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Effects of estradiol and estradiol sulfamate on the uterus of ovariectomized or ovariectomized and hypophysectomized rats

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Abstract

Estradiol sulfamate (J995), estradiol-17β with a substituted sulfamate group in position 3, has much higher systemic estrogenic activity after oral administration than 17β-estradiol (E2) due to reduced hepatic metabolism of the drug. The lower dose necessary for achievement of adequate systemic estrogenic effects results in a substantial reduction of otherwise commonly observed hepatic side-effects. This makes J995 a strong candidate as an estrogen suitable for oral administration. The present study was performed to examine and compare the effects of J995 and E2 on the uterus after oral or subcutaneous administration to ovariectomized or ovariectomized + hypophysectomized female rats, in particular on the levels of the estrogen receptor (ER) (α + β), ERα mRNA and insulin-like growth factor-I (IGF-I) mRNA. The ER levels were determined using a ligand binding assay and the mRNA levels using solution hybridization. The doses of J995 or E2 were chosen to achieve comparable uterotrophic activity. The rats were treated with hormones for 7 days and the treatment was initiated 14 days after surgery. We conclude that there are no major differences in the uterine response to treatment with J995 or E2 with respect to the effects on ER and ERα mRNA levels. The IGF-I mRNA level though, is more affected by J995 than by E2 after 7 days of treatment, indicating a prolonged effect of J995.
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1. Introduction

Oral treatment with estrogen is simple and convenient and has a well-documented therapeutic efficacy, but is also in many ways non-physiological [1,2]. In the intestinal wall and liver most of the ingested 17β-estradiol (E2) is metabolized into estrone, which has a biologic activity corresponding to one third that of E2 [3]. Thus, it is necessary to use much higher doses via the oral route than when estrogens are given parenterally (~ 20-fold, 1 mg E2 vs. 50 μg). Specific differences in hepatic action between the endogenous and synthetic estrogens have been clearly demonstrated [4]. When physiological doses of native estrogens are given parenterally, the effects on liver-derived plasma proteins, coagulation factors, lipoproteins, and triglycerides are very weak or completely abolished [1,2,5–8]. This has encouraged the development of alternative non-oral delivery systems [1,2], and efforts to develop derivatives of natural estrogens lacking the hepatic side-effects [9]. Numerous studies on humans and animals have demonstrated that the hepatic impact of estrogen treatment can be reduced dramatically by modification of the type of estrogen and the route of administration [1,2,5–9].

In a rat model, the estradiol sulfamate J995 has proved to have a 90-fold elevated systemic effect as compared to E2 when given orally, resulting in a reduc-

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tion of hepatic estrogenicity [9]. Systemic estrogenicity was quantitated by assessment of uterine weight, vaginal cornification and measurement of gonadotropins [9]. Thus, the estradiol sulfamate allows oral administration of estrogens with little effect on hepatic metabolism.

Orally administered E2 is effectively extracted from the portal vein blood and excreted via the bile. Only 3% of the dose is found in the venous blood. In contrast to this up to 30% of the orally administered J995 appears in the general circulation [10]. This is possibly due to the fact that almost 99% of [^{3}H]blood levels after [^{3}H]J995 treatment are found in the erythrocyte fraction (RBC) after 3 h and only 1% is retained in the plasma fraction [10]. After [^{3}H]E2 no accumulation of radioactivity in RBC was found. The plasma:RBC ratio was ~ 1:0.7 at several timepoints over 48 h [11]. Pharmacokinetic [11] and toxicological (Elger, unpublished observations) studies revealed complete excretion of the compound and its metabolites. No toxic effects were seen in rats and cynomolgus monkeys at any dose, in spite of their distinct estrogenicity [12].

The much larger portion of J995 than E2 reaching the systemic circulation might be due to protected liver passage in the erythrocyte compartment. It is probably the same mechanism that protects the liver against hormonal effects at first passage. The low level of estrogenic effects in the liver may be explained by a low hepatic tissue concentration. Liver perfusion studies support this hypothesis, since hepatic extraction was very different depending on the presence or absence of erythrocytes in the perfusion medium [13].

The pattern of urinary and fecal elimination of [^{3}H]J995 after oral treatment in rats was very similar to that of [^{3}H]E2 with the exception of a minor delay for J995 as compared to E2 [11].

The hypothesis is that J995 is an inactive pro-drug for E2, since it does not bind to the ER itself. The aim of this study was to compare the effects of J995 and E2 on the uterus, especially regarding the expression of ER and IGF-I, since IGF-I is a mediator of estrogen action in the uterus [14–16].

2. Materials and methods

Doses of E2 and J995 were matched with respect to comparable uterotrophic activity at two dose levels (E2: 0.05 and 0.5 μg subcutaneous (s.c.); 200 μg and 2 mg peroral (p.o.); J995: 0.2 and 2 μg s.c.; 2 and 20 μg p.o.)

Table 1
Experimental design for the different treatment groups ($n = 7$/group)*

Animal operation	100 μg hGH + 10 μg DEX/rat and day	Treatment	Route of administration	Uterine weights (g)	Serum estradiol (pg/ml)
Intact		Vehicle only	p.o.	0.393 ± 0.019^{a}	
Ovx		Vehicle only	p.o.	0.109 ± 0.007^{b}	
Ovx		2 μg J995	p.o.	0.237 ± 0.02^{abc}	
Ovx		20 μg J995	p.o.	0.357 ± 0.027^{ac}	
Ovx		200 μg E2	p.o.	0.233 ± 0.012^{bc}	
Ovx		2 mg E2	p.o.	0.360 ± 0.023^{ac}	
Ovx		Vehicle only	s.c.	0.110 ± 0.006^{a}	13.8 ± 1.84^{ab}
Ovx		0.2 μg J995	s.c.	0.191 ± 0.012^{abc}	17.2 ± 1.21^{ab}
Ovx		2 μg J995	s.c.	0.436 ± 0.037^{b}	47.9 ± 9.36^{c}
Ovx		0.05 μg E2	s.c.	0.171 ± 0.010^{ac}	9.59 ± 1.53^{a}
Ovx		0.5 μg E2	s.c.	0.389 ± 0.028^{c}	29.0 ± 1.38^{bc}
Ovxhx	Vehicle only	Vehicle only	p.o.	0.086 ± 0.007^{ac}	
Ovxhx	Vehicle only	20 μg J995	p.o.	0.234 ± 0.010^{ab}	
Ovxhx	Vehicle only	2 mg E2	p.o.	0.307 ± 0.024^{b}	
Ovxhx	Yes	Vehicle only	p.o.	0.090 ± 0.007^{a}	
Ovxhx	Yes	20 μg J995	p.o.	0.274 ± 0.009^{bc}	
Ovxhx	Yes	2 mg E2	p.o.	0.307 ± 0.021^{b}	
Ovxhx	Vehicle only	Vehicle only	s.c.	0.085 ± 0.008^{ac}	12.5 ± 6.95^{a}
Ovxhx	Vehicle only	2 μg J995	s.c.	0.308 ± 0.010^{b}	40.8 ± 4.96^{ab}
Ovxhx	Vehicle only	0.5 μg E2	s.c.	0.328 ± 0.017^{b}	35.6 ± 5.42^{ab}
Ovxhx	Yes	Vehicle only	s.c.	0.074 ± 0.001^{a}	9.63 ± 2.28^{a}
Ovxhx	Yes	2 μg J995	s.c.	0.274 ± 0.010^{bc}	74.5 ± 5.58^{b}
Ovxhx	Yes	0.5 μg E2	s.c.	0.342 ± 0.020^{b}	46.8 ± 4.82^{b}

* Before blood collection and sacrifice. Values without the same letters are significantly different ($p < 0.05$). Comparisons are done within the different experiments.

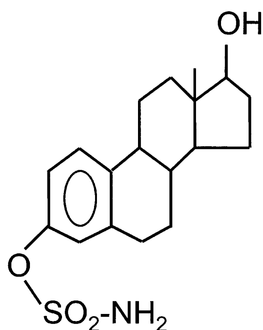

Fig. 1. The structure of estradiol sulfamate (J995).

and administered to female Wistar rats weighing ~200 g, according to Table 1. Intact and vehicle treated operated animals served as controls. The treatment began 2 weeks after operation. The rats ($n = 7$/group) were treated for 7 days and were sacrificed 24 h after the last dose given. Uterine weight was used as a systemic estrogen parameter (Table 1). At 2 μg J995 or 0.5 μg E2/animal per day s.c. not only comparable uterotrophic activity but also comparable E2 plasma levels were recorded (Table 1). This implies that estradiol sulfamate is hydrolyzed to estradiol. The RIA failed to measure E2 appropriately in orally treated animals (data not shown). This may be due to high concentrations of metabolites that are known to lead to massive disturbance of most E2 RIAs [17].

2.1. Chemicals

The estradiol sulfamate (J995) was a preparation from JenaPharm, Jena, Germany (Fig. 1). Diethylstilbestrol (DES) was purchased from Sigma (St. Louis, MO, USA). [2,4,6,7-^{3}H]Estradiol-(3,17-dihydroxy-1,3,5(10)-estratriene), specific activity 92.0 Ci/mmol, was obtained from New England Nuclear (Boston, MA, USA). Radioactivity was determined in a LKB-Wallac 1215 Rackbeta liquid scintillation counter using Readysafe (Beckman Instruments, Fullerton, CA, USA) as scintillation fluid. Dextran T70 was bought from Pharmacia (Uppsala, Sweden). Activated charcoal was purchased from Sigma (St. Louis, MO, USA).

2.2. Preparation of subcellular fractions

The rats were sacrificed by cervical dislocation. The uteri were removed, stripped of adhering fat and connective tissue, weighed, cut in two equal halves and immediately frozen in liquid nitrogen and stored at −70°C until analyzed. After thawing one half was analyzed with respect to receptor content. It was homogenized in TE-buffer in a glass-glass homogenizer, and the homogenate centrifuged at 1200 × *g* for 20 min in a Beckman JA-20 rotor. The supernatant was recentrifuged at 192 000 × *g* for 65 min in a Beckman SW55 rotor and the high-speed supernatant used for determination of 'cytosolic' receptors (cytosolic receptors referring to presumably loosely bound nuclear ER found in the cytosol as a result of cell fractionation procedures). The nuclear pellet obtained after the first centrifugation was used for determination of nuclear receptors. With these techniques both ERα and ERβ were measured.

2.3. Analytical methods

Binding studies of cytoplasmic receptors were performed in the presence of a wide range of [^{3}H]estradiol concentrations (0.4–6.0 nM) ± 100-fold excess of cold DES. Tubes were vortexed and incubated at 4°C for 16 h and measurements of specific estrogen binding were performed by the dextran-coated charcoal method [18]. Determinations of transformed nuclear estrogen receptors were performed by the nuclear exchange method (NEA) as described by Clark and Peck [19]. Data were plotted according to Scatchard [20]. Protein was quantitated spectrophotometrically by the method of Lowry [21].

2.4. RIA analysis

Estradiol in plasma was estimated by RIA (TKSE, Diagnostic Products, Los Angeles, CA, USA), which was adapted and validated for rat plasma. Plasma was extracted with 2 × 2-ml purified diethylether. A calibration curve was established using steroid free rat plasma to which estradiol was added at seven different concentrations. Control plasma with three defined E2 concentrations was evaluated together with experimental samples. Measuring range was 15–1000 pg/ml plasma. Cross-reactivity of estradiol sulfamate and estrone sulfamate was in the order of 0.25 and 0.005%, respectively. Intra assay variation was between 5.1 and 9.2%. Inter assay variation was between 4.5 and 8.2%.

2.5. Preparation of total nucleic acids

Total nucleic acids (TNA) were prepared as described before [16]. In short, the tissues were homogenized and digested with proteinase K in an SDS-containing buffer, followed by subsequent extraction with phenol-chloroform as described by Durnam and Palmiter [22]. The concentration of total DNA in the TNA samples was measured fluorometrically at the wavelength 458 nm with Hoechst Dye 33258 [23].

2.6. Solution hybridization analysis of mRNA

The solution hybridization analysis of specific mRNA was performed as previously described [24,25]. For measurement of mRNA, RNA probes were synthe-

sized in vitro and radiolabeled with [^{35}S]UTP (Amersham, Bucks, UK). The in vitro synthesis of radioactive cRNA was performed essentially as described by Melton et al. [26] using reagents supplied by Promega Biotech, Madison, WI. [^{35}S]UTP cRNA was hybridized (20 000 cpm/incubation) at 70°C to TNA samples as previously described [27]. Incubations were performed in micro-centrifuge vials (Treff, Switzerland) in a volume of 40 μl containing 0.6 mol/l NaCl, 20 mmol/l Tris–HCl, pH 7.5, 4 mmol/l EDTA, 0.1% SDS, 0.75 mmol/l dithiothreitol and 25% formamide. After overnight incubation, samples were treated with RNase for 45 min at 37°C by adding 1 ml of a solution containing 40 μg RNase A and 118 U RNase T1 (Boeringer-Mannheim, Mannheim, Germany) and 100 μg calf thymus DNA to each sample. Radioactivity protected from RNase digestion was precipitated by the addition of 100 μl 6 mol/l trichloroacetic acid and collected on a filter (Whatman GF/C). The radioactivity on the filters was compared with a standard curve of known amounts of in vitro synthesized mRNA complementary to the probe used. Results are expressed as amol (10^{-18}) mRNA/μg DNA in the TNA sample.

2.7. Hybridization probes

The probe used for IGF-I mRNA determinations derived from a genomic clone of mouse IGF-I [27]; a 160-bp Ban I-Pvu I fragment from exon 3 (assuming homology to human IGF-I) was subcloned into the RNA expression vector pSP64.

The probe used for ERα mRNA determinations derived from pMOR101, an ECO RI fragment of 1.9 kb containing the whole open reading frame of the mouse estrogen receptor α [28] inserted into a pSP64 vector. Restriction of this plasmid with Bgl II allows the synthesis of a probe corresponding to nucleotides 1470–2062, which encode the C-terminal, half of the steroid binding domain (E) and all of domain F.

Thus, both ERα mRNA and IGF-I mRNA were detected in the rat tissues using heterologous mouse probes. Rat IGF-I and mouse IGF-I show a high degree of homology. It is feasible to use the mouse IGF-I probe to specifically detect rat IGF-I mRNA as demonstrated by Möller et al. [29]. The rat ERα mRNA and mouse ERα mRNA show 97% homology [30] and it has previously been shown that specific binding is obtained when the mouse ERα probe is hybridized to rat liver RNA on a Northern blot [24].

2.8. Statistics

Statistical evaluation was done with ANOVA on ranks and Kruskal–Wallis test, followed by Dunn's test to determine significance. Comparisons between two groups were done with Mann–Whitney's test on ranks.

3. Results

3.1. Plasma estradiol levels

Recorded plasma levels in treated animals represent the situation 24 h after the last injection (Table 1). Vehicle treated intact controls had mean plasma levels of 18 pg/ml (data not shown). Ovariectomy (ovx) reduced these plasma levels (range 7.5–14 pg/ml) in the various vehicle treated rats, both in pituitary intact and hypophysectomized (hx) groups (Table 1).

3.2. Ovariectomized rats, oral treatment

3.2.1. Uterine weight

The p.o. treated rats, given the high dose of hormone, had a significantly increased uterine weight as compared to the ovx controls (Table 1).

3.2.2. ER

The uterine ER levels increased after ovariectomy and were not affected by treatment with low doses of

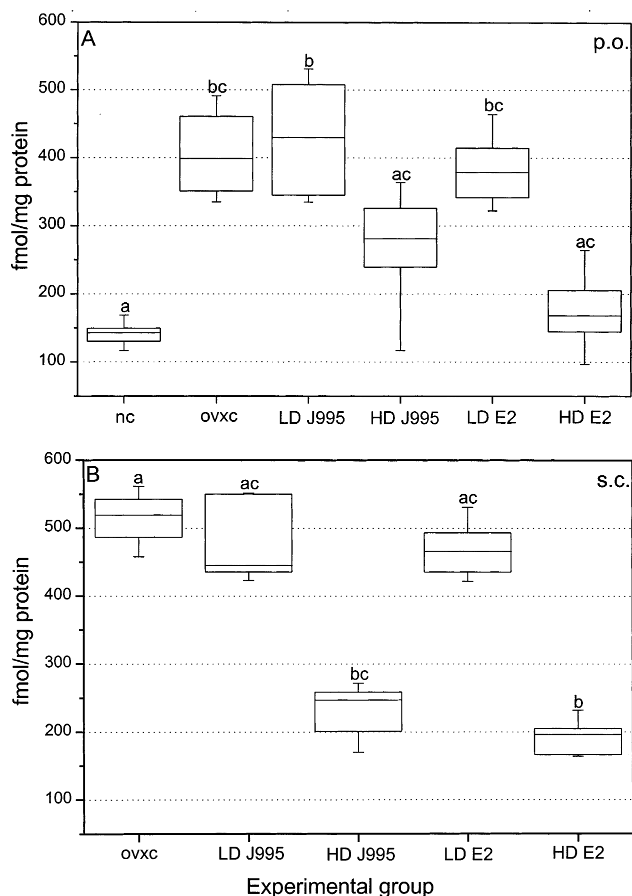

Fig. 2. The levels of ER, determined by a ligand binding assay, in the ovariectomized rats. (A) After oral administration of hormones. (B) After subcutaneous administration of hormones. Boxes without the same letter are significantly different ($P < 0.05$).

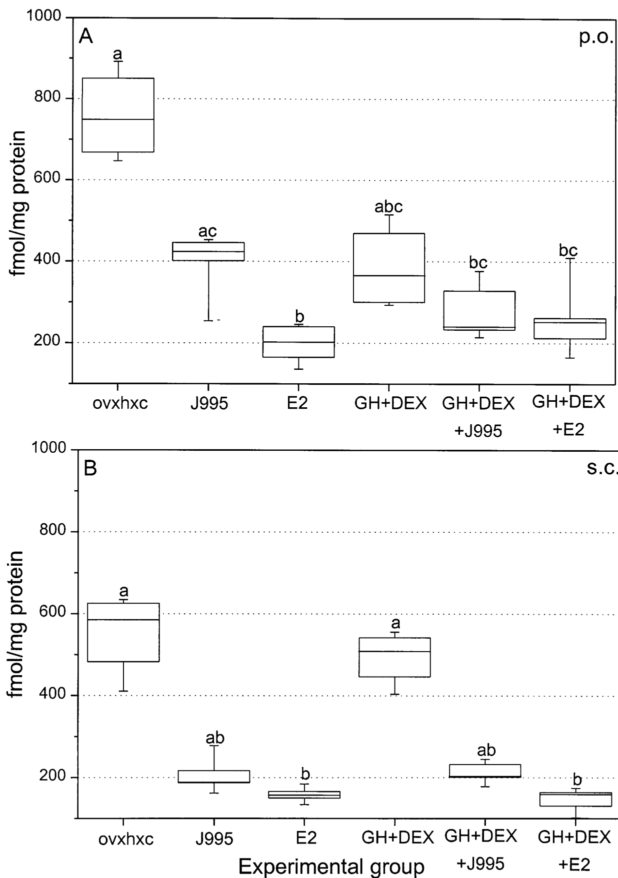

Fig. 3. The levels of ER, determined by a ligand binding assay, in the ovariectomized + hypophysectomized rats. (A) After oral administration of hormones. (B) After subcutaneous administration of hormones. Boxes without the same letter are significantly different ($P < 0.05$).

E2 or J995. Treatment with high doses of E2 and J995 showed a tendency to decrease the ER level again (Fig. 2A).

3.2.3. ERα mRNA

The uterine ER mRNA levels were not affected by ovariectomy. The group of rats treated with the high dose of E2 showed a tendency towards a decreased ER mRNA level as compared to the ovx controls (Fig. 4A).

3.2.4. IGF-I mRNA

The IGF-I mRNA level decreased after ovariectomy as compared to intact animals though not significantly (there is a large variation in the intact group since the IGF-I mRNA varies during the normal estrous cycle [16]). After addition of a low or a high dose of J995 the IGF-I mRNA expression increased again to a level not significantly different from the ovx rats. E2 showed no significant effects on the IGF-I mRNA level in any of the treatment groups (Fig. 6A).

3.3. Ovariectomized rats, subcutaneous treatment

3.3.1. Uterine weight

In the s.c. treated ovx rats both the high doses of J995 and E2 increased the uterine weight significantly (Table 1).

3.3.2. ER

Treatment with high doses of J995 and E2 decreased the levels of ER as compared to the ovx control group (Fig. 2B).

3.3.3. ERα mRNA

The group treated with a low dose of J995 differed from the ovx controls having an increased ER mRNA level. No effects were seen for the high dose J995 or any of the E2 treatments as compared to the ovx controls (Fig. 4B).

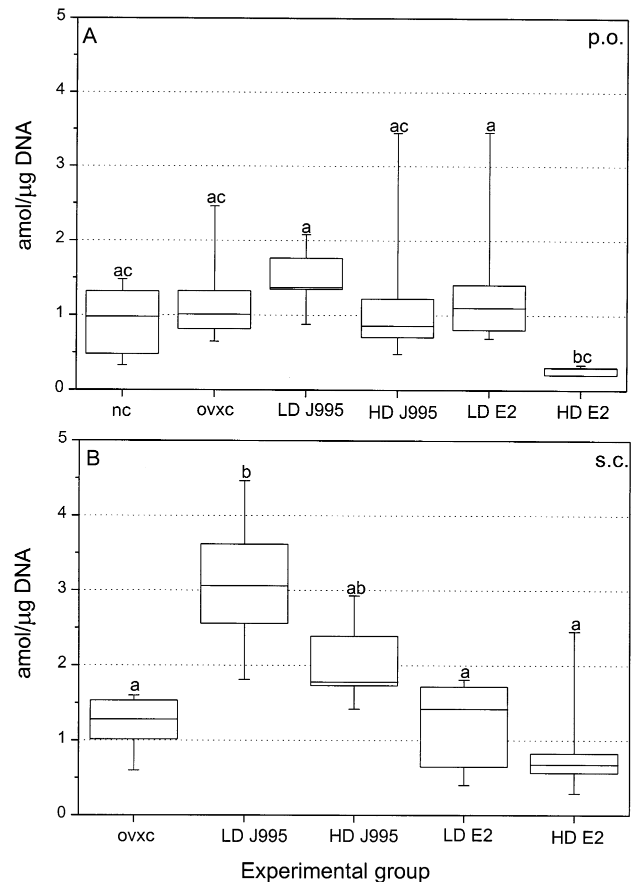

Fig. 4. The levels of ER mRNA, determined by solution hybridization, in the ovariectomized rats. (A) After oral administration of hormones. (B) After subcutaneous administration of hormones. Boxes without the same letter are significantly different ($P < 0.05$).

Table 2
Comparison between two groups with Mann–Whitney's test on ranks

Group	Uterine weight (g)	ER (fmol/mg protein)	ER mRNA (amol/μg DNA)	IGF-I mRNA (amol/μg DNA)
a. GH+DEX substituted versus GH+DEX non-substituted (oral treatment)				
2 mg E2	NS	NS	↑**	↑**
20 μg J995	↑*	↓**	NS	NS
b. GH+DEX substituted versus GH+DEX non-substituted (subcutaneously)				
2 mg E2	NS	NS	↑*	↑*
20 μg J995	↓*	NS	↑**	↑**

* $P<0.05$.
** $P<0.01$.

3.3.4. IGF-I mRNA

The IGF-I mRNA level in the groups treated with J995 increased compared to the ovx control, whereas E2 treatment had no significant effect (Fig. 6B).

3.4. Hypophysectomized and ovariectomized rats, oral treatment

3.4.1. Uterine weights

In the GH (growth hormone) + DEX (dexamethazone) substituted rats J995 and E2 both increased uterine weight, while in the non-substituted animals only E2-exposed animals showed a significant increase (Table 1). The J995 treated group had a higher uterine weight after GH + DEX substitution than without substitution (Table 2a).

3.4.2. ER

E2 treatment decreased the ER level as compared to the ovxhx control group (Fig. 3A). The ER level was decreased after J995 treatment in the GH + DEX substituted rats as compared to the non-substituted (Table 2a).

3.4.3. ERα mRNA

The ER mRNA level was very low in the E2 treated group, but not significantly different from the ovxhx control or the J995 treated group. The GH + DEX substituted and E2 treated group had a significantly increased ER mRNA level as compared to the substituted ovxhx control group (Fig. 5A). The ER mRNA level was higher in the E2 treated GH + DEX substituted rats as compared to the non-substituted (Table 2a).

3.4.4. IGF-I mRNA

The GH + DEX substituted E2 treated group had an IGF-I mRNA level increased compared to the substituted ovxhx controls (Fig. 7A). The GH + DEX and E2 treated group had a significantly increased IGF-I mRNA level as compared to the non-substituted and E2 treated group (Table 2a).

3.5. Hypophysectomized and ovariectomized rats, subcutaneous treatment

3.5.1. Uterine weights

The J995 and E2 treated groups showed increased uterine weights as compared to the ovxhx controls (Table 1). The J995 treated group had a lower uterine

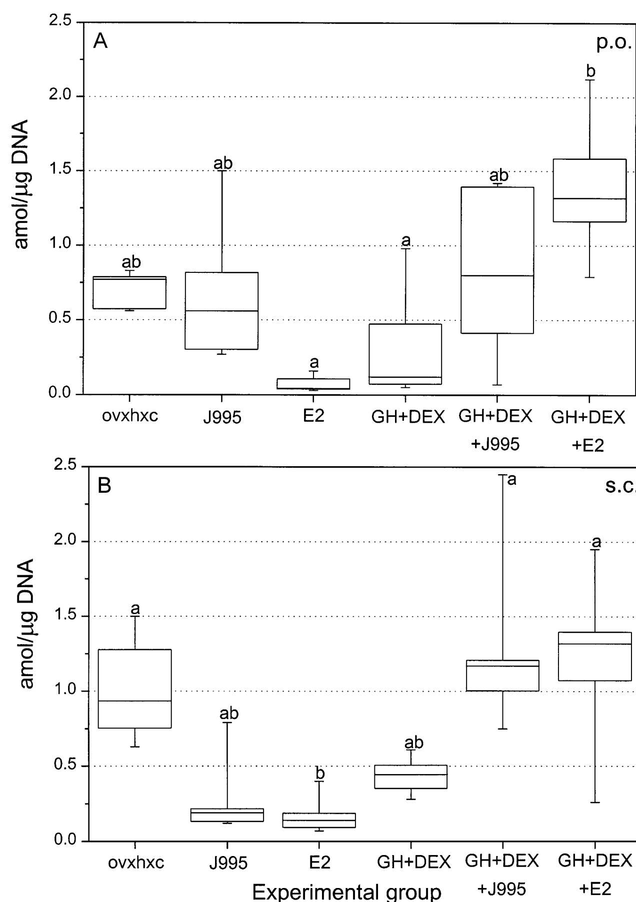

Fig. 5. The levels of ER mRNA, determined by solution hybridization, in the ovariectomized + hypophysectomized rats. (A) After oral administration of hormones. (B) After subcutaneous administration of hormones. Boxes without the same letter are significantly different ($P<0.05$).

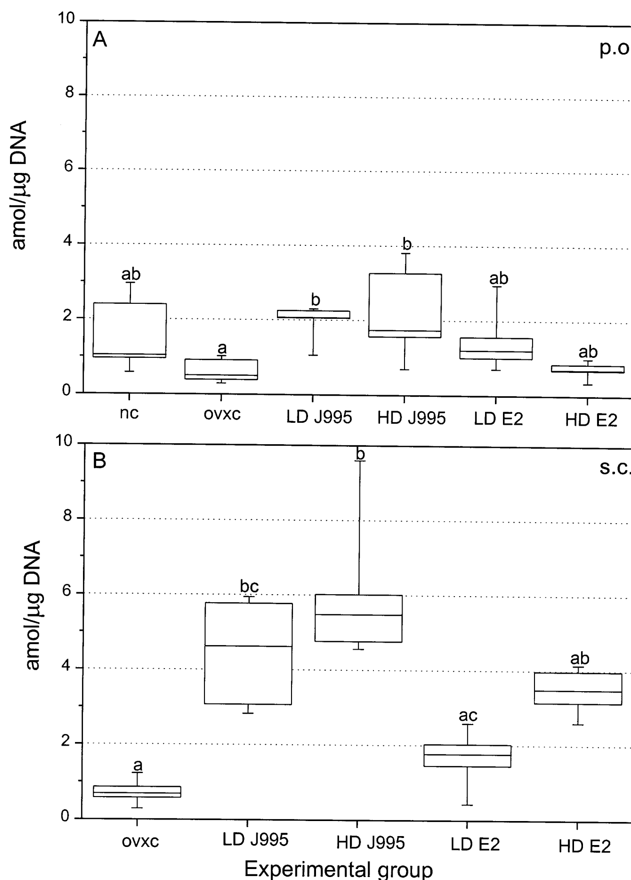

Fig. 6. The levels of IGF-I mRNA, determined by solution hybridization, in the ovariectomized rats. (A) After oral administration of hormones. (B) After subcutaneous administration of hormones. Boxes without the same letter are significantly different ($P < 0.05$).

weight after GH + DEX substitution than without substitution (Table 2b).

3.5.2. ER

The uterine ER level was significantly decreased in the groups that were treated with E2, both when substituted with GH + DEX and when not (Fig. 3B). No differences between the GH + DEX substituted and non-substituted rats were found (Table 2b).

3.5.3. ERα mRNA

Treatment with J995 or E2 decreased the ER mRNA level compared to ovxhx controls, though only E2 to a significant degree. After GH + DEX substitution, J995 or E2 treatment increased the ER mRNA levels, though not significantly (Fig. 5B). The J995 and E2 groups substituted with GH + DEX both had increased ER mRNA levels compared to the non-substituted groups (Table 2b).

3.5.4. IGF-I mRNA

The IGF-I mRNA levels increased after J995 or E2 treatment, both with and without GH + DEX substitution, but only the substituted group receiving J995 to a significant level (Fig. 7B). The IGF-I mRNA levels were increased in both the J995 and E2 treated and substituted groups compared to the non-substituted (Table 2b).

4. Discussion

In the present study the uterine ER levels were shown to increase after ovx and ovxhx, which has been previously shown for hx rats as compared to normal controls [31]. The ER level was not affected in the ovx rats after treatment with the lower doses of substance, whereas the higher doses decreased the level to ~ 50%. This decrease was less pronounced in the J995 as compared to the E2 treated animals. Both substances lowered the ER levels in the s.c. treated ovxhx rats, but only E2 to a significant degree. In the p.o. treated groups the decrease after E2 administration was significant, but only in the groups without GH + DEX substi-

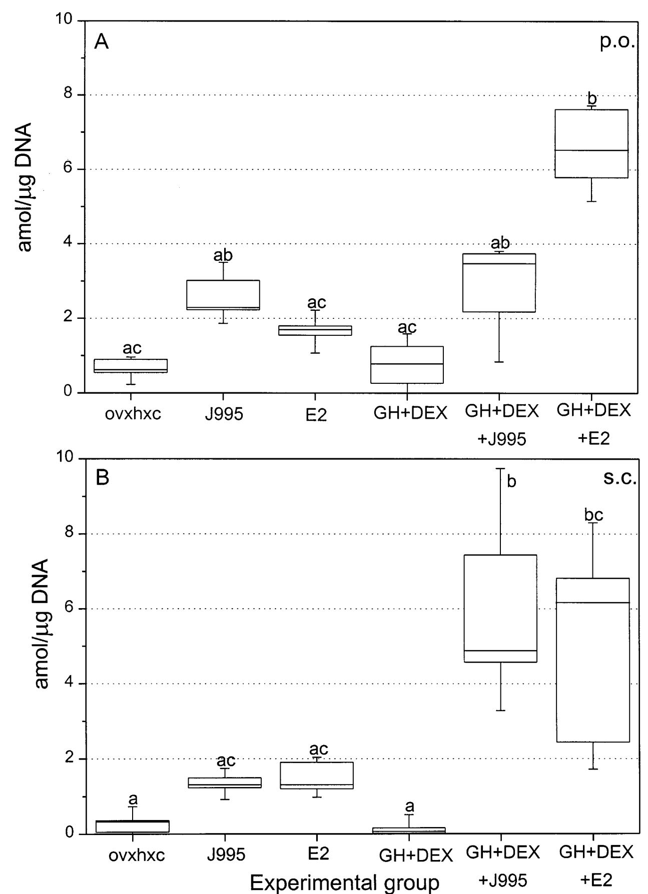

Fig. 7. The levels of IGF-I mRNA, determined by solution hybridization, in the ovariectomized + hypophysectomized rats. (A) After oral administration of hormones. (B) After subcutaneous administration of hormones. Boxes without the same letter are significantly different ($P < 0.05$).

tution. We conclude that E2 affects the ER level more than J995 in the ovx groups and possibly also in the ovxhx groups after 7 days of s.c. treatment.

The ER mRNA level was not different in ovx rats as compared to intact animals. The lower dose of J995 showed an increased level of uterine ER mRNA in the s.c. treated group whereas the high dose of E2 showed a level significantly lower than that of the ovx group. Thus, a higher dose of E2 decreases the ER mRNA level in ovx rats, significantly only in orally treated animals. In s.c. treated rats there was a significant increase in the level of ER mRNA after a low dose of J995, whereas a low dose of E2 had no effect. In a time course study of E2 treatment the ER mRNA level was reported to be increased after 24 h treatment, being maintained approximately at that level after 48 h and then to start decreasing during the rest of the week monitored. After 7 days of continuous treatment, the ER mRNA level was down to 50% of the level seen in the ovx control group [16]. The delay, or absence of a decrease in ER mRNA from J995 could be due to a depot effect with a delayed release of estrogenic activity, or an inability to reach a certain threshold level for estrogen-mediated down-regulation of ER mRNA.

In the ovxhx groups the treatments caused a decrease in the ER mRNA level in the non-substituted groups, significantly for E2 in the s.c. treated rats. On the other hand, in the GH + DEX substituted rats the ER mRNA levels were increased after treatment as compared to the ovxhx + GH + DEX control group, and for p.o. treatment the increase after E2 treatment was significant. Surprisingly, GH + DEX substitution increased the response to the estrogens in the uterus. This effect was not due to increased levels of ER, since they remain unchanged during treatment with GH + DEX. Treatment with GH + DEX does increase the circulating IGF-I levels (Elger et al., in preparation) and the hepatic ER levels [24] (also manuscript in preparation) in hx rats.

The decrease of the uterine IGF-I mRNA level after ovx has previously been described [14–16]. In the ovx rats J995 treatment resulted in a significant increase of the IGF-I mRNA level, both after p.o. and s.c. administration. After 7 days of E2 treatment the increase of the IGF-I mRNA level was not significant, which is in agreement with previous results [16], whereas in this study the J995 treated animals still showed significantly increased IGF-I mRNA levels. A s.c. dose of 2.5 μg E2/24 h administered continuously increased the IGF-I mRNA level in a transient way, with a maximum 24 h after the treatment was initiated [16]. The results of J995 treatment in this experiment suggest a delayed, or prolonged, effect influencing uterine IGF-I expression.

It is the concept of J995 development that this compound is inactive as such. J995 itself has no measurable affinity to the ER, opposed to powerful systemic estrogenic activity at oral and parenteral administration [32]. The pharmacodynamic and toxicological patterns of J995 correspond mainly to those of estradiol and estrone, which arise from it [32]. The main purpose of the development of J995 is the elimination of excessive hepatic estrogenicity of the currently used contraceptives. This cannot be achieved by dose reduction of ethinylestradiol, which would only be possible at the expense of a reasonable bleeding pattern and unlikely to reduce the hepatic impact of this estrogen to a relevant degree. Doses of ethinylestradiol, much below those used in low dose contraceptives, were found to have distinct effects on the above discussed hepatic functions [33]. J995 therapy may combine the convenience of oral treatment with the metabolic advantages of transdermal administration in hormone replacement therapy.

We conclude from this study that E2 and J995 treatment has similar effects with regard to uterine weight and levels of ER, ER mRNA and IGF-I. Whereas E2 seems to cause a more pronounced decrease in ER levels, J995 seems to cause a more marked increase in IGF-I mRNA levels. The relevance of these differences is not yet understood and needs to be studied further. An especially interesting finding is the synergistic effect of simultaneous treatment with estrogens and GH + DEX, which caused a marked increase in uterine ER mRNA and IGF-I mRNA levels as compared to treatment with estrogens alone.

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