Trans-4-hydroxy-3-methoxycinnamic acid (ferulic acid) inhibits the effect of androgens on the rat prostate[1]

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Summary. Trans-4-hydroxy-3-methoxycinnamic acid (ferulic acid, FA) antagonized the effect of exogenous androgens on the ventral prostate (VP) in castrated rats as well as the effect of endogenous androgens in intact rats. FA, however, had no effect on the seminal vesicles (SV) and levator ani muscle (LAM), nor oestrogenic effect in female rats and mice. FA did not antagonize the receptor binding of testosterone nor inhibit the conversion of testosterone into 5α-dihydrotestosterone (DHT).

In the course of screening test for anabolic steroids, we found that the androgenic activity of 19-nortestosterone (19-NT) was completely abolished by the substitution of FA at C-17 position[3]. Therefore, we tested FA itself and have recognized that it has a distinct inhibitory effect on the prostatic gland but not on the other male accessory sex organs. This is an account of such observations.

Materials and methods. FA (Sigma) and steroids (Sigma) were suspended in the aqueous suspending fluid consisting of sodium chloride (0.9%), carboxymethylcellulose (0.5%), polysorbate 80 (0.4%) and benzyl alcohol (0.9%).

Experiment 1. Male Wistar rats (80–90 g) were castrated and FA was injected s.c. once daily for 10 days starting on day 0, either alone or with androgen[4]. For oral assay FA was given p.o. for 7 days either alone or with androgen s.c. 24 h after the last drug treatment, the rats were sacrificed and VP, SV, LAM, adrenals, pituitary, thymus and testes were removed and weighed. For electron microscopic study, the tissues were fixed in 2% glutaraldehyde in phosphate buffer at pH 7.4 for 2 h, followed by 1 h postfixation in 1% osmium tetroxide buffered to pH 7 with 4% sucrose added. After dehydration with ethanol, they were embedded in epon 872.

Experiment 2. Uterotrophic activity was tested by the method of Rubin et al.[5]. Female Wistar rats (50–60 g) were injected s.c. once daily with FA for 3 days, then the uteri were weighed. Oestrogenic effect was tested by the vaginal smear technique[6]. Total doses of FA were 0.6, 1.2 and 1.6 mg/0.3 ml/mouse, which were divided into 3 doses and given at 17.00 h on the 1st day, and 09.00 and 17.00 h on the 2nd day. Each group consisted of 6 spayed mice (c57 bl/6 strain). Vaginal smear was tested twice a day for 3 days after last administration of FA.

Table 1. Antiandrogenic effect of ferulic acid in the castrated rats

Group	Treatment (total dose in mg)			No. of rats	Final mean b.wt	Ventral prostate	Seminal vesicles	Levator ani muscle
1	Control			8	113	8.0±0.5	8.6±0.5	14.0±1.0
	Nandrolon	0.8		5	114	16.9±3.0*	18.6±1.0*	50.7±1.2*
	Nandrolon	0.4		5	109	10.7±0.7*	11.7±1.5**	41.4±3.0*
	19NT-FA	0.8		5	117	8.5±0.5	9.2±0.6	16.0±1.4
2		Nandrolon (s.c.)	FA (s.c.)					
	Control	–	–	6	119	13.3±1.7	6.3±1.0	15.9±1.4
		0.8	–	6	124	22.0±2.4**	16.4±1.6*	51.4±1.9*
		0.8	10	6	124	16.0±2.3	14.5±1.0*	49.7±1.6*
		0.8	25	6	117	17.6±1.8	16.7±1.8*	51.3±3.3*
		–	25	6	111	9.1±0.9**	6.3±0.4	16.8±0.9
3		TP (s.c.)	FA (p.o.)					
	Control	–	–	6	61.7	8.6±1.3	7.4±0.4	13.8±1.2
		0.28	–	6	72.5	40.6±4.4	17.3±1.8	24.8±1.8
		0.28	14	6	64.0	34.6±7.0	13.0±1.4	24.7±1.9
		0.28	42	6	69.7	26.4±2.1*	15.0±0.4	25.5±4.9
4		DHT (s.c.)	FA (s.c.)					
	Control	–	–	10	145	13.6±0.5	14.9±1.6	35.8±1.6
		0.28	–	10	151	42.5±0.9	21.4±0.7	36.8±4.2
		0.28	17.5	10	148	39.8±0.8**	24.1±0.9	37.4±2.5
		0.28	35	10	147	37.9±0.9*	21.9±1.0	38.3±3.1

Mean value (mg wet wt/100 g b.wt)±SE. 19NT-FA: 19 Nortestosterone ferulate, TP: testosterone propionate, FA: ferulic acid, DHT: 5α-dihydrotestosterone. *Statistically different (Student's t-test) from the control (group 1 and 2) or from TP or DHT alone (group 3 and 4), at * $p < 0.01$, ** $p < 0.05$. Group 1 and 2: 10 days assay, group 3 and 4: 7 days assay.

Table 2. The effect of ferulic acid on the organ weights in intact or castrated rats

	Total dose (mg)	No. of rats	Final b.wt (g)	Adrenals	Pituitary	Thymus	Testes
Intact							
Control	–	5	185	21.0±1.2	4.0±0.1	134± 9.3	1100±5.0
FA	10	5	144	21.9±3.0	4.8±0.1	110±24.0*	1300±9.0
Castrated							
Control	–	5	114	27.8±1.3	4.6±0.3	286±36.9	–
FA	10	6	110	27.3±2.3	5.0±0.3	289±21.2	–

Mean (mg/100 g b.wt)±SE. Asterisks denote statistically significant difference from the control. * $p < 0.05$. FA: ferulic acid.

Experiment 3. The effect of FA on the uptake of (^{3}H) testosterone (RCC, Amersham) into VP, SV and LAM was tested in castrated Wistar rats (150–200 g). Immediately after castration FA (25 mg/kg b.wt) was given s.c. for 2 days. Tissues were removed 24 h after last injection, minced in Hanks' solution with 10 mM HEPES-NaOH buffer (pH 7.4), and incubated with 2.5 nM (for whole tissue) or 10 nM (for nuclear and cytosol fractions) (^{3}H) testosterone at 37 °C for 60 min. Nuclear and cytosol fractions were prepared by homogenization in 0.25 M sucrose containing 10 mM Tris-HCl buffer, pH 7.4, 0.1 mM EDTA, 0.5 mM 2-mercaptoethanol, and 1 mM $MgCl_2$ (TEM) with a Polytron, and centrifugation on a sucrose gradient according to Bruchovsky et al.[7]. The purified

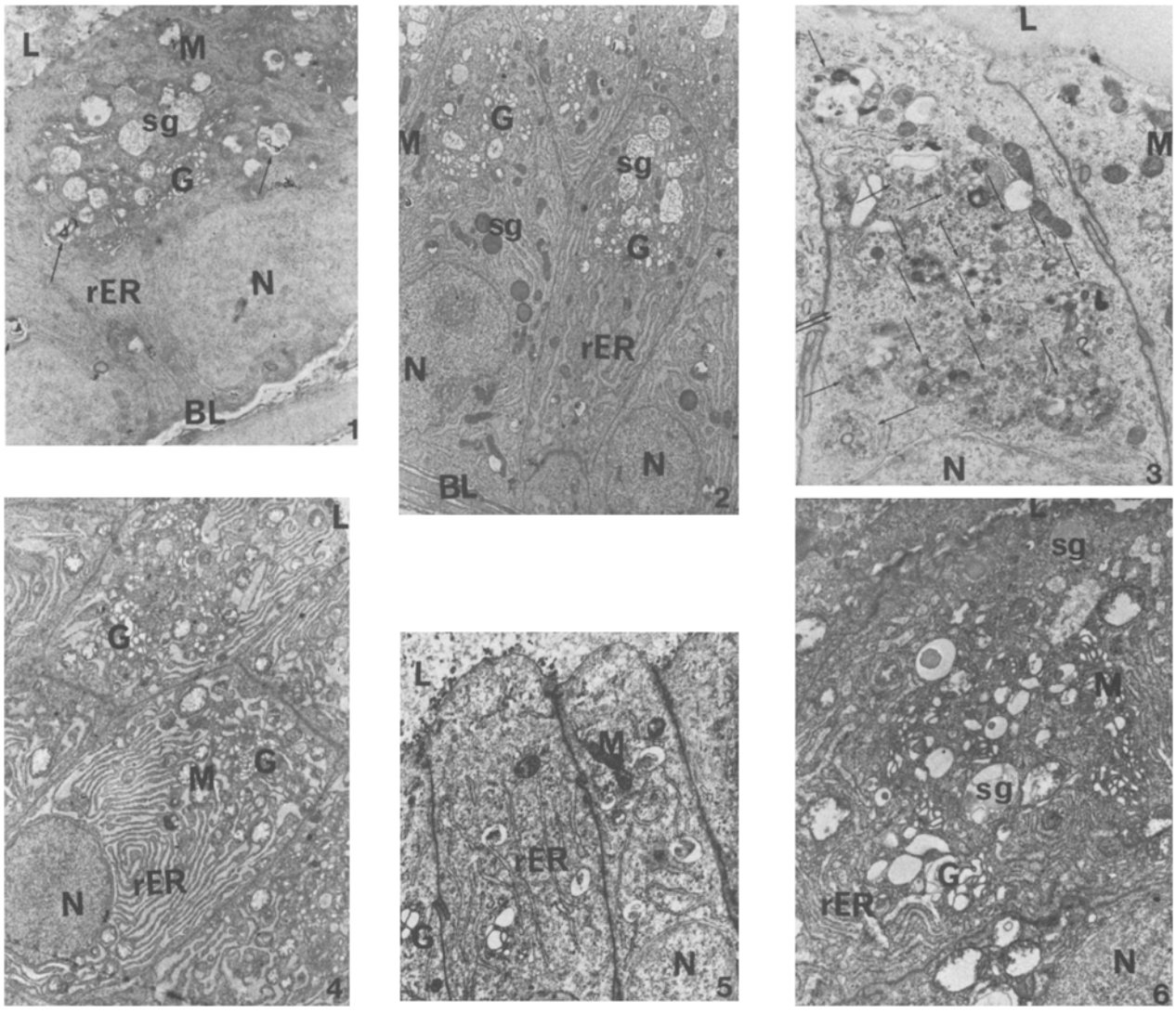

Fig. 1. Electron micrograph of the ventral prostate from the intact rat, which received ferulic acid 3 mg a day for 10 days s.c. Ferulic acid reduces the height of epithelial cells and the numbers of mitochondria and rough surfaced endoplasmic reticulum (rER). Secretory granules (sg) are less electron dense and autophagic vacuoles (arrows) are seen. Nucleus (N) shows surface irregularity. These findings are similar to those of the epithelia from the castrated rats. × 4600. BL, Basal lamina; L, Lumen.

Fig. 2. Control. Golgi regions (G) and rER are well developed. Secretory granules (sg) are electron opaque and after discharge of the contents they became vacuoles which are seen in apical region. Mitochondria (M) are seen in considerable numbers. × 3000.

Fig. 3. 10 days after castration. rER are collapsed and Golgi apparatus (G) is also reduced. Secretory granules almost disappear and autophagic vacuoles (single arrows) occupy the cytoplasmic area. They contain myelin-like membranes, dissoluted cytoplasm and perhaps lysosomal enzymes. Clusters of glycogen granules (double arrow) are scattered in the cytoplasm. × 8700.

Fig. 4. Testosterone propionate-treated (0.04 mg/day) rat, 10 days postcastration. Golgi apparatus (G) and rER are well developed, but mitochondria (M) are swollen. × 3000.

Fig. 5. Ferulic acid, 3 mg daily for 10 days, inhibited the effect of concomitantly injected testosterone propionate (0.04 mg daily). Golgi apparatus (G) and rER undergo a significant collapse and no secretory granules are seen. × 6500. L, Lumen; N, Nucleus.

Fig. 6. Epithelial cells of seminal vesicles from the ferulic acid-treated rat. Ferulic acid were injected s.c. 2 mg a day for 10 days. Secretory granules (sg), rER, mitochondria (M) and Golgi apparatus (G) are well developed. No atrophic changes are seen. × 6900. L, Lumen; N, Nucleus.

nuclei were extracted with 10% trichloroacetic acid, and radioactivities in the supernatant were measured, while the DNA contents in the precipitates were determined using the diphenylamine procedure[8]. Cytosol fraction was mixed with equal volume of dextran-coated charcoal (0.5% dextran T-70 and 5% Norit A in TEM) for 15 min and centrifuged at 1000×g for 10 min. Cytosol protein was determined by the method of Lowry et al.[9]. Effect of FA on 5α-reduction of testosterone was examined according to the method of Lee et al.[10], using a microsomal fraction of rat VP with 0.33 μCi (^{14}C) testosterone (RCC, Amersham) and 0.18 mM NADPH (Sigma). Effects of FA (25 mg/kg b.wt, s.c. for 3 days) on the uptake of 0.5 μCi (^{14}C) amino acid mixture (RCC, Amersham) into subcellular fraction of rat VP were determined by the modified method of Coulson et al.[11].

Results and discussion. Experiment 1. In the castrated rats 19-NT-17β-phenylpropionate (nandrolon), 0.08 mg daily for 10 days, increased the weights of VP, SV and LAM, but 19-NT-17-ferulate did not increase them at all (table 1, group 1). FA, 1.0 or 2.5 mg daily for 10 days, significantly inhibited androgen-induced hypertrophy of VP in castrated rats, whereas the weights of SV and LAM were not reduced by FA (table 1, group 2). FA given p.o. at 6 mg daily for 7 days, significantly inhibited the weight gain of VP by concomitantly injected TP (table 1, group 3), but daily doses of 2 mg did not show significant effect on VP. Weights of SV and LAM, however, were not significantly less than those in testosterone-treated rats, even when daily doses of FA were as high as 6 mg. Similar results were obtained with DHT, 0.04 mg daily for 7 days, and FA, 2.5 or 5 mg daily s.c. (table 1, group 4). By electron microscopy, in the epithelial cells from the FA-treated non-castrated rats many secretory granules were evacuated and became less electron dense (figure 1). Autophagic vacuoles[12] containing myelin membranes and dissoluted cytoplasm were developed. Profiles of rough-surfaced endoplasmic reticulum were collapsed and did not show typical lamellated whirls as seen in the control (figure 2). These findings are similar to those of the VP from the castrated rats[13] (figure 3). TP, 0.04 mg daily for 10 days, could prevent these ultrastructural changes following castration (figure 4), but concomitant injection of FA, 2 mg a day, inhibited the effect of testosterone on the VP (figure 5). However, FA did not show any effect on the ultrastructure of SV (figure 6) as it did not affect the weight of SV described above. FA did not produce the significant change in the weights of adrenals and pituitary glands in both intact and castrated rats (table 2). Thymus was reduced in weight significantly with FA in intact rats. This implies that FA does not reduce the androgen production, because thymus weight is increased by castration[14,15] and reduced by natural androgens[16,17].

Table 3. Uterotrophic activity of ferulic acid

	Total dose (mg)	No. of rats	Final b.wt (g)	Uterine weight Wet	Dry
Control		5	66.0	55.3±5.1	11.6±1.6
FA	3	5	60.6	60.8±7.5	13.1±1.6
FA	1	5	60.3	60.7±2.9	12.6±0.9

Mean (mg/100 g b.wt)±SE. FA: ferulic acid.

Experiment 2. FA had no uterotrophic activity in the spayed rats (table 3), and the vaginal smears of spayed mice showed always the dioestrous stage after FA treatment.

Experiment 3. The uptake of (^{3}H) testosterone by the whole tissue, nuclear and cytosol fractions of VP and SV, and whole tissue of LAM from castrated rats was not inhibited by FA pretreatment (table 4). Conversion of (^{14}C) testos-

Table 4. Effect of ferulic acid on the tissue uptake of (^{3}H)testosterone in the castrated rats

Tissues	Treatment	Whole tissue* (×10^3 dpm/mg wet)	Nuclei (×10^3 dpm/mg DNA)	Cytosol (×10^3 dpm/mg protein)
Ventral prostate	Control	2.75±0.12 (1.81±0.14)	10.1±2.2	5.06±0.69
	Ferulic acid (25 mg/kg s.c. for 2 days)	2.71±0.12 (1.88±0.12)	15.5±4.6	5.52±0.19
Seminal vesicles	Control	4.36±0.22 (2.10±0.15)	14.7±2.7	1.55±0.12
	Ferulic acid (same as above)	4.55±0.21 (2.09±0.05)	21.5±1.3	1.65±0.51
Levator ani muscle	Control	1.57±0.10 (1.18±0.07)		
	Ferulic acid (same as above)	1.62±0.09 (1.43±0.20)		

Mean value±SE. *Values in parenthesis show the non-specific uptake in the presence of 25 μM unlabelled testosterone during incubation. Tissues were removed 24 h after last injection of FA, minced in modified Hanks' solution, and incubated with 2.5 nM (for whole tissue) or 10 nM (for nuclear and cytosol fractions) (^{3}H)testosterone at 37 °C for 60 min.

Table 5. Effect of ferulic acid on the uptake of ^{14}C amino acid into subcellular fractions of the ventral prostate from non-castrated rats

Treatment	No. of rats	^{14}C Amino acid uptake (dpm/μg DNA) Nuclear	Ribosomal	Cytosol Acid precipitable	Acid soluble
Control	7	8.5±1.0	46.2±5.3	36.1±5.9	388±30
Ferulic acid (25 mg/kg b.wt for 3 days, s.c.)	6	6.8±0.8	32.8±4.5	27.5±6.0	289±14*

Mean±SE. * Significantly different from control ($p<0.05$). Ventral prostate was removed 24 h after last injection of FA, minced, and incubated in Hanks' solution with (^{14}C) amino acid mixture for 60 min at 37 °C.

terone into (^{14}C) DHT was determined as radioactivities in DHT and androstanediols fractions after 3–9-min incubation at 37 °C. 5α-Reductase activity of VP from the control animals was 2.97±0.47 pM/mg/protein/min, while those of FA (360 µM)-treated animals was 3.12±0.32. There was no significant difference. The uptake of (^{14}C) amino acid into the nuclear, ribosomal and acid-precipitable cytosol fractions was not inhibited by FA (table 5). However, the uptake of amino acid into the acid-soluble fraction of VP was significantly inhibited. From those results, it seems that FA does not antagonize the binding of androgens with the receptor nor the nuclear acceptor. It does not inhibit the conversion of testosterone to DHT. FA may act on the amino acid uptake at the membrane level. Albeit the real mechanism remains to be solved, it is interesting to note that this compound has a distinct anti-androgenic activity only on the prostatic glands in the rat.

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Are there cyclic variations in estradiol secretion in the non-pregnant rabbit?*

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Summary. Plasma estradiol-17β (E2) concentration was measured in 5 adult non-pregnant rabbits each in 3 different seasons (January, April and September). Blood samples were taken from each rabbit every other day. There was a considerable variation in plasma E2 levels from one sampling day to another, irrespective of the season. The pattern of variation in E2 levels in individual rabbits tended to be cyclic and this cycle was roughly of the order of 8 days. There was no correlation between changes in E2 levels and those in the vaginal appearance.

The non-pregnant adult rabbit is generally considered to be in continuous estrus, since it is believed that the female is capable of breeding more or less at any time of the year[1,2]. There is a general belief that the variation in plasma estrogen levels in the rabbits is relatively minor since the animals are in continous heat[2]. This is somewhat surprising in view of the fact that as early as 1934, it was shown[3,4] that in the rabbit ovary, mature follicles do not survive indefinitely but regress within 7–10 days during the estrous period.

During the course of our studies on the ovarian steroids concentrations in rabbit plasma and myometrium under a variety of conditions[5–7], we observed a great variation in the plasma concentration of estradiol-17β (E2) in the control (non-pregnant) group. These observations, together with the fact that data of the kind presented here were not available, led us to examine the variations in plasma E2 levels not only in different rabbits but in the same rabbit on different days.

Material and methods. Plasma estradiol-17β (E2) concentrations were measured in 5 adult non-pregnant rabbits, each in 3 different seasons (January, April and September). Blood samples from the marginal ear vein were collected in heparinized syringes from conscious, unrestrained does at about the same time in the morning of each sampling day.

The concentration of E2 was determined by radioimmunoassay of Lindberg et al.[8] as detailed previously[5–7]. Furthermore, to check the relative specificity for E2, the concentration of E2 in plasma extract was compared before and after Sephadex LH-20 chromatography. There was excellent agreement in the concentration of E2 between chromatographed and non-chromatographed extracts of plasma samples[7].

Results. Table 1 shows that in each of the 5 rabbits studied in January there was a considerable variation in plasma E2 concentration from one sampling day to another in the same rabbit, and it ranged between 14 and 54 pg/ml in all samples from 5 rabbits measured over a period of 15 days. Due to our inability to obtain blood samples from a particular rabbit on a particular day, or loss of the sample, some values in table 1 (and table 2) are missing. This minor incompleteness, however, was insignificant to have an influence on our general conclusion.

As can be seen in the figure (A), where data from 2 rabbits (1 and 3, table 1) are depicted, there was a tendency for a cyclic pattern in these variations. A similar variation was observed in rabbits studied in April (table 2). The variation and the range of plasma E2 concentrations taken at different days was comparable from one rabbit to another and the lowest and the highest values among all samples assayed in this group was 14 and 62 pg/ml, respectively. A pattern reminiscent of cyclic variation (figure, B) was more conspicuous, and this cycle was roughly of 8 days duration. Rabbits studied in September gave almost identical results with those studied in April, and variation in plasma E2 levels seemed to be cyclic in nature (figure, C). The patterns of variations in individual rabbits were very similar, but for clarity, data from only 2 rabbits in each group