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Sulfamates of Various Estrogens are Prodrugs with Increased Systemic and Reduced Hepatic Estrogenicity at Oral Application

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Oral therapy with natural or synthetic estrogens, like ethinylestradiol, suffers from low, suboptimally defined bioavailability and excess hepatic estrogen actions. *N,N*-alkylated and non-alkylated sulfamates of ethinylestradiol, estradiol and estrone overcome these deficiencies. Ovariectomized Wistar rats ($n = 6$–7/group) were orally treated for 7 days, and killed on day 8, plasma was gained on days 0, 4, and 8. Systemic estrogenicity was quantified by assessment of uterine weight, vaginal cornification, and measurement of gonadotropins by homologous RIA. Estrogenicity in the liver was analysed. Angiotensinogen was estimated by RIA of angiotensin-1 after incubation of EDTA-plasma with porcine renin. Total and high-density cholesterol were measured by enzymatic methods. Preliminary biotransformation studies were performed after oral administration of 10 μg, 5 μCi [2,4,6,7-^{3}H]estradiol sulfamate. Ethinylestradiol led to distinct elevation of angiotensin-1 and dramatic depression of cholesterol fractions, reflecting hepatic estrogen effects, already at doses with marginal systemic effects. Estradiol and estrone had systemic and hepatic estrogenic activity at much higher doses only. Estrogen sulfamates had systemic estrogen activity 10–90-fold above that of their parent estrogen. Non-alkylated sulfamates of given estrogens were more active than *N*-alkylated ones. Elevation of systemic estrogen activity was always combined with a dramatic *reduction* of hepatic estrogenicity. Estradiol sulfamate had a 90-fold elevated systemic estrogen activity vs estradiol, but lacked hepatic activity including the 30-fold dose inducing vaginal response. Three hours after administration no unchanged estradiol sulfamate was detectable in plasma. Rather peaks, probably representing estradiol and estrone, were found. Estrogen sulfamates are considered prodrugs of their parent estrogen, which do not interact with any liver function during the first-pass. They represent a new strategy of oral hormone administration. Their main potential seems to be the systemic generation of natural estrogens when used in oral contraceptives.

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INTRODUCTION

The main therapeutically used estrogens comprise natural estrogens and a few "synthetic" ones, ethinylestradiol, mestranol and the non-steroidal diethylstilbestrol. Natural as well as non-natural estrogens dominate certain therapeutic areas for good reasons. The use of synthetic estrogens is restricted to contraception and tumor therapy. Their preference for these indications is certainly due to their superior and more reliable inhibitory effect on the hypothalamic–pituitary–gonadal axis. Their advantage versus natural estrogens is a much higher, i.e. better defined, oral bioavailability and a longer retention in target tissues [1, 2]. Successful attempts to develop hormonal contraceptives with natural estrogens are not known. Very careful pharmacokinetic studies with orally administered estradiol led to the conclusion that inter- and intra-individual variability of pharmacokinetic parameters is so substantial that it is *theoretically*

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impossible to develop estradiol-containing oral contraceptives [3].

The disadvantage of synthetic estrogens are effects on metabolic functions. Their physiological background are sexually dimorphic liver functions which are determined by male and female gonadal hormones. The pharmacology of these dimorphisms is complex due to interaction of pituitary hormones as growth hormone or prolactin [4, 5] and other hormones as insulin, glucocorticoids, thyroid hormones. The role of these factors for the transcription of the estrogen modulated angiotensinogen gene in the liver is well established [6, 7].

The synthesis of important factors of the blood clotting system is estrogen-modulated. This also applies to that of various lipoproteins, angiotensinogen, CBG, SHBG, thyroxin-binding globulin, coeruloplasmin, and various enzymes of the cytochrome *P*450-system [1, 8]. Ethinylestradiol or DES have much higher hepato-cellular than systemic estrogenicity: in relation to FSH-secretion inhibitory activity these estrogens are 4–18 times more active in the liver than estrone sulfate [8]. A similar ratio of systemic and hepatic effects was estimated comparing ethinylestradiol and estradiol [1].

The main identified risk arising from the use of combined oral contraceptives is an increased cardiovascular morbidity. The use of natural estrogens in hormone replacement therapy was the opposite effect [9, 10]. There is consensus that oral contraception related cardiovascular risks are not dependent on the duration of contraception and cease with the end of their use. This was considered as strong evidence of a thrombotic origin of complications like myocardial infarction. It was concluded that the estrogen component in contraceptives is to be blamed for this problem [11].

The discussed problems of *oral* estrogen therapy were the main object of our research. The conventional oral treatment with natural estrogens overcomes the barrier represented by the liver by mere mass of the administered hormone. The pharmacodynamic implication of this strategy are distinct alterations of estrogen regulated liver functions. These can be avoided, when an equivalent systemic estrogen action is achieved by parenteral administration [10, 12].

Natural estrogens are extensively metabolized at first liver passage [13]. The only *established* means to obtain *high* oral bioavailability of steroidal estrogens is the prevention of their biotransformation in the liver at first passage. Oxidation of the tertiary 17β-hydroxy group of ethinylestradiol is not possible. Impaired oxidation of 17β-hydroxyl group compared to estradiol-17β was seen in case of a 14α,15α-methylene estradiol (J 824) using human placental 17β-OH-steroid dehydrogenase (G. Kaufmann, Jena*pharm*, unpublished data). This compound is orally active, with pronounced effects on hepatic functions [14]. It thus seems that impairment of 17-hydroxy oxidation, independent of its mechanism, leads to increased oral activity, but implies profound alteration of pharmacodynamic and pharmacokinetic properties of corresponding molecules compared to systemically generated estradiol.

There is a much prolonged retention of ethinylestradiol at the cellular level [1, 15]. Prolonged retention of ethinylestradiol in target cells, together with high concentrations in the portal vein blood during resorption from the gastro-intestinal tract are the likely cause of excessive hepatic estrogenicity. The contribution of first liver passage versus inherent properties of ethinylestradiol are not undisputed. Studies comparing oral and vaginal administration of ethinylestradiol [16] demonstrated excessive hepatic estrogen effects with both routes of administration.

The question has to be raised, whether elevated hepatic estrogenicity is an unavoidable problem of good oral activity. This paper demonstrates that chemically modified, *per se* inactive derivatives of sulfates of natural and artificial estrogens, have an as yet unrecognized potential to overcome the liver barrier without compromising important liver functions. Estrogen sulfamates (see Fig. 1) may be used for a new treatment strategy to achieve an efficient estrogen treatment which avoids the above described liver–hormone and hormone–liver interactions. The presented material from studies with 3-sulfamates of estradiol, estrone, and ethinylestradiol is selected from results of ongoing research on novel estrogens for oral contraceptives and hormone replacement therapy. In particular, various steroidal sulfamates have been investigated since *N*,*N*-dialkylated sulfamates of ethinylestradiol, the first steroid sulfamates described, were found to be strong estrogens with a prolonged effect and antifertility activity upon oral administration [17, 18].

For electronic reasons the sulfamate moiety was suggested to act as a hydrogen acceptor, thus fulfilling prerequisites for a considerable binding with blood proteins. In fact erythrocytes were found to bind a substantial amount of administered *N*,*N*-alkylated ethinylestradiol sulfamates or their metabolites, respectively [17]. Pharmacological evaluation and synthesis of new compounds was resumed when it was found that one compound (J 271) had dissociated hepatocellular and systemic estrogenic activity [19].

Manifestation of hepatic estrogenicity varies determined by systemic or oral route of administration and by the chemical constitution of estrogen. Humans and rats show analogous responses in this respect. Strong evidence for direct hepato-cellular effects with respect to a given estrogen responsive parameter is a pronounced decrease of the ratio of systemic to hepatic activity at oral administration. The induction of angiotensinogen secretion represents a proven hepatocellular effect of estrogens [7, 20, 21]. The decrease of total and HDL cholesterol in the blood of estrogen treated ovariectomized rats is very prominent at

oral treatment suggesting a direct estrogen effect on lipoproteins in the liver [18, 19].

MATERIALS AND METHODS

Chemistry

The *N,N*-dimethyl and *N,N*-diethyl sulfamates of estrone (J 922, J 804) and the 3-*N,N*-dimethyl, 3-*N,N*-diethyl and 3-*N,N*-tetramethylene sulfamates of ethinylestradiol (J 665, J 271, J 272) were synthesized from the parent steroids and the corresponding sulfamoyl chlorides by phase transfer technique (X). Thus, at 60–80°C, a solution of the steoid and the *N,N*-disubstituted sulfamoyl chloride (1.5 equiv.) in toluene was intensively stirred with an aqueous solution containing triethyl benzylammonium chloride (0.1 equiv.) and sodium hydroxide (0.75 equiv.). Additional aqueous sodium hydroxide solution (1.5 equiv.) was added within a range of 2 h. Conventional work-up after TLC control and crystallization gave the desired esters.

Estrone *N,N*-dimethyl sulfamate (J 992): mp. 190–194°C (AcOEt); $[\alpha]_D^{20}$ + 116° (*c* = 1, Chf).
Estrone *N,N*-diethyl sulfamate (J 804): mp. 176–180°C (MeOH); $[\alpha]_D^{23}$ + 109° (*c* = 1, Chf).
Ethinylestradiol 3-*N,N*-dimethyl sulfamate (J 665): mp. 156–158°C (MeOH); $[\alpha]_D^{23}$ + 5° (*c* = 1, Chf).
Ethinylestradiol 3-*N,N*-diethyl sulfamate (J 271): mp. 113–115°C (*i*-Pr_2O); $[\alpha]_D^{23}$ + 3° (*c* = 1, Chf).
Ethinylestradiol 3-*N,N*-tetramethylene sulfamate (J 272): mp. 121–122°C (Et_2O); $[\alpha]_D^{23}$ + 10° (*c* = 1, Chf).

Estrone sulfamate (J 994) was prepared by treating a solution of estrone in dimethyl formamide with sulfamoyl chloride (5 equiv.) at 20–22°C for 4 h. Upon addition of water the compound was isolated by filtration and crystallized from methanol.

Estrone sulfamate (J 994): mp. 199–202°C (Ac/n-Hx); $[\alpha]_D^{20}$ + 128° (*c* = 1, Dx).

The 3-sulfamate as well as the 3-*N,N*-dimethyl and 3-*N,N*-diethylsulfamates of estradiol (J 995, J 991, J 981) were obtained by sodium borohydride reduction of respectively J 994, J 992 and J 804 in a mixture of methanol and tetrahydrofuran at +5°C.

Estradiol-3-sulfamate (J 995): mp. 204–206°C (Ac); $[\alpha]_D^{20}$ + 61° (*c* = 1, Dx).
Estradiol 3-*N,N*-dimethyl sulfamate (J 991): mp. 204–208°C (Chf/MeOH); $[\alpha]_D^{20}$ + 61° (*c* = 1, Chf).
Estradiol 3-*N,N*-diethyl sulfamate (J 981): mp. 176–178°C; $[\alpha]_D^{20}$ + 57°C (*c* = 1, Chf).

Ethinylestradiol-3-sulfamate (J 1028) was synthesized from ethinylestradiol-17-trimethyl silylether. Treatment of the silylether at 20–22°C with sulfamoyl chloride (5 equiv.) in dichloromethane solution in the presence of 2,6-tert.butyl-4-methylpyridine (3.5 equiv.) gave the esterified silylether which in turn was cleaved by stirring the reaction mixture with aqueous hydrochloric acid (18%) overnight. Upon work-up J 1028 was purified by flash chromatography on silica (eluent chloroform/ethyl acetate) and crystallization.

Ethinylestradiol-3-sulfamate (J 1028): mp. 209–211°C (Ac/n-Hx); $[\alpha]_D^{20}$ − 15° (*c* = 1.02, Py).

Satisfactory spectral data (^{1}H-NMR, ^{13}C-NMR, IR, UV, high resolution MS) and elemental analyses were obtained for all compounds. The purity of the sulfamates was determined by HPLC and was found to be 99.5–99.95%.

Animal studies

Ovariectomized adult female Wistar rats, 2 weeks after ovariectomy were used in experiments. The systemic and hepatic estrogenicity was evaluated. Animals

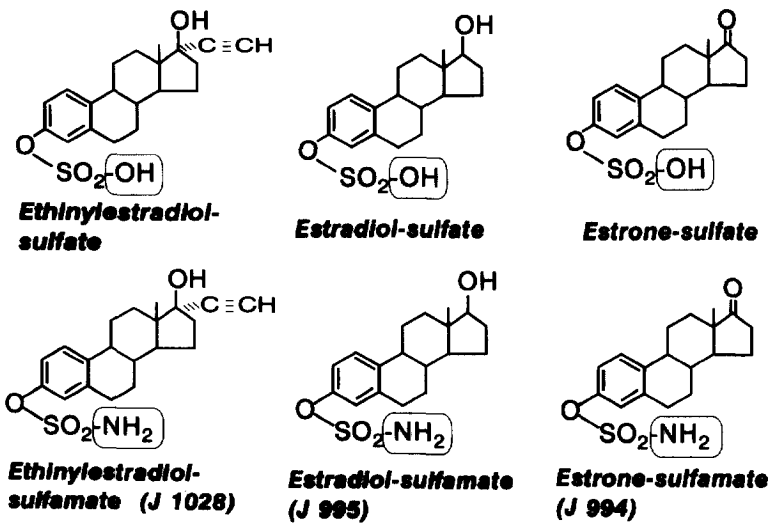

Fig. 1. Chemical structure of sulfates and sulfamates of estradiol, estrone and ethinylestradiol.

Table 1. Comparison of ethinylestradiol, estrone and estradiol and their 3-sulfamates with respect to systemic and hepatic estrogen parameters at oral administration

Parent steroids and tested sulfamates	Code	Equipotent oral dose (μg/animal/day) Uterus Δ100%↑	Angiotensin I Δ50%↑	HDL-cholesterol Δ50%↓
Ethinylestradiol*	EE	7.0–17	3.0–14	2.0–6.0
~ S	J 1028	0.2	7.0	3.0
~ DME-S	J 665	≪10	> 10	> 10
~ DET-S	J 271	0.7	55	32
Estrone	E1	115	100	250
~ S	J 994	3.8	300	40
~ DME-S	J 992	7	> 100	> 100
~ DET-S	J 804	11	> 100	> 100
Estradiol	E2	150	190	210
~ S	J 995	1.7	> 30	> 30
~ DME-S	J 991	9.5	> 30	> 30
~ DET-S	J 981	11	> 100	> 100

S, 3-sulfamate; DME-S, 3-dimethylsulfamate; DET-S, 3-diethylsulfamate.
*ET 94.003; ET 94.048; ET 94.059; ET 95.015; ET # = file number of experiment.

were treated from day 1–7, and killed on day 8 of the study. The animal number per group was 7 as a rule. Ethinylestradiol was tested as standard substance in all studies.

Assessment of systemic estrogenicity. The vaginal response was microscopically scored (stage 1–3) checking unstained smears days 1–8. The lowest dose inducing cornification by day 8 (stage 3) in 100% of animals was determined. The dose per day, inducing a 100% increase of uterine weights compared to ovariectomized vehicle-treated controls, was calculated on the basis of dose–response curve. In some of the studies the lowest dose which prompted a drop of plasma gonadotropin levels was determined by RIA for rat LH and FSH (Amersham).

Assessment of hepatic estrogenicity. Blood was collected on days 0, 4, and 8 of the study under light ether anesthesia. Maximum amount of blood was collected when the animals were bled on morning of day 8. EDTA plasma was prepared, shock frozen and kept in a refrigerator at −70°C until analysis.

Changes of angiotensinogen were estimated by RIA of angiotensin-1 (RIA RENCTK, Sorin/Biomedica) after treatment of plasma with renin. Steps: EDTA-plasma was diluted with phosphate buffer pH 6.5, 0.1 M and kept at a temperature of 0°C. To 100 μl of this dilution 2.5 mU porcine renin in 25 μl solution (R2761, Sigma), and 2.5 μl PMSF (a converting enzyme inhibitor, Sorin/Biomedica kit) and 12.5 μl reaction buffer (kit) were added. This preparation was then incubated at 37°C for 3 h. For one determination 50 μl of medium were used in the above angiotensin-1 RIA.

Cholesterol fractions, HDL and total cholesterol, were determined by enzymatic colorimetric method (Dr Lange tests LNC 026, LNC 526, HDL-cholesterol precipitation with BCZ 526). The dose-level which led to a 50% increase of angiotensin-1 and a 50% decrease of HDL or total cholesterol was determined.

The presented material is selected from several studies in the course of compound finding as compounds became available. All evaluated parameters cannot be reported in the volume of this article. The presented data focus on studies with the non-alkylated 3-sulfamates of each of the above parent estrogens and their *N,N*-dimethyl- and *N,N*-diethyl-derivatives (see Table 1). In a separate controlled study three sulfamates of ethinylestradiol were compared at a molar equivalent dose of 10 μg steroid/animal/day vs 10 μg ethinylestradiol/animal/day at oral administration. This study included the *N,N*-dimethyl- (J 665), *N,N*-diethyl- (J 271) and the *N,N*-tetramethylene-sulfamates (J 272) of ethinylestradiol. The file numbers (ET) identify individual experiments.

Formulation and administration of test compounds

Saline, containing 85 mg polyoxyethylen(50)-stearat (MYRJ, ICI Speciality Chemicals Essen, Germany), a surface active agent per 100 ml, was used as vehicle. A suspension of compounds was prepared in a 25 ml Erlenmayer flask by addition of Zirconium beads during formulation and ultrasound treatment in a water bath for 30 min. The daily dose was orally administered in 0.2 ml of vehicle. Control animals received the same volume of vehicle orally.

Preliminary studies on the metabolism of estrodial-3-sulfamate (J 995) after oral administration in female rats

Estrone- and estradiol-3-sulfamates were synthesized using [2,4,6,7-^{3}H]estrone (103 Ci/mMol, Amersham) diluted with cold estrone as starting material. Specific activity of the final material (J 995) was 182 μCi/mMol. Two ovariectomized female rats were orally treated with 10 μg J 995 (= 5 μCi ^{3}H) and completely bled in anesthesia 3 h later. A total volume of approx. 3 ml Heparin plasma per animal was gained. Each plasma was extracted twice with ether.

Approximately 30% of plasma radioactivity was ether extractible. Labeled J 995 added to control plasma was found to be more than 90% extractible. The non-extractible activity in plasma of treated rats may represent native estrogen conjugates. These were not further analysed as yet. Chromatographic methods were used to analyse the extracted activity. These allow the complete separation of J 995 from estradiol and estrone which are expected products of J 995 hydrolysis. Column chromatography using Lipidex 5000 (Canberra/Packard). Conditioning and elution medium was trichloro-methane:n-hexane:methanol (50:50:1; v/v/v). 5 ml pipettes (inner diameter 6 mm) were filled with Lipidex 5000, length 8.5 cm. For separation of steroids a total volume of 40 ml eluent was used. Estradiol is eluted in fraction 5–13, J995 in fraction 20–40. Estradiol and J 995 are eluted with a recovery of more than 80%.

For the further differentiation of estradiol—and J 995—fraction TLC on silica gel [Polygram-Sil G/UV 254, Macharay-Nagel DC-system. Trichloro-methane (Roth)/acetic acid methyl ester (Merck) (7:3)] was used: System 200 imaging scanner (Packard Canberra) was used in the evaluation of studies with ^{3}H-labeled substance J 995.

All reported studies were revised by the official ethical committee for animal experiments and approved by the government of Thueringen.

RESULTS

Evaluation of systemic estrogen activity

Estrone and estradiol had low oral activity. Oral doses of approx. 115 and 150 μg/day, respectively, were needed to increase the uterine weight by 100% compared to the vehicle controls (see Table 1). Ethinylestradiol was much more active than these estrogens. A corresponding elevation of uterine weights was already seen at doses between 7 and 17 μg/day in 4 studies using this estrogen as reference substance. Various sulfamates of these estrogens exceed the oral activity of their parent steroid by far. The most active compound was the non-alkylated sulfamate of a given steroid in all investigated cases. With respect of uterine growth ethinylestradiol sulfamate (J 1028) was approx. 40 times more potent than ethinylestradiol. Estrone sulfamate (J 994) was approx. 30 times more potent than estrone, estradiol sulfamate (J 995) was approx. 90 times more potent than estradiol. This increase of systemic oral estrogenic activity was also seen using vaginal cornification or depression of LH and FSH as indicators of activity. Vaginal cornification was seen near the threshold dose with respect to uterine growth inducing activity [see Fig. 2(A)]. A controlled study was performed comparing J 994 and J 995 versus estrone and estradiol with respect to antigonadotropic activity. This was seen at a dose of 10 μg/day of the sulfamate estrogens. Estradiol and estrone had corresponding effects at doses of 100–1000 μg/day only (see Fig. 3). Lower doses of estrogen sulfamates (< 10 μg/day) went along with FSH- and LH-levels above those of ovariectomized control animals. The latter findings need to be confirmed and analysed in more depth.

Evaluation of hepatic estrogenic activity

Effects on hepatic functions as defined in the description of methods can be induced with doses of estrone and estradiol above those which double the uterine weight vs controls. Estrone was found gradually more active in the liver than estradiol (see Table 1). Under estrone the threshold for changes of angiotensin-1 was below that of uterine weight changes as defined in this paper. In case of estradiol the reverse situation was found. The dose of ethinylestradiol which elevated

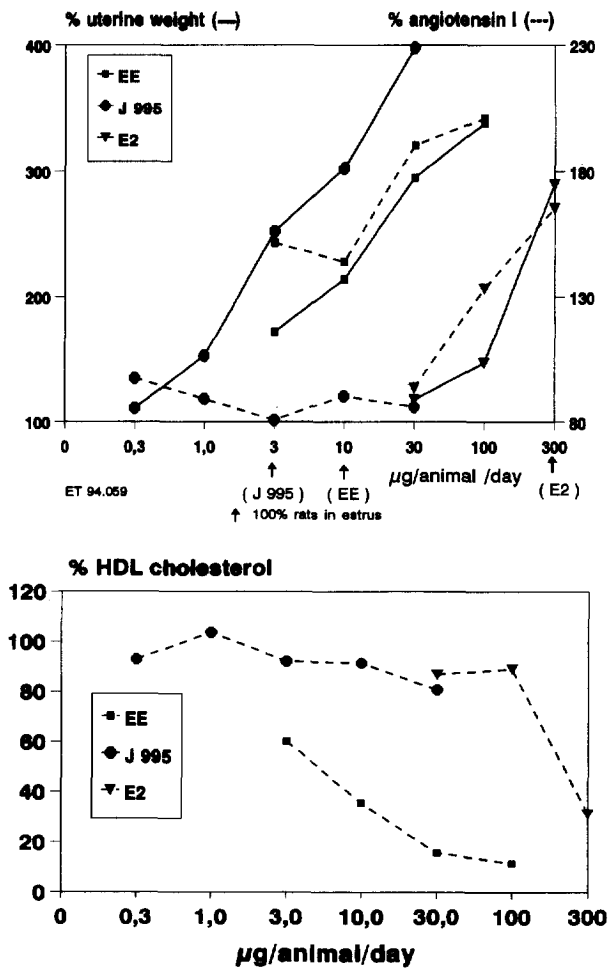

Fig. 2. Comparison of systemic and hepatic estrogen action of ethinylestradiol (EE) estradiol (E_2 and estradiol sulfamate (J 995) in ovariectomized rats: oral treatment days 1–7, measurements on day 8 (autopsy). (A) Mean uterotropic effect, vehicle controls set 100%. Arrow heads indicate lowest dose at which 100% of rats had vaginal estrus. Angiotensin-1 concentration: mean increase versus pretreatment value, which is set 100%. (B) Effects on HDL-cholesterol levels in the same study. Mean values, vehicle controls day 8 set 100%. ET # = file number of experiment.

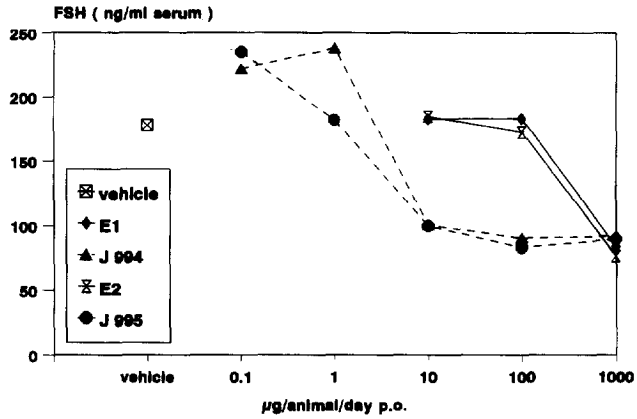

Fig. 3. Depression of FSH in serum of ovariectomized rats by estradiol sulfamate and estrone sulfamate and their parent estrogens at oral administration. Treatment day 1–7, blood collection on morning of day 8. ET # = file number of experiment.

uterine weight by 100% had substantial effects on angiotensin-1 and cholesterol fractions. Even lower doses still had distinct effects on these parameters (see Table 1). There is a different behaviour of hepatic parameters under sulfamate estrogens. Ethinylestradiol sulfamate (J 1028), which has 40 times higher systemic estrogen activity than ethinylestradiol had similar or lower hepatic estrogenicity than this estrogen. An even more pronounced dissociation of estrogen actions in the liver versus systemic estrogen parameters was found for the diethylsulfamate of ethinylestradiol (J 271) versus ethinylestradiol itself. This compound combines a nearly 10-fold increase of systemic with a nearly 10-fold reduction of hepatic estrogenicity.

The reduction of hepatic estrogenicity is even more impressive in the case of sulfamates of natural estrogens versus their parent estrogens. Distinct changes of hepatic parameters could not be detected at doses that led to maximum stimulation of uterine growth [see Fig. 2(A, B) and Table 1].

Comparison of sulfamates of ethinylestradiol (J 271, J 272, J 665) at a molar dose of 10 µg steroid/day at oral administration with ethinylestradiol

Ranking the compounds according to their effects on uterine growth the following can be seen (see Fig. 4). All sulfamates had much higher uterotropic activity than ethinylestradiol, however, less effects on total cholesterol and angiotensin-1 levels on day 8. These differences were statistically significant. The higher the induced uterine growth, the *lower* were the effects on liver parameters. The systemically most active J 665 had no significant effect on total cholesterol and angiotensin-1 compared to vehicle treated controls.

Preliminary studies on biotransformation of estradiol sulfamate (J 995)

The recorded profiles in Fig. 5(A, B) demonstrate that labelled estradiol and J 995 yield distinct peaks in their fractions. Findings in plasma 3 h after administration of [2,4,6,7-^{3}H]estradiol sulfamate (J 995) suggest a very rapid decomposition of this compound *in vivo*. Only 30% of radioactivity were found to be ether extractible as the administered steroid itself. No peak was detectable in the J 995 fraction of plasma extracts at this time point, whereas distinct peaks appeared in the estradiol/estrone fraction [see Fig. 5(C and D)].

DISCUSSION

The evaluated *N,N*-alkylated and non-alkylated sulfamate substituents in C-3 have a profound effect on the pharmacology of their parent estrogen. The non-alkylated sulfamates of estrone, estradiol and ethinylestradiol had much higher systemic estrogen activity than the alkylated derivatives tested. Ethinylestradiol standard is hampered by an enormous inter-assay variation of effects of ethinylestradiol. The lowest and the highest value

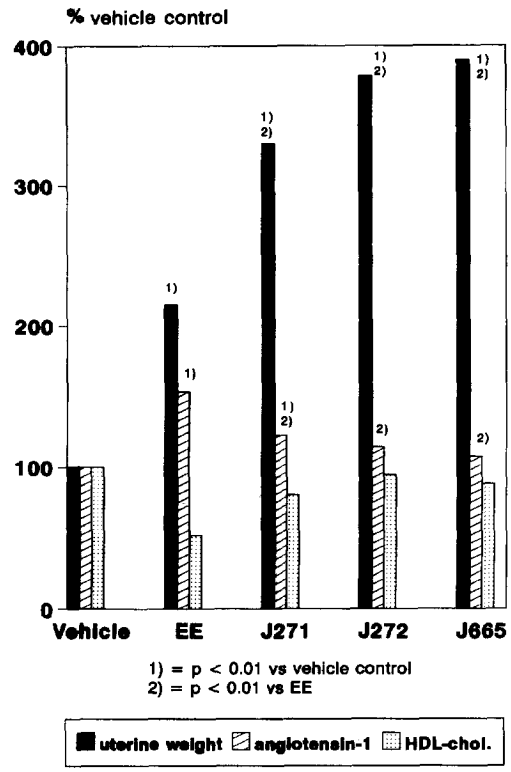

Fig. 4. Relative change of uterine weight, plasma angiotensin-1 and total cholesterol in ovariectomized rats as measured on day 8 after oral treatment days 1–7 with 10 µg ethinylestradiol (EE) per animal per day or molar equivalent dose of ethinylestradiol sulfamates J 271, J 272 and J 665. Mean values, values in vehicle treated controls on day 8 set 100%. All differences between controls vs EE were statistically significant ($P < 0.01$). J 271, J 272 and J 665 induced significantly more uterine growth than EE ($P < 0.01$). Cholesterol and angiotensin-1 levels under J 665 were significantly different vs EE but not from those in controls. ET # = file number of experiment.

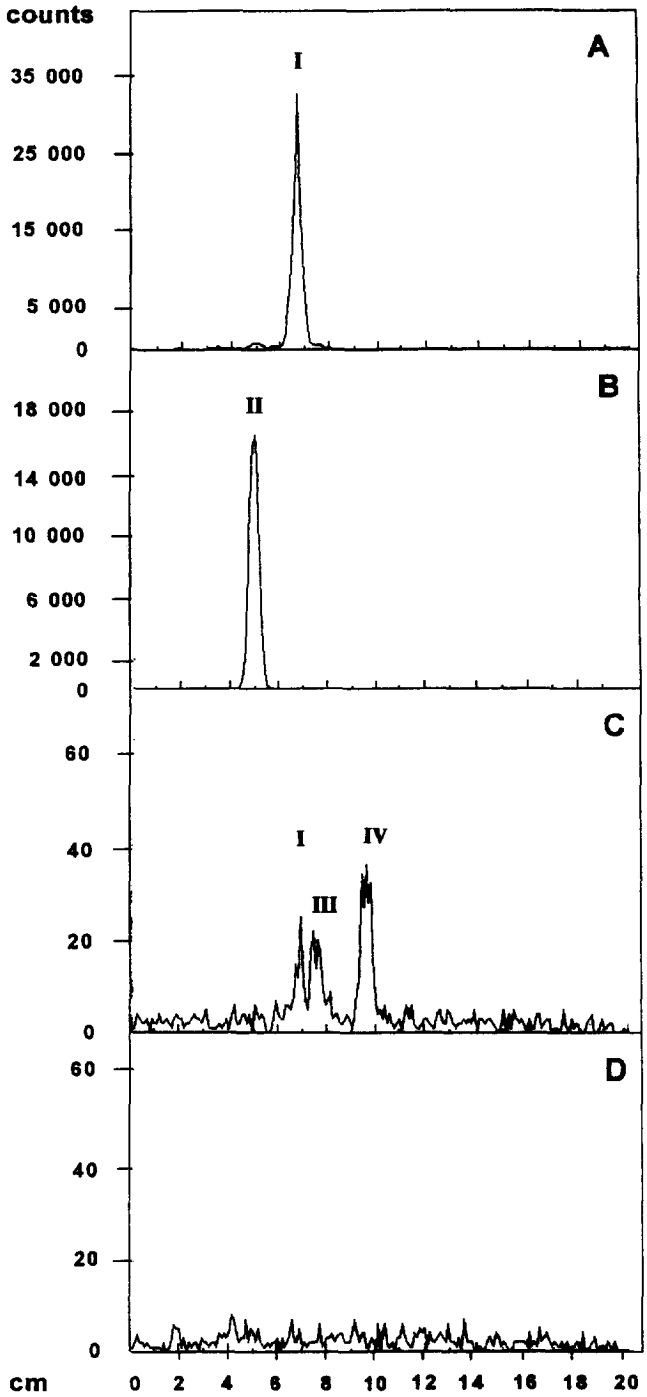

Fig. 5. Silica gel TLC of [2,4,6,7-^{3}H]estradiol and [2,4,6,7-^{3}H]estradiol sulfamate. For location of estradiol (I) and estradiol sulfamate (II), see peaks in (A) and (B). Peaks in (C) and (D) show activity in ml 7–14 ("estradiol fraction") and ml 20–40 ("estradiol sulfamate fraction") of lipidex 5000 column chromatography of ether extractible activity from plasma 3 h after oral administration of 10 µg/5 µCi of [2,4,6,7-^{3}H]estradiol sulfamate to an ovariectomized rat. Note that three discernible peaks were only present in C: Peak I is very likely estradiol, peak III and IV are in the position of 17α-OH-estradiol and estrone, respectively. There was no peak in position of estradiol sulfamate in D.

found in four assays using ethinyl-estradiol as reference compound differ by a factor of 2.4 (see Table 1). Varying rates of responding and non-responding animals were found at a defined dose level. Due to the erratic behaviour of the reference compound relative activity of test compounds versus this estrogen was not calculated. An extraordinary inter- and intraindividual variation of pharmacokinetic parameters as bioavailability (more than factor 3 between lowest and highest values), C_{max}, T_{max} and half-life at oral application which prevails in women [2] may exist in the rat as well. In spite of the described biostatistical problem, there can be no doubt that all tested sulfamate derivatives of ethinylestradiol, and the "free" sulfamates of estrone and estradiol (i.e. J 994 and J 995) exceed ethinylestradiol with respect to systemic estrogen activity by a factor up to 40 in case of ethinylestradiol sulfamates or by factor 3–10 in case of the latter.

Surprisingly, there was no correlation of systemic effects with those on estrogen modulated liver functions. Alterations of these factors in the blood at *parenteral* administration of estrogens can only be seen at dose levels several times above those which induce substantial uterine growth [14, 19, 21]. The presented studies with *oral* administration of ethinylestradiol or the tested natural estrogens show effects on liver function as defined in this paper close to the dose which doubles uterine weights. This is evidence that the selected parameters indeed reflect hepatic estrogen actions. Gradual differences with respect to the ratio of systemic and hepatic estrogenicity among estrone and estradiol were seen in the study but were not analyzed in detail.

Orally administered estrogen sulfamates behave to both the systemic and the hepatic estrogen parameters as parenterally administered natural estrogens. As far as evaluated with respect to a broader spectrum of reproductive functions, they exert powerful effects on uterus, vagina and gonadotropin secretion. However, very consistently, they have no or very moderate effects on the liver over a wide range of doses. The threshold for hepatic effects as defined in this paper may be 100 times above a 100% uterine growth inducing dose. It could not be identified for some analogues in spite of the fact that a broad range of doses was tested.

The observed dissociation of effects is evidence of an as yet unknown mode of passage of estrogen through the liver. It is generally agreed that steroid sulfates are not active hormones [22]. It seems very likely, that this applies to estrogen sulfamates as well. Studies with the dextran charcoal assay of estrogen receptors in MCF-7 cytosol (donated by Abbott) revealed very little displacement of specific [^{3}H]estradiol (0.5–3 nM) binding with a 200-fold excess of unlabeled J 271, J 994, J 995 (personal communication, A. Hedden and E. V. Jensen, IHF Hamburg). A hydrolysis of the estrogen sulfamate releasing the parent estrogen has to take place *systemically* in a very efficient manner, otherwise the high systemic activity cannot be explained. The low level of

estrogen activity in the liver indicates that this orgn is unlikely to play a major role in this activation process. The site or sites of prodrug activation and its mechanism are not yet known.

The virtual absence of J 995 in plasma 3 h after administration and the appearance of ether extractible metabolites, which probably represent estradiol and estrone at this time point are consistent with this notion. However, the chemical identity of identified peaks remains to be studied. Incubation of [2,4,6,7-^{3}H]J 995 in plasma at 37°C over 24 h failed to generate corresponding peaks in the estradiol fraction (data not shown). This finding indicates that the observed peaks are not due to spontaneous degradation of J 995 in plasma and that the relevant activating enzymes are not present there. The substantial level of non-extractible radioactivity in plasma is unlikely to represent unchanged J 995, since this compound itself is easily ether extractible. This fraction of metabolites might consist of sulfates and glucuronides formed as secondary metabolites.

Lacking extraction of administered non-activated sulfamate steroids during the first passage through liver is probably the explanation of both, high systemic activity and the absence of hepatic estrogen action. The investigated sulfamates of ethinylestradiol, which are likely to act estrogenic via the release of ethinylestradiol, show a reverse correlation of systemic and hepatic activity. This indicates a profound effect on ethinylestradiol concentrations in the liver and systemic estrogen target tissues depending on administration as sulfamate or in a direct form.

The significance of the presented findings for the human is unknown. The oral bioavailability of ethinylestradiol is much lower in rats than in humans (4 vs 40%) [3, 23]. Sulfamates of this steroid could lead to an improved oral bioavailability of the parent steroid close to 100% and overcome its awkward variability of pharmacokinetic parameters in humans. These prevent a precise definition of estrogen dosing [2]. An increase of activity by the same factor as found in the rat is not very likely in humans since only the much lower first pass loss could be avoided. It goes without saying that a reduction of hepatic estrogen effects of ethinylestradiol, including those on factors of the clotting system and on angiotensinogen secretion would contribute to the safety of hormonal contraception at a most crucial point.

An even higher therapeutic potential of estrogen sulfamates lies in the option to replace the ethinylestradiol in contraceptives, but also estrogens in hormone replacement therapy by more efficient prodrugs of natural estrogens. The presented data suggest that sulfamates of estradiol and estrone generate natural estrogens in the system without the well known [8, 10] hepatic impact or orally administered estradiol. Their systemic activity in the human may reach or even exceed that of ethinylestradiol. The exclusion of hepatic estrogen effects beyond the physiological sex dimorphism would be an obvious advantage. The lack of side effects might also help to optimize the dose of estrogen in contraceptives with respect to the control of bleeding pattern. Today's reductions of ethinylestradiol doses may be seen as dubious attempts to reduce metabolic side effects at the expense of a well controlled endometrium.

Sulfamates of estrogens and other steroids were synthesized and evaluated by various groups with different pharmacological intentions [24–27]. The latter quoted group [27, 28] found that steroid sulfamates including estrone sulfamate are potent sulfatase inhibitors. A therapeutic strategy was discussed to prevent the generation of estrogen from estrogen sulfates at the tissue level in hormone-dependent tumor disease. The powerful estrogen activity of the given estrogen sulfamates demonstrated is certainly unfavourable for estrogen ablative strategies. It remains to be evaluated whether sulfamate estrogens interfere with sulfatases and the generation of estrogens from endogenous precursors, i.e. estrone sulfate. Another aspect is their potential hydrolysis of sulfamates by these enzymes. Tissues might be exposed to locally released estrogens depending on their enzyme activity modulating the response of receptive tissues. There is as yet no evidence for or against tissue specific effects, these however have to be taken into account.

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