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Uterine Estrogen Sulfatase May Play a More Important Role than the Hepatic Sulfatase in Mediating the Uterotropic Action of Estrone-3-Sulfate

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The estrogenic activity of sulfonated estrogens results from the release of active estrogens via desulfonation (hydrolysis) catalyzed by estrogen sulfatase. In this study, the relative importance of uterine or hepatic estrone (E_1)-3-sulfatase in mediating the uterotropic action of E_1-3-sulfate is evaluated by comparing its hormonal potency in animals that have comparable uterine E_1-3-sulfatase activity but markedly different hepatic enzyme activity. Liver microsomes from immature or adult female Sprague-Dawley rats contained 12- or 55-fold higher E_1-3-sulfatase activity, respectively, than the liver microsomes from immature or adult female CD-1 mice. In contrast, uterine whole homogenates from immature female Sprague-Dawley rats contained approx twofold higher E_1-3-sulfatase activity than was detected in the uterine whole homogenates from immature female CD-1 mice. It is estimated that the total E_1-3-sulfatase activity in the liver of an immature female rat or mouse is approx 1080- or 260-fold higher, respectively, than the activity in the uterus. The ED_{50} values for the uterotropic effect of E_1-3-sulfate and E_1 in immature female CD-1 mice were 240 and 8 pmol/g body wt, respectively, and the corresponding ED_{50} values in immature female Sprague-Dawley rats were 840 and 60 pmol/g body wt, respectively. The difference in the ratios of the uterotropic ED_{50} for E_1-3-sulfate over that for E_1 in immature rats and mice (14 and 30, respectively) is 1.14-fold, which correlates very closely with their difference in the uterine E_1-3-sulfatase activity (approx twofold), but not their difference in the hepatic sulfatase activity (approx 12-fold). The results of this study provide evidence suggesting that E_1-3-sulfatase in the uterus (an estrogen target organ) may play a more important role than the hepatic sulfatase in mediating the uterotropic action of sulfonated estrogens.

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Key Words: Estrogen sulfatase; estrogen sulfate; uterotropic action; estrogen metabolism in target tissues.

Abbreviations: E_1, estrone; E_2, estradiol; E_1-3-sulfate, estrone-3-sulfate; E_1-3-sulfatase, estrone-3-sulfatase; ED_{50}, the dose that elicits a 50% of the maximum response.

Introduction

In postmenopausal women, circulating levels of unmetabolized estradiol (E_2) or E_1 drop markedly as a result of diminished biosynthesis of estrogens in their gonads. However, the concentration of unmetabolized E_2 in some target tissues (e.g., uterine endometrium and breast tissues) of postmenopausal women is at least 10-fold higher than its circulating concentration *(1,2)* and is almost comparable to the tissue concentration found in premenopausal women *(3)*. It is believed that estrogen biosynthesis in extragonadal target tissues may contribute to the high levels of active estrogens found in these sites. Since E_1-3-sulfate is the most important form of circulating estrogens in postmenopausal women *(4–6)* and high levels of E_1-3-sulfate are also present in their breast tissues *(7,8)*, it is thought that desulfonation of estrogen sulfates may be an important source of active estrogens in addition to the *in situ* aromatization of androgens *(9–12)*. Several studies have suggested that estrogen formation in breast tumors of postmenopausal women through the desulfonation pathway may be more important than the aromatase pathway of estrogen formation *(13,14)*.

The sulfonated estrogens themselves have little or no estrogen receptor binding affinity, and their estrogenic activity is believed to result from the release of unconjugated estrogens catalyzed by estrogen sulfatase (depicted in Fig. 1). In a tissue or cell, the availability of E_1-3-sulfate (the substrate) and the amount of E_1-3-sulfatase (the enzyme) are two important factors that determine the rate of E_1 formation from E_1-3-sulfate. Given the large amounts of estrogen sulfates available in the blood *(4–6)* and target tissues *(7,8)* of postmenopausal women, the amount of E_1-3-sulfatase becomes a major variable for the formation

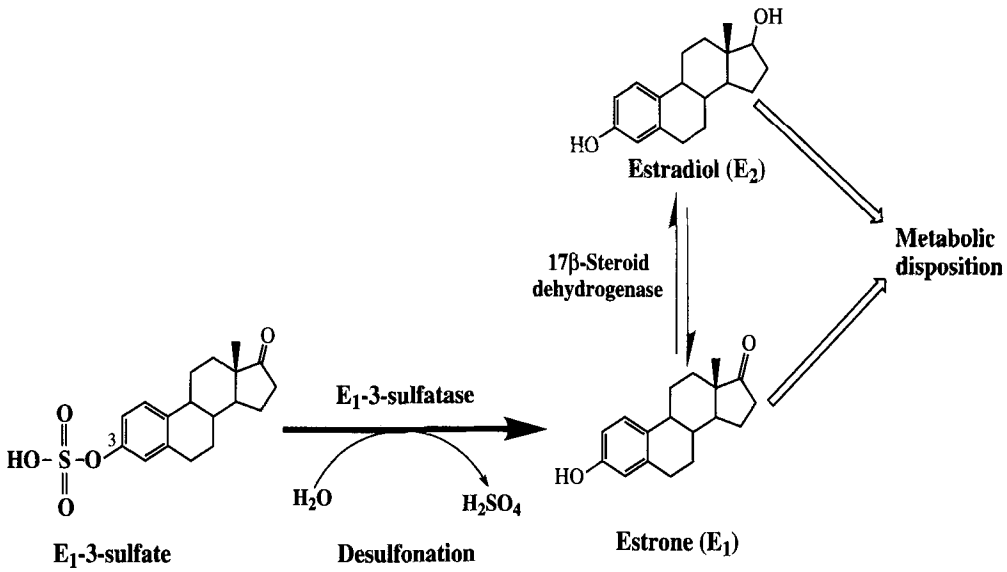

Fig. 1. Enzymatic desulfonation (hydrolysis) of E_1-3-sulfate catalyzed by E_1-3-sulfatase. As shown, the released E_1 can be further converted to E_2 (a more potent estrogen) by 17β-steroid dehydrogenase. The enzymatic desulfonation is a critical step for the formation of hormonally active estrogens (such as E_1 and E_2) from hormonally inactive estrogen sulfates. In a given tissue or cell, the availability of E_1-3-sulfate (the substrate) and the amount of E_1-3-sulfatase (the enzyme) are two important factors that determine the rate of E_1 formation from E_1-3-sulfate. The estrogenic potency of exogenously administered E_1-3-sulfate in a given target organ of different strains/species of animals would be affected by many factors, including: (1) the rate of metabolic desulfonation of E_1-3-sulfate; (2) the rate of interconversion between the E_1 and E_2; (3) the rate of metabolic disposition of E_1 and E_2, such as metabolism and excretion; and/or (4) the sensitivity of uterine estrogen receptors to E_1 and/or E_2. However, determining the ratio of the estrogenic potency of E_1-3-sulfate relative to that of E_1 will provide a parameter that grossly takes into account the above-mentioned variables in different strains/species of animals after E_1 is metabolically formed from E_1-3-sulfate. This parameter may be used to indicate the extent of desulfonation of E_1-3-sulfate in vivo.

of active estrogen. Previous studies have shown that liver and many extrahepatic target tissues or cells contain estrogen sulfatase activity *(8–12,15)*, but the enzyme activity in extrahepatic tissues is much lower than that in liver. The relative importance of E_1-3-sulfatase in a target organ vs liver for the hormonal action of E_1-3-sulfate was not known and has been investigated in this study. We compared the uterotropic potency of E_1-3-sulfate (relative to E_1) in Sprague-Dawley rats and CD-1 mice, which were found to have comparable levels of uterine E_1-3-sulfatase activity, but very different levels of hepatic E_1-3-sulfatase activity. Our data suggest that the uterine E_1-3-sulfatase may play a more important role than the hepatic sulfatase in mediating the uterotropic action of E_1-3-sulfate.

Results and Discussion

E_1-3-Sulfatase Activity in the Liver and Uterus of Female Mice and Rats

Earlier studies showed that the E_1-3-sulfatase activity in rat liver is mainly contained in the microsomal fraction *(9)*. We observed that liver microsomes from adult female CD-1 mice, Sencar mice, or C3H/OuJ mice contained similar levels of E_1-3-sulfatase activity (V_{max} = 35–96 pmol E_1 formed/mg protein/min), but their activity was only approx 1–2% of the activity found in liver microsomes from adult female Sprague-Dawley rats or Long-Evans rats (Table 1). Liver

Table 1
Kinetic Parameters (V_{max}, K_m, and V_{max}/K_m) for Liver Microsomal E_1-3-Sulfatase Activity in Three Strains of Adult Female Mice and Two Strains of Adult Female Rats[a]

Species/strains	V_{max} pmol E_1 formed/ mg protein/min	K_m μM	V_{max}/K_m
CD-1 mice	92.1	1.6	57.6
Sencar mice	96.0	2.0	48.0
C3H/OuJ mice	34.8	3.5	9.9
Sprague-Dawley rats	4969	14.9	333.5
Long-Evans rats	6144	10.1	608.3

[a]The V_{max} and K_m values were calculated based on the Eadie-Hofstee analysis of the liver microsomal desulfonation of increasing concentrations (from 1.25–40 μ*M*) of [6,7-^{3}H]E_1-3-sulfate (kinetic curves not shown). Liver microsomes were prepared from pooled liver tissues from adult female rats or mice. The incubation mixtures consisted of 0.1–0.2 mg microsomal protein and different concentrations of E_1-3-sulfate (containing 0.2 μCi [6,7-^{3}H]E_1-3-sulfate) in a final volume of 1.0 mL Tris-HCl buffer (100 m*M*, pH 7.4). The incubation conditions and assay methods are described in the Materials and Methods section. The rate of microsomal desulfonation of E_1-3-sulfate at each substrate concentration was determined based on replicate measurements. The average intraassay variation was 3.7%.

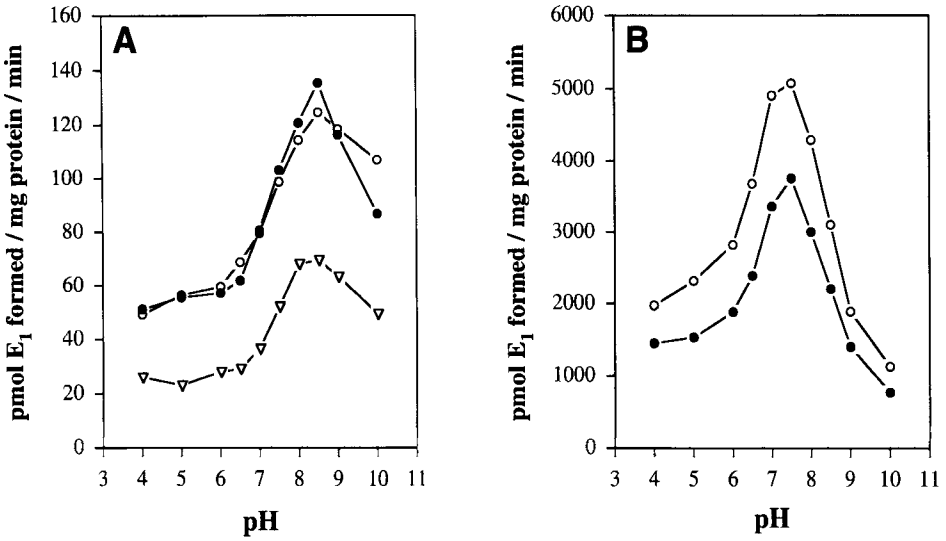

Fig. 2. Different pH optima for the desulfonation of E_1-3-sulfate in vitro by liver microsomes from adult female mice (**A**) and adult female rats (**B**). The incubation mixture consisted of 0.1–0.2 mg microsomal protein and 30 μ*M* E_1-3-sulfate (containing 0.2 μCi [6,7-^{3}H]E_1-3-sulfate) in a final volume of 1.0 mL Tris-HCl buffer (100 m*M*, pH 7.4). The incubation conditions and assay methods are described in the Materials and Methods section. Each point is the mean of duplicate determinations. (A) ○ Female CD-1 mice, ● female Sencar mice, ▽ female C3H/OuJ mice; (B) ○ female Long-Evans rats, ● female Sprague-Dawley rats.

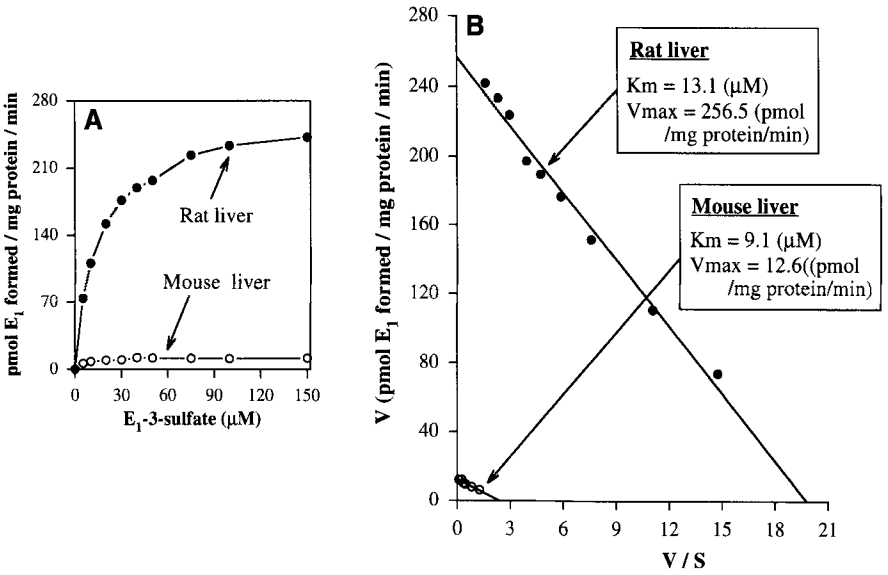

Fig. 3. Eadie-Hofstee analysis of the desulfonation of E_1-3-sulfate in vitro by crude liver homogenates from immature female CD-1 mice and immature female Sprague-Dawley rats (**B**). Panel (**A**) shows the rates of enzymatic desulfonation as a function of E_1-3-sulfate substrate concentration. The incubation mixture consisted of 0.2 mg cellular protein and 5–150 μ*M* E_1-3-sulfate (containing 1.0 μCi [6,7-^{3}H]E_1-3-sulfate) in a final volume of 0.5 mL Tris-HCl buffer (100 m*M*, pH 7.4). The incubation conditions and assay methods are described in the Materials and Methods section. Each point represents the mean of triplicate determinations.

microsomal E_1-3-sulfatase of these three strains of adult female mice had consistently lower K_m values than that of the two strains of female rats (Table 1). Moreover, the pH optima and the pH-dependence curves for liver microsomal E_1-3-sulfatase of the three strains of mice were consistently different from those for liver microsomal sulfatase of the two strains of rats (Fig. 2). These data suggest that the enzyme content and the biochemical characteristics of liver microsomal E_1-3-sulfatase in adult female rats and mice are substantially different. It is noteworthy that the K_m values determined in this study for liver microsomal E_1-3-sulfatase of female rats are in close agreement with those reported earlier *(9)*, but few studies have been published on characterizating the hepatic microsomal E_1-3-sulfatase of female mice.

We also evaluated E_1-3-sulfate activity in the liver and uterus (a classical estrogen target organ) of immature female CD-1 mice and immature female Sprague-Dawley rats. The desulfonation of increasing concentrations of E_1-3-sulfate (from 5–150 μ*M*) catalyzed by hepatic whole homogenates of immature female mice or rats displayed

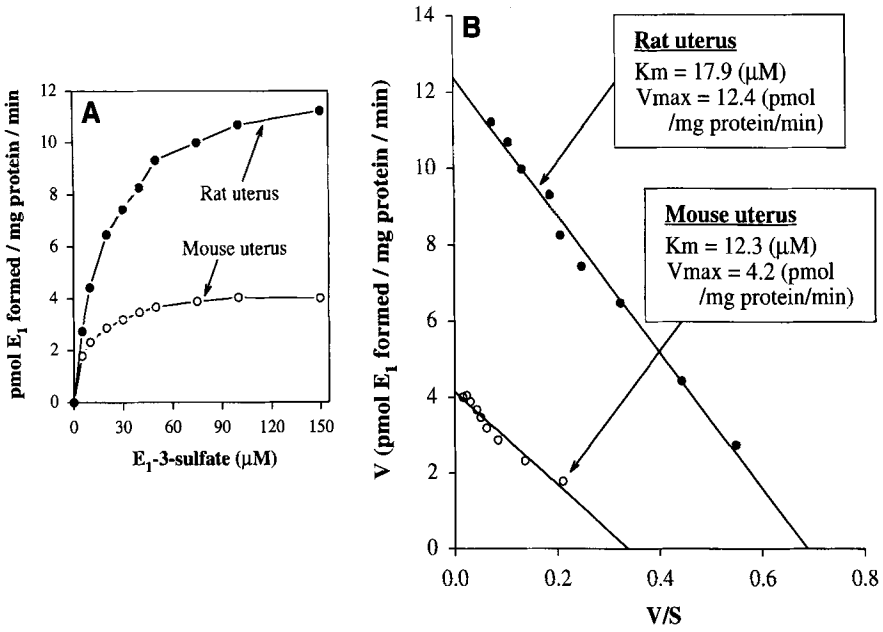

Fig. 4. Eadie-Hofstee analysis of the desulfonation of E_1-3-sulfate in vitro by crude uterine homogenates from immature female CD-1 mice and immature female Sprague-Dawley rats (**B**). Panel (**A**) shows the rates of enzymatic desulfonation as a function of E_1-3-sulfate substrate concentration. The incubation mixture consisted of 0.3–0.5 mg cellular protein and 5–150 μ*M* E_1-3-sulfate (containing 1.0 μCi [6,7-^{3}H]E_1-3-sulfate) in a final volume of 0.5 mL Tris-HCl buffer (100 m*M*, pH 7.4). The incubation conditions and assay methods are described in the Materials and Methods section. Each point represents the mean of triplicate determinations.

typical Michaelis-Menton's curve patterns (Fig. 3). The V_{max} and K_m values for the hepatic E_1-3-sulfatase of CD-1 mice were 12.6 pmol E_1 formed/mg protein/min and 9.1 μ*M*, respectively, and the corresponding values for Sprague-Dawley rats were 256.5 pmol E_1 formed/mg protein/min and 13.1 μ*M*, respectively (Fig. 3). The substrate concentration-dependent curves and the V_{max} and K_m values for the uterine E_1-3-sulfatase of immature CD-1 mice and immature Sprague-Dawley rats are shown in Fig. 4. The V_{max} for E_1-3-sulfatase activity in the crude hepatic homogenates of immature rats is about 12-fold higher than that of immature mice.

It is estimated that the total E_1-3-sulfatase activity (calculated based on the V_{max}) in the liver of an immature female rat or mouse is approx 1319 or 91 pmol E_1 formed/min/g body wt, respectively. In contrast, the total activity of E_1-3-sulfatase in the uterus of an immature rat or mouse is only 1.22 or 0.35 pmol E_1 formed/min/g body wt, respectively. These data suggest that the total hepatic E_1-3-sulfatase activity of an immature female rat or mouse is approx 1080- or 260-fold higher, respectively, than their total activity in uterus.

In summary, liver microsomes from immature or adult female Sprague-Dawley rats contained 12- or 55-fold higher E_1-3-sulfatase activity, respectively, than liver microsomes from immature or adult female CD-1 mice. In contrast, uterine whole homogenates from immature female Sprague-Dawley rats contained about twofold higher E_1-3-sulfatase activity than was detected in the uterine whole homogenates from immature female CD-1 mice. It is estimated that the liver of an immature female rat or mouse may contain approx 1080- or 260-fold higher E_1-3-sulfatase activity, respectively, than the uterus.

Uterotropic Activity of E_1-3-Sulfate in Immature Female Mice and Rats

The estrogenic activity of E_1-3-sulfate results from desulfonation of E_1-3-sulfate to E_1 catalyzed by E_1-3-sulfatase (depicted in Fig. 1). Earlier studies showed that rat liver contains very high levels of E_1-3-sulfatase activity compared with other organs of the same animal *(9)*. Consistent with this earlier report, results of our present study showed that a whole liver of a female Sprague-Dawley rat or a CD-1 mouse contains approx 1081- or 260-fold higher E_1-3-sulfatase activity, respectively, than the uterus. If the liver is the major site for E_1-3-sulfate desulfonation in vivo, then the extent of hydrolysis of exogenously administered E_1-3-sulfate in female Sprague-Dawley rats and female CD-1 mice would be markedly different (which, in turn, would affect the estrogenic potency of E_1-3-sulfate in their target organs). In this study, by determining the uterotropic potency of E_1-3-sulfate relative to that of E_1 in female Sprague-Dawley rats and female CD-1 mice, we evaluated the relative importance of metabolic hydrolysis of E_1-3-sulfate in uterus vs liver for its uterotropic activity.

We first compared the uterotropic potency of E_1 in immature CD-1 mice and Sprague-Dawley rats. The control uterine wet weights of these mice and rats (body wt-adjusted) were 1.11 ± 0.11 and 1.04 ± 0.13 mg/g body wt, respectively (Fig. 5). Twenty-four hours after receiving an sc injection of increasing doses of E_1, the uterine wet weights of immature female mice and rats were increased in a dose-dependent manner, and the maximum increase (% over control) was similar (Fig. 5A,C). However, the ED_{50} values

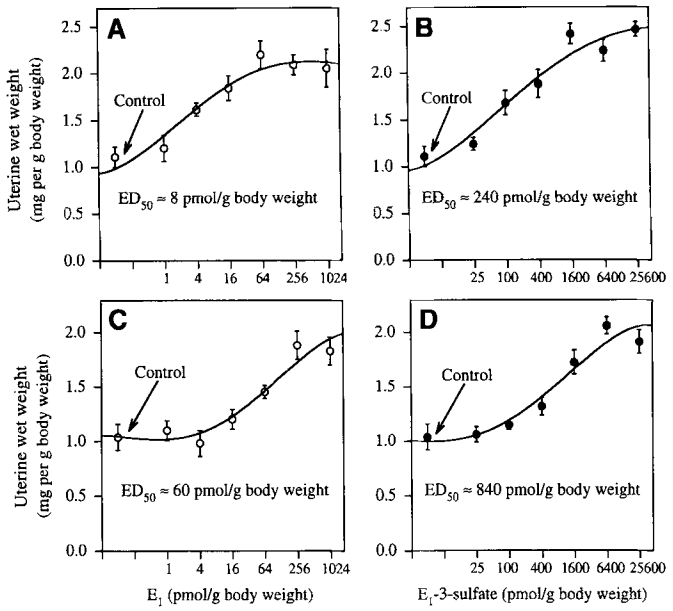

Fig. 5. The uterotropic effect of E_1 and E_1-3-sulfate in immature female CD-1 mice (panels **A** and **B**) and immature female Sprague-Dawley rats (panels **C** and **D**). Each dose group contained 10–12 mice or 8 rats. Twenty-four hours after an sc injection of increasing doses of E_1 or E_1-3-sulfate, the animals were sacrificed. The uterus was immediately removed from the animal, the surrounding connective tissues were trimmed, and the wet wt of each uterus was measured. The ED_{50} values were determined based on the computer-assisted simulation of the sigmoid dose–response curves shown above.

(body wt-adjusted) for E_1 in stimulating the uterine growth in immature rats and mice were approx 60 and 8 pmol/g body wt, respectively (Fig. 5A,C), suggesting that the in vivo uterotropic activity of E_1 in immature female rats is markedly less potent than that in immature female mice. The different uterotropic potency of E_1 in female rats and mice (Fig. 5A,C) may be attributed to these following factors:

1. Differences in the metabolic interconversion of E_1 and E_2 (a more potent estrogen than E_1) catalyzed by 17β-steroid dehydrogenase (depicted in Fig. 1);
2. Differences in the metabolic inactivation (e.g., hydroxylation and conjugation metabolism) of E_1 and E_2;
3. Differences in the tissue distribution and excretion of E_1 and E_2; and/or
4. Differences in the sensitivity of uterine estrogen receptors to E_1 and/or E_2.

Because these possible differences could markedly affect the potency of E_1 in stimulating uterine growth in mice and rats, assaying the uterotropic activity of administered E_1-3-sulfate in these animals would not provide a parameter that directly implies the extent of E_1 formation in vivo via desulfonation of E_1-3-sulfate. However, determining the ratio of the ED_{50} for E_1-3-sulfate relative to that for E_1 may provide a parameter that directly tells how many fold of an E_1-3-sulfate dosage (relative to E_1) is required to elicit the same magnitude of a uterotropic response produced by E_1. Moreover, since this parameter grossly takes into account the possible differences after E_1 is metabolically formed in vivo from E_1-3-sulfate, it may be useful for indicating the extent of E_1-3-sulfate desulfonation in vivo.

Twenty-four hours after an sc injection of increasing doses of E_1-3-sulfate to the mice and rats, their uterine wet weights were increased in a dose-dependent manner, and the curve patterns were very similar to those of E_1 (Fig. 5B,D). The maximum increase of uterine wet weight in mice and rats was 118 and 81%, respectively, over the corresponding controls, and the ED_{50} values for E_1-3-sulfate in mice and rats were approx 240 and 840 pmol/g body wt, respectively (Fig. 5B,D). The ratio of the ED_{50} for E_1-3-sulfate over that for E_1 is 30 in female CD-1 mice and 14 in female Sprague-Dawley rats.

To understand the above data with respect to the extent of in vivo desulfonation of E_1-3-sulfate, let us first assume that the uterine E_1-3-sulfatase were of negligible importance in determining the concentrations of unconjugated estrogens in the uterus (since its total activity is only <1/1081 of the activity in a rat liver and <1/260 of that in a mouse liver). Under this assumption, E_1 would be formed from E_1-3-sulfate in liver (the major site) and other extrahepatic tissues (probably to a much less extent), then released into systemic circulation, and subsequently transported to the uterus to elicit a uterotropic response mediated by estrogen receptors. If this assumption correctly implies what is really happening in vivo, then our data on the ratios of the uterotropic potency of E_1-3-sulfate relative to that of E_1 would suggest that approx 1 out of every 30 administered E_1-3-sulfate molecules is converted to E_1 in female mice and 1 out of every 14 E_1-3-sulfate molecules is converted to E_1 in female rats. Accordingly, the estimated overall extent of in vivo desulfonation of E_1-3-sulfate (body wt-adjusted) in female Sprague-Dawley rats would be approx onefold higher than that in female CD-1 mice. This conclusion (based on the above assumption) is not in agreement with the ~12-fold higher liver microsomal E_1-3-sulfatase activity found in female Sprague-Dawley rats than that in female CD-1 mice, which suggests that the original assumption likely is incorrect.

An alternative possibility would be that the unconjugated E_1 contained in the uterus is, to a very significant extent, formed *in situ* by the uterine E_1-3-sulfatase (illustrated in Fig. 6). If this is true, then the ratio of the uterotropic ED_{50} for E_1-3-sulfate relative to that for E_1 would primarily reflect the desulfonation of E_1-3-sulfate in the uterus (which determines the amount of E_1 formed in the uterus and therefore the uterotropic activity), and this ratio may not be in agreement with the overall extent of E_1-3-sulfate hydrolysis in the body. The results of this study showed that the difference in the ratios of the ED_{50} for E_1-3-sulfate over that for E_1 in female rats and mice (difference 114%; Fig. 5) positively correlates with their approx twofold differ-

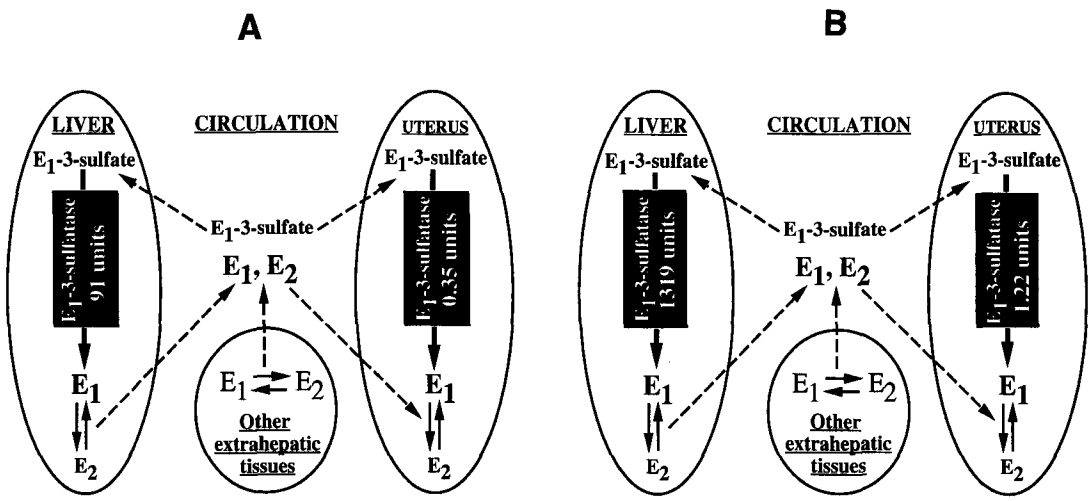

Fig. 6. The relative contributions of the hepatic and uterine E_1-3-sulfatase for the concentrations of E_1 plus E_2 in the uterus of immature female CD-1 mice (**A**) and immature female Sprague-Dawley rats (**B**). The units for the total hepatic or uterine E_1-3-sulfatase activity in mice and rats are calculated based on the V_{max} values and expressed as pmol E_1 formed/min/g body wt. If the uterine E_1-3-sulfatase was of negligible importance for the *in situ* formation of $E_1 + E_2$, then E_1 (and E_2) would be produced mainly in liver (catalyzed by hepatic E_1-3-sulfatase), then released into systemic circulation, and subsequently transported to the uterus. Under this assumption, the ratio of the uterotropic ED_{50} for E_1-3-sulfate over that for E_1 would largely reflect the hepatic desulfonation of E_1-3-sulfate in vivo, which governs the concentration of $E_1 + E_2$ in circulation and uterus, and ultimately the uterotropic activity of E_1-3-sulfate. Alternatively, if the majority of $E_1 + E_2$ in uterus originates from the *in situ* desulfonation of E_1-3-sulfate by uterine E_1-3-sulfatase, then the ratio of the uterotropic ED_{50} for E_1-3-sulfate relative to that for E_1 would primarily reflect the hydrolysis of E_1-3-sulfate in this target organ. Theoretically, there is another possibility that the uterine concentration of $E_1 + E_2$ is contributed equally by both hepatic and uterine hydrolysis of E_1-3-sulfate. A comparison of the uterotropic potency of E_1-3-sulfate in CD-1 mice and Sprague-Dawley rats with their E_1-3-sulfatase activities in uterus and liver suggests that the uterine E_1-3-sulfatase is largely responsible for the active estrogens formed in this organ.

ence in uterine E_1-3-sulfatase activity, but not with their ~12-fold difference in hepatic sulfatase activity. The close correlation of the uterotropic activity of E_1-3-sulfate (relative to E_1) with uterine E_1-3-sulfatase activity led us to suggest that the uterine E_1-3-sulfatase may play a major role in mediating the uterotropic action of E_1-3-sulfate, although its total activity is much less than the total hepatic E_1-3-sulfatase activity. Figure 6 depicts the above two possibilities in female CD-1 mice and female Sprague-Dawley rats.

Several reasons (discussed below) may explain why the uterine E_1-3-sulfatase plays a more important role than hepatic sulfatase for the uterotropic action of E_1-3-sulfate.

1. Active estrogens formed in estrogen target cells are immediately available for interaction with the intracellular estrogen receptor leading to the expression of hormonal responses. In contrast, the situation is very different in liver, because the released estrogens need to be transported through systemic circulation to target sites where they exert their action. During this process, active estrogens are diluted as a result of systemic distribution. Moreover, a majority (>95%) of unconjugated E_1 and E_2 in the blood are tightly bound to plasma proteins. Consequently, only a very small fraction of the active estrogens formed in liver may reach a given target tissue.
2. The free estrogens formed in liver are subject to hepatic metabolic inactivation and also inactivation in extrahepatic tissues (probably to a much lesser extent) during systemic distribution. Therefore, the active estrogens formed in the target site would have much higher bioavailability to the estrogen receptor (where the hormonal responses are initiated), although the total amount of active estrogens formed in a given target organ may be much less than that formed in liver.
3. Earlier studies reported that E_1-3-sulfate can be taken up by several human breast cancer cell lines in culture *(8)* and by rabbit uterus in vivo *(16)*. Consistent with these observations, very high concentrations of estrogen sulfates were found to be present in breast tissues of postmenopausal women *(7,8)*. The available high concentrations of E_1-3-sulfate (a substrate for enzymatic desulfonation) could be another important factor favoring the *in situ* formation of E_1 from E_1-3-sulfate.

Finally, it is noteworthy that although the interspecies differences were grossly taken into consideration in this study by comparing the uterotropic potency of E_1-3-sulfate relative to E_1 in mice and rats, the data presented would be more convincing if the comparison were made in the same species/strain of animals. It will be, therefore, of interest to selectively modulate the levels of E_1-3-sulfatase in estrogen target organs or liver and then compare the hormonal potency of E_1-3-sulfate in these animals. Comparing the pharmacokinetics of radiolabeled E_1-3-sulfate (such as the levels of E_1-3-sulfate and E_1 in blood and tissues) in female mice and rats may also be useful in verifying the relative importance of uterine E_1-3-sulfatase as revealed in this study.

Conclusions

The results of our present study showed that liver microsomes from immature or adult female Sprague-Dawley rats contained approx 12- or 55-fold higher E_1-3-sulfatase activity (pmol E_1 formed/mg protein/min), respectively, than liver microsomes from immature or adult female CD-1 mice, but their uterine E_1-3-sulfatase activity was quite comparable (difference approx twofold). It is estimated that the liver of a female rat or mouse may contain at least 260- to 1081-fold higher E_1-3-sulfatase activity than their uterus. The difference in the ratios of the uterotropic ED_{50} for E_1-3-sulfate relative to E_1 in immature rats and mice correlates very closely with their difference in the uterine E_1-3-sulfatase activity, but not with their difference in the hepatic sulfatase activity. The results of this study provide evidence suggesting that although the activity of E_1-3-sulfatase in a target organ is much lower than that in liver, it may play a more important role than the hepatic sulfatase in mediating the hormonal action of estrogen sulfates in this target organ.

Materials and Methods

Chemicals

E_1-3-sulfate (sodium salt) and E_1 were purchased from Sigma Chemical Co. (St. Louis, MO). [6,7-^{3}H]E_1-3-sulfate (SA ~57 Ci/mmol) was obtained from Du Pont New England Nuclear Research Products (Boston, MA). All solvents (HPLC-grade) used in this study were obtained from Fisher Scientific Co. (Springfield, NJ).

Animals

Adult (8-wk-old) female Long-Evans rats and adult (8-wk-old) or immature (18-d-old) female Sprague-Dawley rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Adult (8-wk-old) female Sencar mice and adult (8-wk-old) or immature (18-d-old) female CD-1 mice were purchased from Charles River Laboratories (Wilmington, MA). Adult (8-wk-old) female C3H/OuJ mice were purchased from Jackson Laboratory (Bar Harbor, ME). The animals were housed in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care, kept on a 12-h light–12-h dark cycle, and allowed free access to drinking water and Purina Laboratory Chow (prepared by Ralston Purina Co., St. Louis, MO). The animals were allowed to acclimatize for 5 d before use in the experiment. The animal experiments reported in this study were performed in adherence to the guidelines established in the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health (NIH).

Preparation of Liver Microsomes or Uterine Whole Homogenates

In this study, liver microsomes from two strains (Sprague-Dawley or Long-Evans) of adult female rats and three strains (CD-1, Sencar, and C3H/OuJ) of adult female mice were prepared. Liver samples from 4 adult female rats or from 10 adult female mice of each strain were pooled together and homogenized with a Brinkmann homogenizer in 4 vol of 20 m*M* Tris-HCl buffer (ice-cold, pH 7.4) containing 0.25 *M* sucrose. Hepatic microsomes were prepared by differential centrifugations according to the procedures described previously *(17)*.

The hepatic whole homogenates were prepared from pooled liver tissues from 5 immature female Sprague-Dawley rats or 10 immature female CD-1 mice. The pooled liver tissues were homogenized with a Tri-R homogenizer (Model K41) in 4 vol of ice-cold 20 m*M* Tris-HCl/0.25 *M* sucrose solution. Aliquots of the hepatic whole homogenates (in 20 m*M* Tris-HCl/0.25 *M* sucrose solution) were then stored at –80°C.

The uterine whole homogenates were prepared from pooled uterine tissues from 20 immature female Sprague-Dawley rats or 40 immature female CD-1 mice. The uterine tissues (after trimmed off connective tissues) were well minced with a sharp eye-surgery scissor, and then homogenized with a Tri-R homogenizer (Model K41) in 4 vol of ice-cold 20 m*M* Tris-HCl/0.25 *M* sucrose solution. Aliquots of the uterine whole homogenates (in 20 m*M* Tris-HCl/0.25 *M* sucrose solution) were then stored at –80°C. Immediately prior to the assay of E_1-3-sulfatase activity, the uterine whole homogenates were quickly thawed in a 37°C water bath, diluted with 0.2 *M* Tris/HCl buffer (ice-cold, pH 7.4) to a protein concentration of 0.6 mg/mL, and homogenized once again with a Tri-R homogenizer (Model K41) for about 2 min.

The protein concentrations in liver microsomes and in hepatic or uterine whole homogenates were determined by the Bio-Rad protein assay kit (Hercules, CA) with bovine serum albumin (BSA) as a standard.

Assay of E_1-3-Sulfatase Activity

E_1-3-sulfatase activity in liver microsomes or uterine whole homogenates of the rats and mice was determined by measuring the formation of ^{3}H-labeled nonpolar metabolites (mainly [6,7-^{3}H]E_1) from [6,7-^{3}H]E_1-3-sulfate (the substrate) according to the method described in a previous study *(18)*. The incubation mixture consisted of 0.1–0.5 mg cellular protein (liver microsomes or whole tissue homogenates) and 1.25–150 μ*M* E_1-3-sulfate (containing 0.2–1.0 μCi [6,7-^{3}H]E_1-3-sulfate) in a final volume of 1.0 mL Tris-HCl buffer (25 m*M*, pH 7.4). The incubation was carried out at 30°C for 15 min. After incubation, the test tubes were immediately placed on ice and were extracted with 5 mL ice-cold toluene. Aliquots of the organic supernatants were removed for radioactivity measurement. Blank values were determined for each individual assay in the absence of microsomes or whole-tissue homogenates and were subtracted. The estrogen sulfatase activity in microsomes or whole-tissue homogenates was expressed as pmol E_1 formed/mg protein/min.

Assay of Uterotropic Response of Female Rats and Mice to E_1 and E_1-3-Sulfate

Female 23-d-old Sprague-Dawley rats (weighing 50–60 g) and female 23-d-old CD-1 mice (weighing 10–13 g) received an sc injection of E_1 (0, 1, 4, 16, 64, 256, or 1024 pmol/g body wt, dissolved in ~100 µL of 30% aqueous ethanol) or E_1-3-sulfate (0, 25, 100, 400, 1600, 6400, or 25,600 pmol/g body wt, dissolved in ~100 µL of 0.9% saline). Each dose group contained 8 female Sprague-Dawley rats or 10–12 female CD-1 mice. Twenty-four hours after an injection of E_1 or E_1-3-sulfate, the animals were sacrificed by euthanasia in a CO_2 chamber. The uterus was immediately removed, the surrounding connective tissues were trimmed, and the wet wt of each uterus was measured. We chose to measure the uterine wet wt at 24 h after an ip injection of E_1 or E_2, because an earlier study showed that the maximal increase of the uterine wet wt occurred at 24–30 h after a single injection of an estrogen *(19)*. The ED_{50} values for E_1-3-sulfate and E_1 were determined based on the computer-assisted curve simulation of the sigmoid dose–response curves in which the *x*-axis was the dose of E_1-3-sulfate in a logarithmic scale and the *y*-axis was the uterotropic response in a linear scale.

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