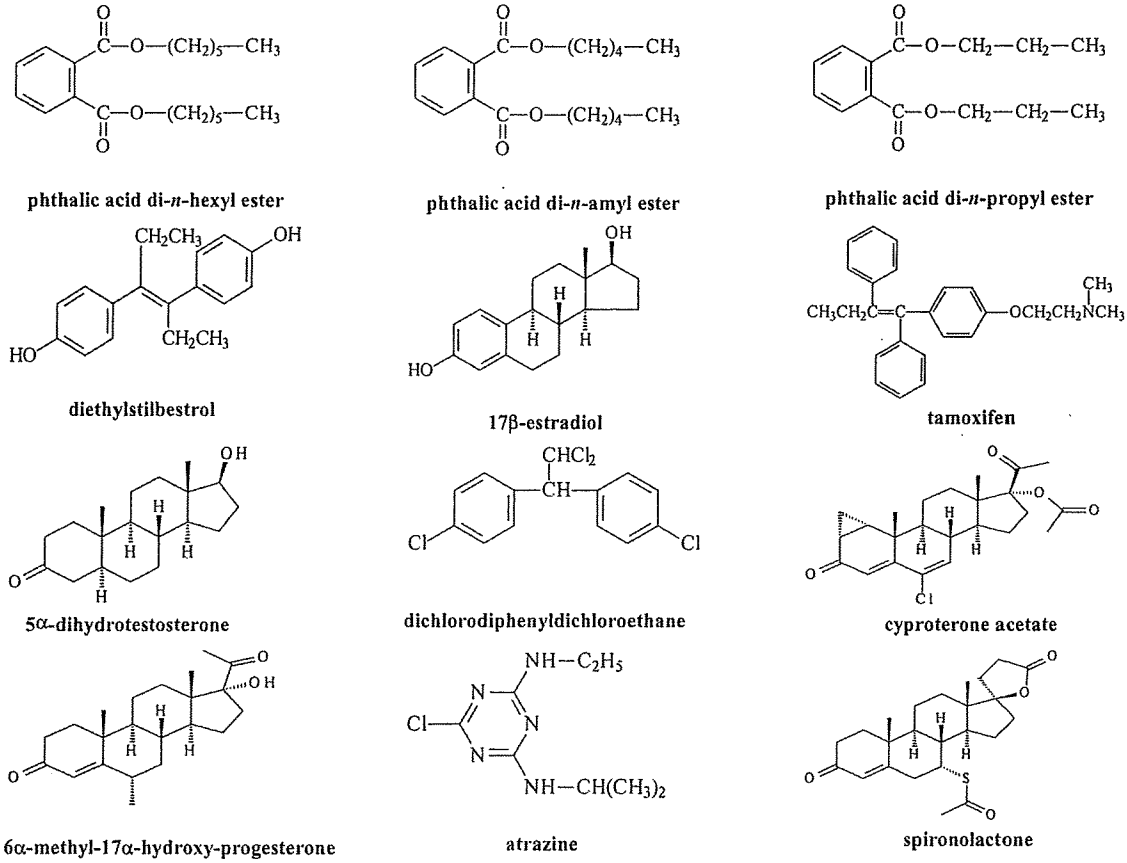

Fig. 1. Chemical structures of text chemicals.

acetate, 6α-methyl-17α-hydroxy-progesterone, and spironolactone have an androgenic steroid structure and are known to have androgen antagonistic properties; and atrazine is a reference endocrine disruptor (Friedmann, 2002; Gray et al., 2001; Laws et al., 2000). All chemicals were dissolved in olive oil (Fujimi Pharmaceutical, Company, Osaka, Japan) before use.

2.1.2. Animals

Male Brl Han: WIST Jcl (GALAS) rats castrated at 42-days of age were purchased from Clea Japan, Inc. (Shizuoka, Japan) and housed three per cage in stainless steel wire-mesh cages throughout the study. After allowing 14-days to recover from the operation, the rats were weighed, weight-ranked, and randomly assigned to each of the experimental and control groups. Body weight and clinical signs were recorded daily throughout the study. Rats were provided with water automatically and given ad libitum access to a commercial diet (MF, Oriental Yeast Co., Tokyo, Japan). The animal room was maintained at a temperature of 23 ± 2 °C and relative humidity of 55 ± 5%, and was artificially illuminated with fluorescent light on a 12-h light/dark cycle (0600–1800 h). All animals were cared for according to the principles outlined in the guide for animal experimentation prepared by the Japanese Association for Laboratory Animal Science.

2.1.3. Study design

Each chemical was orally administered via a stomach tube for 10 consecutive days beginning on postnatal day 56. A vehicle control group given only olive oil was also established. Testosterone propionate (TP,

CAS No. 57-63-6, 98% purity, Sigma Chemical Co.), 0.2 mg/kg/day, was administered to some rats by subcutaneous injection in the back after oral administration of each chemical, and a positive control injected with TP was also established. A group given the androgen antagonist flutamide, 10 mg/kg/day, plus TP was established to confirm the reliability of the study. Each group consisted of six rats. The doses of each chemical were selected based on the results of a preliminary study. In the preliminary study, each chemical was orally administered to castrated rats of the same strain for 7-days beginning on postnatal day 56, and the no observed adverse effect level (NOAEL) in the preliminary study was selected as the highest dosage in the 10-day administration study. However, increased body weight gain was observed in rats given some chemicals in the 10-day administration study. The volume of the olive oil solution containing TP was 0.2 ml/kg/day, and the volume of the olive oil solution containing the chemical was 5 ml/kg. The animals were killed by bleeding from the abdominal vein under deep ether anesthesia approximately 24 h after the final dose. The ventral prostate with fluid, seminal vesicle with fluid, bulbocavernosus/levator ani muscle (BC/LA), glans penis, and Cowper's gland were carefully dissected free of adhering fat and weighed.

2.1.4. Statistical analysis

Differences in body weight and organ weight between the vehicle group and each of the chemical groups and between the vehicle-plus-TP group and each of the chemicals plus-TP groups were assessed for statistical significance by the two-tailed Student's *t*-test.

2.2. Receptor binding assay

A recombinant human androgen receptor ligand binding domain (hAR-LBD), which expressed in *Escherichia coli* as a fused protein with maltose binding protein, was purchased from Toyobo Co., Ltd (Tokyo, Japan). It was supplied as a solution of 5.13 nM, determined from binding affinity of tritium-labeled mibolerone, in 50 mM pipes (piperazine-1,4-bis(2-ethanesulfonic acid)) buffer (pH 7.4).

The stock solutions (10 mM) of the test substance and dihydrotestosterone as a standard ligand were prepared with DMSO and diluted to 10 times higher concentration than test solution with tris–HCl (pH 7.4) containing 1 mM EDTA, 1 mM EGTA, 1 mM $NaVO_3$, 10% glycerol, 10 mg/ml γ-globulin, 0.5 mM phenylmethylsulfonyl fluoride, and 0.2 mM leupeptin (binding buffer). Each 10 μl of the test substance and radiolabeled ligand ([1,2,4,5,6,7-3H]dihydrotestosterone, Amersham Biosciences K.K.) solutions and 30 μl of the hAR-LBD solution were mixed with 50 μl of the binding buffer. After the solution was incubated for 1 h at 25 °C, free radiolabeled ligand was removed by incubation with 100 μl of 0.4% dextran-coated charcoal (SIGMA-Aldrich Co., USA) suspension in the binding buffer for 10 min at 4 °C, followed by filtration. The radioactivity in the filtrate was measured using liquid scintillation counter. More than four final concentrations were set in the suitable range in 10^{-11}–10^{-4} M to determine the IC_{50} value for each test substance depending on the binding affinity. The final concentration of hAR-LBD was 1.5 nM.

The percent ratio (B/B_0 (%)) of radiolabeled ligand bound to the receptor was calculated from the radioactivities of the solutions with (B) and without (B_0) the test substance subtracting the radioactivity due to nonspecifically bound ligand. The B/B_0 value versus concentration of test substance curve was fit to the logistic equation and IC_{50} value of each chemical was calculated by least-squares method using the computer program GraphPad Prism®. The binding abilities of test chemicals to the receptor were evaluated by relative binding affinity (RBA), ratio of IC_{50} values to standard ligand (dihydroteststeron).

3. Results

3.1. Hershberger assay

3.1.1. Clinical signs and body weights

Final body weights are shown in Table 2.

No abnormal clinical signs were detected in any of the rats.

A significant decrease in body weight gain was observed in rats given 0.05 mg/kg diethylstilbestrol and 0.05 mg/kg diethylstilbestrol plus TP, 2.0 mg/kg 17β-estradiol and 2.0 mg/kg 17β-estradiol plus TP, 0.1 mg/kg tamoxifen and 0.1 mg/kg tamoxifen plus TP, 10 mg/kg cyproterone acetate plus TP, 100 mg/kg

Table 2
Body weight and absolute accessory sex organ weight

Chemicals	RAB (% of DHT)	Dosages (mg/kg/day)	Body weight (g)	Ventral prostate (mg)	Seminal vesicle (mg)	BC/LA (mg)	Glans penis (mg)	Cowper's gland (mg)
Phthalic acid di-*n*-hexyl ester	not binding	Vehicle control	270.8 ± 9.0	16.4 ± 2.7	22.4 ± 3.7	147.9 ± 24.7	39.8 ± 3.2	4.7 ± 1.3
		40	274.4 ± 11.2	12.0 ± 4.5	26.1 ± 6.1	126.1 ± 21.7	40.5 ± 4.7	4.6 ± 2.1
		200	268.2 ± 9.6	17.1 ± 6.3	25.3 ± 3.2	146.4 ± 17.1	42.7 ± 1.8	4.7 ± 0.4
		1000	275.0 ± 9.7	14.8 ± 2.6	24.1 ± 4.5	130.8 ± 22.7	41.7 ± 5.4	4.2 ± 1.1
		Vehicle + TP	282.5 ± 12.7	111.0 ± 18.6	216.8 ± 59.2	321.5 ± 39.1	71.7 ± 6.2	20.3 ± 4.8
		40 + TP	281.6 ± 11.0	114.6 ± 26.3	271.6 ± 44.8	325.9 ± 39.8	67.4 ± 3.7	22.2 ± 4.0
		200 + TP	284.1 ± 13.9	105.6 ± 25.4	212.0 ± 45.1	337.2 ± 16.0	71.5 ± 7.0	20.0 ± 2.0
		1000 + TP	277.8 ± 12.6	94.1 ± 23.2	178.2 ± 31.0	315.0 ± 20.1	68.2 ± 6.0	19.8 ± 1.1
		FT + TP	279.2 ± 12.2	16.9 ± 2.9**	26.0 ± 4.7**	146.5 ± 25.6**	41.4 ± 4.3**	4.7 ± 0.7**
Phthalic acid di-*n*-amyl ester	not binding	Vehicle control	271.8 ± 10.5	13.1 ± 2.8	21.9 ± 4.5	145.7 ± 31.8	37.7 ± 4.5	3.6 ± 1.1
		40	269.4 ± 10.6	15.3 ± 3.8	25.0 ± 3.2	135.7 ± 19.1	41.3 ± 3.9	4.1 ± 0.8
		200	268.1 ± 10.3	13.1 ± 4.1	22.9 ± 4.5	143.6 ± 30.8	39.7 ± 3.7	4.2 ± 1.4
		1000	275.2 ± 13.2	13.0 ± 2.4	24.8 ± 3.5	136.9 ± 21.3	41.2 ± 3.6	4.3 ± 1.7
		Vehicle + TP	274.7 ± 15.3	108.3 ± 10.5	189.9 ± 25.6	343.2 ± 32.4	72.1 ± 5.0	25.2 ± 5.2
		40 + TP	280.5 ± 11.2	98.8 ± 21.7	190.6 ± 36.3	315.6 ± 49.9	69.4 ± 6.2	17.1 ± 3.4**
		200 + TP	282.0 ± 6.2	108.3 ± 19.0	158.0 ± 28.1	297.4 ± 27.5*	68.0 ± 4.3	20.4 ± 2.0
		1000 + TP	279.7 ± 8.3	76.7 ± 15.2**	147.6 ± 13.3**	285.9 ± 20.9**	70.7 ± 4.6	18.4 ± 4.7*
		FT + TP	271.4 ± 15.8	16.8 ± 3.9**	23.2 ± 4.1**	154.8 ± 21.6**	40.6 ± 4.1**	4.3 ± 0.9**
Phthalic acid di-*n*-propyl ester	not binding	Vehicle control	262.4 ± 11.6	14.0 ± 3.0	28.9 ± 5.8	134.1 ± 23.3	37.4 ± 3.7	4.2 ± 1.5
		40	261.4 ± 11.6	11.3 ± 3.5	24.0 ± 6.3	127.7 ± 10.5	36.8 ± 1.1	3.7 ± 0.9
		200	261.0 ± 11.2	14.5 ± 4.6	23.4 ± 5.1	139.8 ± 16.9	37.4 ± 2.7	4.3 ± 1.1
		1000	262.0 ± 14.9	11.7 ± 3.9	24.2 ± 5.4	115.9 ± 9.2	37.1 ± 3.3	3.8 ± 0.8
		Vehicle + TP	265.0 ± 6.0	87.9 ± 12.8	140.6 ± 22.0	298.4 ± 39.5	65.3 ± 5.6	16.6 ± 2.7
		40 + TP	270.1 ± 16.1	92.0 ± 17.2	140.3 ± 27.6	321.6 ± 62.1	64.6 ± 4.3	19.7 ± 4.9
		200 + TP	267.9 ± 10.3	92.4 ± 13.5	168.2 ± 31.7	338.8 ± 31.7	68.7 ± 4.9	21.4 ± 0.9**
		1000 + TP	272.2 ± 7.8	88.7 ± 22.1	156.0 ± 46.4	284.2 ± 20.1	67.4 ± 5.8	17.4 ± 3.3
		FT + TP	270.2 ± 9.9	15.9 ± 2.7**	28.3 ± 5.4**	139.3 ± 23.1**	39.6 ± 2.2**	3.9 ± 1.1**
Diethylstilbestrol	0.00539	Vehicle control	267.7 ± 12.0	13.2 ± 1.9	24.7 ± 3.1	134.3 ± 17.3	38.5 ± 4.6	4.3 ± 1.0
		0.002	268.4 ± 9.3	14.9 ± 1.8	27.2 ± 4.1	150.9 ± 21.1	38.3 ± 4.8	5.1 ± 0.9
		0.01	259.9 ± 6.7	14.4 ± 2.2	24.6 ± 4.3	143.9 ± 26.8	40.1 ± 4.2	4.0 ± 0.7
		0.05	248.2 ± 11.9****	14.6 ± 3.4	31.0 ± 4.0****	122.6 ± 18.9	41.6 ± 4.3	5.4 ± 1.1
		Vehicle + TP	280.2 ± 8.8	105.8 ± 19.9	221.6 ± 27.0	341.9 ± 42.3	68.8 ± 5.9	21.2 ± 3.1
		0.002 + TP	273.8 ± 12.1	109.8 ± 15.4	239.8 ± 37.0	338.6 ± 43.7	71.2 ± 6.1	22.8 ± 3.7
		0.01 + TP	269.2 ± 14.6	102.5 ± 16.6	226.5 ± 31.8	328.5 ± 16.4	70.9 ± 3.4	21.3 ± 4.7
		0.05 + TP	252.1 ± 12.0**	76.4 ± 10.2**	209.0 ± 50.9	289.1 ± 48.0	67.7 ± 5.8	19.3 ± 3.5
		FT + TP	269.3 ± 11.7	20.1 ± 2.2**	34.6 ± 8.3**	141.5 ± 18.1**	43.7 ± 6.1**	5.7 ± 1.3**

Table 2 (*Continued*)

Chemicals	RAB (% of DHT)	Dosages (mg/kg/day)	Body weight (g)	Vental prostate (mg)	Seminal vesicle (mg)	BC/LA (mg)	Glans penis (mg)	Cowper's gland (mg)
17β-Estradiol	2.56	Vehicle control	275.3 ± 14.1	14.3 ± 4.5	25.8 ± 3.6	150.9 ± 19.3	40.4 ± 3.4	4.0 ± 0.7
		0.1	276.3 ± 15.1	15.7 ± 2.4	26.9 ± 4.6	138.6 ± 11.3	41.2 ± 4.9	4.9 ± 1.1
		0.4	271.3 ± 9.2	15.3 ± 4.2	28.9 ± 8.6	145.4 ± 22.4	39.6 ± 1.4	5.0 ± 0.8****
		2.0	253.2 ± 8.2***	16.3 ± 1.7	28.1 ± 3.3	126.4 ± 11.6****	41.2 ± 5.4	4.1 ± 0.9
		Vehicle + TP	283.8 ± 13.8	121.8 ± 21.9	258.0 ± 39.7	349.2 ± 27.8	73.2 ± 3.1	23.9 ± 5.3
		0.1 + TP	285.1 ± 10.3	122.0 ± 9.9	255.2 ± 45.8	350.0 ± 31.4	71.1 ± 1.3	22.3 ± 4.8
		0.4 + TP	277.3 ± 9.9	102.3 ± 11.2	232.5 ± 31.5	365.3 ± 50.6	70.7 ± 7.6	21.0 ± 3.3
		2.0 + TP	257.0 ± 13.7**	99.0 ± 22.2	208.1 ± 61.3	294.0 ± 59.8	64.0 ± 4.2**	19.4 ± 6.7
		FT + TP	277.9 ± 13.5	18.1 ± 2.3**	24.7 ± 5.7**	150.0 ± 16.8**	38.9 ± 2.6**	4.6 ± 0.8**
Tamoxifen	0.0129	Vehicle control	279.4 ± 17.8	14.0 ± 3.6	26.2 ± 5.1	132.0 ± 18.9	42.1 ± 3.7	3.7 ± 1.6
		0.004	275.8 ± 13.8	16.8 ± 3.2	27.8 ± 6.9	139.7 ± 29.9	41.1 ± 5.2	4.8 ± 1.6
		0.02	265.0 ± 12.7	14.8 ± 4.5	23.7 ± 5.2	135.0 ± 11.0	38.5 ± 4.3	4.5 ± 1.4
		0.1	253.2 ± 11.5****	14.0 ± 3.0	22.6 ± 1.8	125.9 ± 21.0	39.5 ± 4.0	5.0 ± 0.8
		Vehicle + TP	289.3 ± 14.9	110.8 ± 14.5	237.0 ± 53.1	349.4 ± 48.9	68.2 ± 3.2	22.5 ± 3.1
		0.004 + TP	288.9 ± 14.9	120.9 ± 10.8	244.3 ± 35.9	349.2 ± 39.8	70.9 ± 6.3	23.6 ± 3.4
		0.02 + TP	279.2 ± 14.9	123.9 ± 13.2	250.3 ± 39.5	337.6 ± 36.5	71.3 ± 6.0	21.2 ± 5.6
		0.1 + TP	258.6 ± 7.9**	112.6 ± 22.2	268.7 ± 50.7	318.5 ± 50.8	71.0 ± 6.0	22.4 ± 3.3
		FT + TP	289.8 ± 10.2	18.1 ± 5.7**	23.1 ± 3.8**	150.8 ± 19.3**	42.5 ± 3.6**	4.7 ± 0.5**
5α-Dihydrotestosterone	100	Vehicle control	262.3 ± 17.2	16.2 ± 1.5	26.8 ± 5.9	141.2 ± 20.0	40.4 ± 2.0	5.0 ± 1.3
		8	261.0 ± 11.0	16.1 ± 1.9	23.5 ± 3.7	125.6 ± 8.9	40.9 ± 3.2	4.5 ± 0.8
		40	258.3 ± 9.1	27.9 ± 9.9****	27.4 ± 6.3	143.7 ± 19.1	44.3 ± 6.4	4.4 ± 1.1
		200	252.2 ± 19.1	63.6 ± 18.9***	90.8 ± 44.3****	230.3 ± 59.0****	58.2 ± 6.4***	11.9 ± 2.7***
		Vehicle + TP	266.6 ± 11.1	102.5 ± 16.5	231.6 ± 42.4	362.7 ± 28.2	70.2 ± 4.4	25.3 ± 3.7
		8 + TP	272.8 ± 13.4	110.1 ± 20.5	237.8 ± 56.6	325.0 ± 33.1	67.4 ± 1.8	20.3 ± 3.5*
		40 + TP	270.2 ± 14.9	108.6 ± 6.1	235.9 ± 43.1	333.2 ± 49.5	70.5 ± 3.7	21.5 ± 3.5
		200 + TP	266.3 ± 18.0	105.9 ± 17.8	274.0 ± 33.5	357.6 ± 33.4	73.5 ± 4.3	26.9 ± 4.3
		FT + TP	267.2 ± 14.4	20.4 ± 2.9**	27.8 ± 5.3**	147.8 ± 17.8**	42.1 ± 4.5**	5.1 ± 2.3**
Dichlorodiphenyl-dichloroethane	0.0139	Vehicle control	268.3 ± 14.5	19.7 ± 5.8	28.9 ± 7.6	131.8 ± 13.3	42.1 ± 4.5	4.9 ± 1.5
		8	270.9 ± 16.7	14.4 ± 2.8	26.2 ± 10.0	144.8 ± 14.3	37.6 ± 4.2	4.6 ± 0.5
		40	271.3 ± 12.7	14.5 ± 2.3	23.2 ± 3.7	144.4 ± 12.9	39.0 ± 3.0	3.8 ± 0.7
		200	263.6 ± 14.3	15.9 ± 3.1	26.1 ± 5.7	122.9 ± 11.7	40.2 ± 3.0	4.4 ± 0.5
		Vehicle + TP	276.8 ± 15.6	106.8 ± 14.4	235.0 ± 34.9	328.7 ± 53.4	71.3 ± 3.4	23.2 ± 4.0
		8 + TP	281.1 ± 13.0	121.5 ± 14.4	231.2 ± 63.0	349.3 ± 44.7	72.0 ± 5.7	23.1 ± 3.5
		40 + TP	272.7 ± 11.3	99.2 ± 23.6	202.7 ± 31.7	305.5 ± 39.4	69.5 ± 4.1	22.0 ± 2.0
		200 + TP	270.0 ± 11.3	95.9 ± 10.0	155.0 ± 19.1**	272.7 ± 24.7*	68.1 ± 2.3	21.5 ± 3.4
		FT + TP	268.3 ± 18.9	17.9 ± 1.8**	24.0 ± 2.3**	136.1 ± 29.8**	40.2 ± 3.3**	4.3 ± 1.6**

Cyproterone acetate	11.5	Vehicle control	270.0 ± 16.2	13.8 ± 3.9	24.9 ± 4.0	142.8 ± 8.2	39.3 ± 3.3	4.5 ± 1.0
		0.4	261.8 ± 14.3	15.8 ± 2.2	25.4 ± 1.9	131.0 ± 16.8	39.2 ± 3.7	4.7 ± 1.0
		2	250.7 ± 14.9	16.6 ± 2.1	26.8 ± 5.5	114.9 ± 17.2***	39.1 ± 2.4	4.6 ± 0.8
		10	242.3 ± 19.0	13.6 ± 1.8	22.3 ± 3.1	120.7 ± 8.5***	38.7 ± 1.4	3.1 ± 0.8****
		Vehicle + TP	271.3 ± 18.8	91.9 ± 11.0	153.2 ± 25.6	315.8 ± 35.8	67.3 ± 2.9	21.4 ± 3.6
		0.4 + TP	269.7 ± 15.8	74.3 ± 9.2*	119.1 ± 30.7	261.6 ± 20.7**	59.6 ± 3.0**	17.2 ± 1.6*
		2 + TP	252.4 ± 13.2	41.4 ± 7.7**	64.0 ± 10.2**	175.7 ± 28.7**	54.7 ± 9.0**	9.5 ± 1.7**
		10 + TP	248.1 ± 10.6**	23.8 ± 2.4**	32.2 ± 5.9**	129.2 ± 15.3**	42.3 ± 6.5**	5.4 ± 1.4**
		FT + TP	272.6 ± 19.0	20.2 ± 1.8**	25.2 ± 2.6**	133.4 ± 26.5**	41.2 ± 3.4**	5.0 ± 0.7**
6α-Methyl-17α-hydroxy-progesterone	5.86	Vehicle control	274.3 ± 14.9	17.2 ± 1.7	25.2 ± 3.0	140.2 ± 11.6	39.0 ± 2.9	5.8 ± 0.7
		20	260.8 ± 13.6	16.5 ± 0.9	26.9 ± 2.9	128.6 ± 21.7	41.5 ± 2.6	5.5 ± 2.1
		100	253.2 ± 13.2****	14.4 ± 2.3****	23.2 ± 2.6	119.1 ± 21.8	37.5 ± 1.6	4.4 ± 1.2****
		500	257.3 ± 14.0	16.5 ± 2.5	25.2 ± 4.0	120.9 ± 12.4****	39.9 ± 2.3	4.7 ± 1.2
		Vehicle + TP	276.6 ± 6.6	104.8 ± 8.2	201.4 ± 32.7	328.7 ± 43.7	70.3 ± 2.0	22.8 ± 3.2
		20 + TP	271.5 ± 15.8	96.8 ± 12.3	200.0 ± 23.6	308.9 ± 28.1	70.2 ± 3.5	21.4 ± 5.1
		100 + TP	271.0 ± 11.2	83.3 ± 17.8*	188.3 ± 39.7	259.6 ± 35.3*	65.1 ± 5.5	18.0 ± 2.0*
		500 + TP	262.1 ± 12.8*	61.9 ± 15.0**	105.8 ± 19.9**	219.7 ± 51.1**	60.3 ± 4.5**	14.9 ± 4.9**
		FT + TP	280.8 ± 14.0	19.7 ± 1.4**	27.6 ± 3.5**	147.6 ± 22.0**	43.3 ± 3.0**	5.0 ± 0.4**
Atrazine	not binding	Vehicle control	277.2 ± 16.5	15.1 ± 2.6	25.2 ± 4.5	141.9 ± 37.7	38.6 ± 3.0	5.2 ± 1.5
		3.2	273.5 ± 19.9	16.4 ± 1.8	26.7 ± 3.5	134.1 ± 20.5	38.8 ± 3.0	5.0 ± 1.8
		16	276.2 ± 13.2	17.2 ± 2.6	26.1 ± 3.0	147.9 ± 23.3	41.1 ± 3.4	4.6 ± 0.6
		80	258.5 ± 10.2****	15.1 ± 2.6	24.4 ± 2.3	127.2 ± 16.3	40.3 ± 3.1	4.9 ± 0.9
		Vehicle + TP	284.5 ± 11.0	108.5 ± 29.2	211.2 ± 73.4	314.0 ± 39.9	69.8 ± 6.2	21.9 ± 3.4
		3.2 + TP	292.3 ± 16.8	107.8 ± 14.0	206.3 ± 26.8	328.9 ± 25.4	70.2 ± 8.4	22.1 ± 2.2
		16 + TP	276.3 ± 12.2	103.2 ± 13.4	218.1 ± 44.9	319.3 ± 45.0	69.4 ± 4.7	23.8 ± 4.2
		80 + TP	272.4 ± 14.3	97.5 ± 8.1	231.1 ± 70.3	320.7 ± 42.0	69.5 ± 3.4	20.1 ± 3.6
		FT + TP	293.6 ± 12.5	17.5 ± 4.4**	25.3 ± 2.8**	153.6 ± 16.7**	41.1 ± 3.1**	4.7 ± 1.0**
Spironolactone	2.70	Vehicle control	275.2 ± 14.0	17.0 ± 2.4	29.3 ± 8.5	155.8 ± 26.3	41.0 ± 4.2	5.3 ± 2.0
		8	276.0 ± 11.1	16.5 ± 1.4	27.7 ± 3.4	158.8 ± 30.6	39.4 ± 2.1	5.2 ± 1.4
		40	276.6 ± 11.9	16.7 ± 2.6	26.8 ± 5.4	141.2 ± 13.7	40.2 ± 2.5	4.6 ± 0.5
		200	263.3 ± 14.2	17.5 ± 2.2	27.5 ± 4.9	144.9 ± 35.1	41.3 ± 2.4	4.8 ± 1.1
		Vehicle + TP	280.1 ± 16.6	109.7 ± 12.0	231.1 ± 39.8	326.3 ± 40.2	69.7 ± 4.7	22.6 ± 5.0
		8 + TP	276.7 ± 14.4	89.8 ± 9.9*	192.3 ± 32.9	305.2 ± 43.9	67.2 ± 4.2	18.8 ± 2.7
		40 + TP	275.9 ± 16.8	69.9 ± 12.1**	146.8 ± 36.0**	277.6 ± 24.1*	65.0 ± 2.4	17.6 ± 1.9
		200 + TP	269.4 ± 16.5	34.2 ± 7.6**	49.0 ± 14.3**	184.3 ± 13.8**	51.3 ± 4.2**	8.7 ± 1.6**
		FT + TP	279.8 ± 12.6	17.0 ± 2.2**	24.3 ± 4.2**	152.5 ± 13.8**	40.0 ± 2.9**	5.2 ± 2.2**

RBA, receptor binding affinity; DHT, dihydrotestosterone; TP, testosterone propionate; FT, flutamide; BC/LA, Bulbocavernosus and levator ani muscles.

* Significantly different from vehicle control + TP at $P < 0.05$.

** Significantly different from vehicle control + TP at $P < 0.01$.

*** Significantly different from vehicle control at $P < 0.01$.

**** Significantly different from vehicle control at $P < 0.05$.

6α-methyl-17α-hydroxy-progesterone and 500 mg/kg 6α-methyl-17α-hydroxy-progesterone plus TP, and 80 mg/kg atrazine.

3.1.2. Organ weight

Absolute accessory sex organ weights are shown in Table 2, and these were essential the same as the relative organ weight changes.The weight of all accessory sex organs increased significantly in the rats given 5α-dihydrotestosterone, but they decreased significantly in the rats given cyproterone acetate, 6α-methyl-17α-hydroxy-progesterone, and spironolactone. The weight of the accessory sex organs except the glans penis decreased significantly in rats given phthalic acid di-*n*-amyl ester, and the weight of some accessory sex organs decreased significantly in the rats given dichlorodiphenyldichloroethane.

Seminal vesicle weight increased significantly in the high diethylstilbestrol dosage group.

Despite the significant decrease in absolute ventral prostate weight in the high diethylstilbestrol dosage plus TP group, decrease in absolute BC/LA weight in the high 17β-estradiol dosage group, decrease in absolute weight of glans penis in the high 17β-estradiol dosage plus TP group, decrease in absolute BC/LA weight in the middle and high cyproterone acetate dosage groups, decrease in absolute weight of the Cowper's gland in the high cyproterone acetate dosage group, and decrease in absolute BC/LA in the high 6α-methyl-17α-hydroxy-progesterone dosage group, no relative weight changes in these organs were detected within the same dosage group (relative weight changes not shown). The decreases in absolute weight of some accessory sex organs in response to some chemicals without any change in the relative organs were considered to be attributable to the decreases in body weight gain induced by these chemicals.

No organ weight changes or dose-related changes were detected in the rats given phthalic acid di-*n*-hexyl ester, phthalic acid di-*n*-propyl ester, tamoxifen, and atrazine.

3.2. Receptor binding assay

The results of the receptor binding assays are shown in Table 2.

Eight of 12 chemicals, diethylstilbestrol, 17β-estradiol, tamoxifen, 5α-dihydrotestosterone, dichlorodiphenyldichloroethane, cyproterone acetate, 6α-methyl-17α-hydroxy-progesterone, and spironolactone, were positive in the androgen receptor binding assay.

4. Discussion

The OECD proposed the Hershberger assay as a screening method to detect the androgenic properties of endocrine disrupting chemicals, and this has been reported to be useful in this regard (OECD, 2000, 2003). In the present study, we performed Hershberger assays of 12 various chemicals. The weights of the accessory sex organs of rats given TP were higher than those of rats given the vehicle alone, and the organ weights of rats given flutamide plus TP were lower than those of rats given TP alone, thereby confirming the reliability of this study.

The weight of all or some of the accessory sex organs decreased significantly in rats given cyproterone acetate plus TP, spironolactone plus TP, 6α-methyl-17α-hydroxy-progesterone plus TP, and dichlorodiphenyldichloroethane plus TP, and thus these chemicals are thought to have androgen antagonist affinity. The antagonist affinity of cyproterone acetate and spironolactone was higher than that of the other chemicals. The receptor binding affinity of 6α-methyl-17α-hydroxy-progesterone and spironolactone was 5.86 and 2.70, respectively, and weight changes in accessory sex organs were detected at 100 mg/kg/day 6α-methyl-17α-hydroxy-progesterone and at 8 mg/kg/day spironolactone. These findings indicate that (anti)androgen potency according to the receptor binding assay does not completely correspond to potency according to the Hershberger assay. Metabolism and other unknown factors present in the in vivo tests are thought to be related to this phenomenon, and differences in pharmaco/toxicokinetics between the in vitro and in vivo tests are also suggested. Hershberger assay and receptor binding assay using many chemicals are needed to clarify the relationship between two assays.

Increased seminal vesicle weight was detected in the high estrogenic compound "diethylstilbestrol" group in this study. However, whether diethylstilbestrol has an androgenic effect remains uncertain. In our previous Hershberger assay, the weight of some male accessory sex organs increased in rats given the

typical estrogenic compounds ethinyl estradiol, equilin, norgestrel, or estrone, but we could not determine whether these chemicals exhibit androgen agonistic properties (Yamasaki et al., 2003), because estrogen and androgen receptors are said to be present in the accessory sex organs of male rats and mice (Re et al., 2001; Weihua et al., 2001; Williams et al., 2001). On the other hand, the receptor binding affinity of other estrogenic compounds, i.e., 17β-estradiol and tamoxifen, was higher than that of diethylstilbestrol, but no androgenic properties of these two estrogenic compounds were detected in the Hershberger assays. The androgenic affinity detected in the Hershberger assay may not be present in diethylstilbestrol.

Di(*n*-butyl)phthalate has been reported to inhibit the endocrine mediated effects in male rats after in gestational and lactational exposure without the androgen receptor affinity (Mylchreest et al., 1999). In the present study, the Hershberger assays of three phthalates, phthalic acid di-*n*-hexyl ester, phthalic acid di-*n*-amyl ester, and phthalic acid di-*n*-propyl ester, were performed at the same dosages, and the results showed weak androgen antagonistic affinity only in the assay of phthalic acid di-*n*-amyl ester. We did not investigate why only phthalic acid di-*n*-amyl ester has androgen antagonist affinity. The chemical structure of these compounds is similar, and only their number of CH_2 groups differs. Phthalic acid di-*n*-amyl ester contains many more CH_2 groups than phthalic acid di-*n*-propyl ester and fewer than phthalic acid di-*n*-hexyl ester, and there may be some relationship between the number of CH_2 groups and androgen antagonistic affinity. On the other hand, it is noteworthy that no receptor binding affinity was detected in these three phthalates. The positive result of phtalic acid di-*n*-amyl ester in the Hershberger assay may be related to the testosterone metabolism in the liver.

A clear androgen agonistic effect was detected in 5α-dihydrotestosterone in the present study. Androgen agonistic affinity of androgen derivatives testosterone enanthate and methyltestosterone were also detected in the Hershberger assay in our previous study (Yamasaki et al., 2003). Therefore, it is suggested that the androgen agonistic effect in androgenic chemicals is detected in the Hershberger assay.

Atrazine has been reported to inhibit testosterone production in rat males following peripubertal exposure (Friedmann, 2002), and atrazine is said to affect the pituitary/hypothalamic axis (USEPA, 2002). Thus, it was not surprising that no endocrine disrupter property of atrazine was detected in the Hershberger assay in castrated rats.

Acknowledgements

This work was supported by a grant from Ministry of Economical Trade and Industry.

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