vitamin K, was a gift from Dr. John T. Matschiner, University of Nebraska School of Medicine, and the nonradioactive vitamin K, used in our experiments was AquaMEPHYTON, kindly given to us by Merck Sharp & Dohme, Rahway, N.J. Ware and Stragnell anticoagulant, obtained from Hyland Division, Travenol Laboratories, Costa Mesa, Calif., was used with all blood samples for prothrombin assay. Dade plasma test reagents, scintillation chemicals, and solvents for thin layer chromatography were obtained from Fisher Scientific Co., Maryland Heights, Mo. The thin-layer Silica Gel-G-coated plastic chromatographic plates were obtained from Brinkmann Instruments, Inc., Westbury, N.Y.

RESULTS

Development of Prothrombin-Proconvertin Deficiency in the Intact Male and Female Rat

The effect of substitution of a vitamin K-free diet on intact male and female rats for a period of 19 days is shown in Fig. 1, A and B. In this experiment males exhibit a precipitous decline in plasma prothrombin-proconvertin levels whereas females maintained a much higher plasma prothrombin-proconvertin activity throughout the experiments. Only 30% of the intact females died during the 19-day experiment (Fig. 1, A), whereas 80% of the intact males had expired before day 16. In the second experiment (Fig. 1, C and D), under nearly identical conditions as possible with the exception that the vitamin K-free diet was fortified with ethynylestradiol to deliver an approximate dose of 5–10 µg/day, male rats did not succumb as rapidly as before. In the first experiment (Fig. 1, A and B), intact males showed a gradual increase in plasma prothrombin-proconvertin to as much as 140% of normal levels after initiation of the vitamin K-free diet, in contrast to the precipitous drop seen in the males. When the vitamin K-free diet was fortified with ethynylestradiol, both males and females responded differently. Comparison of the growth patterns shown in the two experiments suggests that the estradiol reduced the food intake while it acted to protect the animals from the hemorrhagic effects of vitamin K deficiency. All deaths in both experiments were due to hemorrhage, either spontaneous or associated with cardiac puncture during blood sampling.

Effect of Castration on Development of Prothrombin-Proconvertin Deficiency in Female and Male Rats

The development of prothrombin deficiency in the castrated male rat (Fig. 2) is less severe than in the intact male rat (Fig. 1, A). The castrate males show a more
FIG. 1. A: effect of vitamin K-free diet on plasma prothrombin-proconvertin levels in intact male and female rats. Animals were fed a vitamin K-free diet starting on day 0 and continuing for duration of experiment. Blood samples (1 ml) were drawn by cardiac puncture from each animal on days indicated and plasma prothrombin-proconvertin activity (% prothrombin) was determined and expressed as a percentage of individual animal's control level (day 0). Each point represents mean ± SE of number of surviving animals, indicated in parentheses. B: mean weight gain ± SE of animals represented in A, indicating continued growth and positive nutritional balance on this diet. Deviation in growth curve indicates temporary withdrawal of food for 24 h. C: experiment similar to A, except that vitamin K-free diet contained ethynylestradiol (5-10 μg/day). D: mean weight change ± SE for animals represented in C.

Effect of Ethynylestradiol on the Development of Prothrombin-Proconvertin Deficiency in the Castrate Male and Female Rat

If estrogen serves a protecting function, then one would expect the development of prothrombin deficiency to be very similar, if not identical, in castrate male and female rats given a vitamin K-free diet supplemented with ethynylestradiol (5-10 μg/day). The responses of castrate male and female rats are indeed remarkably similar, as shown in Fig. 3, A. Both groups showed very little development of prothrombin deficiency and very few deaths due to hemorrhage. Again, this would suggest a protective function of estrogen in the maintenance of plasma prothrombin levels during dietary vitamin K deficiency.

We established the acceptance of the estrogen-fortified diet by the animals by monitoring their weight (Fig. 3, B). The castrate animals accepted the diet (illustrated by weight gain) after a short lag period lasting up to 4 days.

Effect of Estrogen on Intestinal Absorption of Vitamin K₁ (Phylloquinone)

Although estrogen has not been shown to substitute for vitamin K₁, it does demonstrate, as we have seen, some type of prothrombogenic action. Estrogen might act by modifying the metabolism of vitamin K or by facilitating its absorption from the gastrointestinal tract so that more vitamin K₁ would be present at the site of synthesis.
The possibility that estrogen might facilitate vitamin K absorption was tested by cannulating the thoracic lymph duct of castrate female and male rats, both with and without estrogen treatment. Thirty minutes after establishment of a control lymph flow, 10 mg of unlabeled vitamin K1 (AquaMEPHYTON) was injected intragastrically. The total lymph collected was analyzed colorimetrically for vitamin K content. The untreated, castrate males had no detectable vitamin K1 in their lymph during the 210 min of lymph collection, as shown in Fig. 4, whereas the control castrate females absorbed a cumulative 1-2 µg of vitamin K1 during the last 60 min of lymph collection. Both estrogen-treated, castrate female and male rats showed significant levels of vitamin K1 in their lymph in contrast to the very low values in control animals. The estrogen-treated, castrate females absorbed a cumulative 10-13 µg during the experiment, but the estrogen-treated, castrate males showed the greatest total absorption of vitamin K1, 24-27 µg in 210 min. From the summary of the data (Table 1) it can be seen that all of the animals demonstrated similar body weight and lymph flows. The data obtained from the colorimetric lymph assays indicate that estrogen treatment increased the rate of vitamin K1 absorption.

A similar set of experiments was performed with 14C-labeled vitamin K1 added to unlabeled carrier vitamin to enable us to determine the radioactivity of the vitamin K content of the lymph. The total injection of vitamin K1 was reduced to 1 mg in order to simulate more accurately the physiologic concentrations. Unlike the nonradioactive experiments, the vitamin K1 was injected intraduodenally to minimize differences in stomach-emptying time. Castrate males, rather than both sexes, were used in the four isotope experiments because males had responded with the highest rate of absorption in the colorimetric experiments. Again, the estrogen-treated, castrate male rats absorbed much more vitamin K during the experiment than the control animals, as shown in Fig. 5. The control rats absorbed only 8-10 µg, cumulatively, in 175 min, whereas the...
TABLE 1. Effect of estrogen treatment on in vivo intestinal absorption of vitamin K₁ in castrate male and female rats

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Body Wt, g</th>
<th>Treatment</th>
<th>Mean Lymph Flow, g/min</th>
<th>Duration of Expt, min</th>
<th>Total Lymph Collected, g</th>
<th>Total Vitamin K Absorbed, µg</th>
<th>Mean Vitamin K Absorbed, µg/g lymph</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>360</td>
<td>Control</td>
<td>0.013</td>
<td>240</td>
<td>2.900 ± 0.13</td>
<td>0.0 ± 0.0</td>
<td>0.00</td>
</tr>
<tr>
<td>Female</td>
<td>376</td>
<td>Control</td>
<td>0.014</td>
<td>240</td>
<td>3.293 ± 0.09</td>
<td>1.2 ± 0.1</td>
<td>0.36</td>
</tr>
<tr>
<td>Male</td>
<td>393</td>
<td>Estradiol</td>
<td>0.015</td>
<td>240</td>
<td>3.603 ± 0.44</td>
<td>25.8 ± 3.0</td>
<td>7.16</td>
</tr>
<tr>
<td>Female</td>
<td>355</td>
<td>Estradiol</td>
<td>0.011</td>
<td>240</td>
<td>2.603 ± 0.21</td>
<td>11.8 ± 3.5</td>
<td>4.53</td>
</tr>
</tbody>
</table>

All values represent the mean of three animals ± SE (where given). Physiologic data of the animals represented in Fig. 4 is tabulated for each experimental group.

TABLE 2. Effect of estrogen treatment on in vivo intestinal absorption of ¹⁴C-labeled vitamin K₁ in castrate male rat

<table>
<thead>
<tr>
<th>Animal</th>
<th>Body Wt, g</th>
<th>Treatment</th>
<th>Mean Lymph Flow g/min</th>
<th>Duration of Expt, min</th>
<th>Total Lymph Collected, g</th>
<th>Total Vitamin K Absorbed, µg</th>
<th>Mean Vitamin K Absorbed, µg/g lymph</th>
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<tr>
<td>A</td>
<td>497</td>
<td>Control</td>
<td>0.012</td>
<td>165</td>
<td>1.986</td>
<td>11.60</td>
<td>5.84</td>
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<tr>
<td>B</td>
<td>502</td>
<td>Control</td>
<td>0.016</td>
<td>225</td>
<td>3.586</td>
<td>22.65</td>
<td>6.32</td>
</tr>
<tr>
<td>C</td>
<td>487</td>
<td>Estradiol</td>
<td>0.015</td>
<td>195</td>
<td>2.850</td>
<td>113.02</td>
<td>39.66</td>
</tr>
<tr>
<td>D</td>
<td>501</td>
<td>Estradiol</td>
<td>0.019</td>
<td>165</td>
<td>3.131</td>
<td>102.89</td>
<td>32.86</td>
</tr>
</tbody>
</table>

Physiologic data of the animals represented in Fig. 5 is tabulated for each individual animal.

cestrone-treated rats absorbed 60-70 µg in the same period. The continuation of the experiment for an additional 60 min did not significantly alter the results, as shown by control animal B (Table 2). Although the animals were quite similar in body weight and lymph flow (Table 2), the total vitamin K₁ absorbed within the time constraints of our experiment differed by as much as 50%. These results would seem to indicate some type of facilitatory action of estrogen with respect to vitamin K₁ absorption in the castrate rat.

DISCUSSION

It has been reported that intact male rats and castrate male and female rats are much more susceptible to uncontrolled hemorrhage due to coumarin anticoagulants and vitamin K deficiency than are intact female rats (4-6, 8). These results seem to indicate a protective function of estrogen in the development of hypoprothrombinemia in the rat.

We have further investigated this protective phenomenon in the rat and shown that ethynylestradiol (5-10 µg/day) with the diet does indeed affect the prothrombin-proconvertin levels during dietary vitamin K deficiency. Intact male and female rats placed on a vitamin K-free diet respond quite differently. The female rat maintains a plasma prothrombin-proconvertin level compatible with life whereas the male rat responds with a dramatic, precipitous drop in plasma prothrombin-proconvertin, predisposing the animals to hemorrhage. Castration of the animals 1 wk prior to administration of the vitamin K-free diet greatly reduces the hypoprothrombinemic response of the male rat, and the castrate males do not exhibit the rapid life-threatening plasma prothrombin depletion seen in the intact rat. However, the castrate females respond in much the same manner as the intact females.

Castrate male and female rats, when supplemented with ethynylestradiol (5-10 µg/day orally) appear to respond identically (within experimental error).

Because vitamin K is an essential vitamin to the rat for prothrombin synthesis and is normally acquired by enteral absorption, it was of great interest to study the effects of estrogen on the intestinal absorption of vitamin K₁. Analysis of thoracic duct lymph collected from control and estrogen-treated castrate male and female rats after intragastric injection of unlabeled vitamin K₁ or intraduodenal injection of ¹⁴C-labeled vitamin K₁ was rewarding. There is little doubt that estrogen treatment increases the absorption of vitamin K₁, as determined by either colorimetric or radioactivity spectrophotometric assay and values were converted to cumulative total micrograms of vitamin K₁ absorbed. Each curve represents a single animal.
treated, castrate male rats absorbed 24-27 μg of vitamin K, whereas nontreated controls absorbed essentially no detectable vitamin K.

In support of the colorimetric observations, the experiments using radioactive vitamin K, (¹⁴C uniform label in the phytol side chain) in castrate male rats probably show a more accurate physiologic response in that significant absorption is also detected in the nontreated controls. The estrogen-treated castrate rats absorbed 102-113 μg, whereas the nontreated controls absorbed only 22 μg during a longer period than that recorded for the estrogen-treated rats.

These data suggest that estrogen can assist in maintaining plasma prothrombin-proconvertin levels during vitamin K deficiency. Furthermore, one possible mechanism by which this may be accomplished is through a facilitatory action of estrogen on the intestinal absorption of vitamin K, permitting larger quantities of the vitamin to be absorbed in the presence of similar intraluminal concentrations.

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REFERENCES