Table 1. Effect on *in vivo* anticonvulsant drugs on production of *N*-acetylserotonin (NAS), hydroxyindole acetic acid (HIAA), hydroxytryptophol (HTOL), melatonin (MTN), methoxyindole acetic acid (MIAA) and methoxytryptophol (MTOL) (ng produced/pineal/24 hr ± S.E.M.)

	Dosage	Concentration	NAS	HIAA	HTOL	MTN	MIAA	MTOL
No drug	—	—	5.3 ± 0.5	69 ± 11	12 ± 4	4.6 ± 0.4	5.2 ± 0.3	1.6 ± 0.3
Acetazolamide	100	10	3.0 ± 0.4†	61 ± 10	14 ± 5	2.2 ± 0.4†	5.8 ± 0.5	1.7 ± 0.5
Beclamide	250	5	3.3 ± 0.3†	63 ± 12	10 ± 3	2.1 ± 0.6†	5.5 ± 0.9	2.0 ± 0.4
Carbamazepine	25	6	3.6 ± 0.5*	57 ± 9	15 ± 6	2.8 ± 0.5*	5.4 ± 1.1	2.1 ± 0.5
Clonazepam	0.1	0.005	3.4 ± 0.4*	71 ± 13	14 ± 4	3.0 ± 0.5*	5.5 ± 0.9	1.7 ± 0.3
Diazepam	10	0.2	4.6 ± 0.8	61 ± 11	13 ± 4	4.2 ± 0.5	5.3 ± 0.7	1.8 ± 0.5
Diphenyhydantoin	100	10	4.4 ± 0.6	63 ± 12	10 ± 4	4.0 ± 0.6	5.6 ± 0.5	1.9 ± 0.4
Ethosuximide	25	40	5.2 ± 0.7	77 ± 13	14 ± 5	4.3 ± 0.6	5.3 ± 0.8	2.1 ± 0.6
Pheneturide	150	5	4.9 ± 0.6	68 ± 11	11 ± 5	4.5 ± 0.4	5.2 ± 0.7	1.5 ± 0.4
Phenobarbitone	12.5	10	4.0 ± 0.4	79 ± 14	16 ± 6	3.2 ± 0.5	5.5 ± 0.8	2.3 ± 0.8
Primidone	142	5	4.2 ± 0.4	81 ± 13	14 ± 5	3.4 ± 0.4	5.3 ± 0.6	2.2 ± 0.4
Sulthiame	50	5	3.9 ± 0.3*	78 ± 11	15 ± 4	3.4 ± 0.3†	5.6 ± 0.5	2.0 ± 0.5
Valproate	64	50	5.1 ± 0.5	71 ± 15	13 ± 3	4.3 ± 0.5	5.0 ± 0.7	2.3 ± 0.6

Dosage of drugs administered to rats (mg/kg) and concentration in the culture medium (μg/ml) are given.
* $P = 0.05$, † $P = 0.025$.

is also apparent, as the drugs reduced melatonin production, that this pineal hormone does not play a role in control of seizure states following anticonvulsant drug administration.

In conclusion, therefore, it is apparent that the pineal gland is capable of compensating to a certain extent for drug-induced changes in indole production and only five of the drugs tested caused significant reduction in melatonin output after chronic administration. It is possible that treatment with these drugs for longer periods may allow for compensatory mechanisms to return pineal indole output to normal and studies are at present underway to examine this possibility.

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Effect of 6-methylene-4-pregnene-3,20-dione treatment on hepatic bile acid sulfotransferase activity in male rats

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6-Methylene-4-pregnene-3,20-dione has been shown recently *in vitro* to be an irreversible inhibitor of prostatic Δ^4-3-ketosteroid-5α-reductase (5α-reductase) [1]. Detailed kinetic studies suggest that inhibition is dependent upon the presence of NADPH and involves two phases of interaction between the compound and the enzyme. In the first phase, 6-methylene-4-pregnene-3,20-dione binds reversibly to the enzyme; this binding can be shown to be competitive with the binding of the enzyme's natural substrate, testosterone. In the second interaction, the enzyme is irreversibly inhibited, presumably as the result of covalent binding of 6-methylene-4-pregnene-3,20-dione at the active site.

Treatment of male rats with 6-methylene-4-pregnene-3,20-dione results in marked regression of the weight of the ventral prostate and seminal vesicles after only 11 days [2]. These data suggest that 6-methylene-4-pregnene-3,20-dione also may be an effective antiandrogen drug *in vivo*.

Studies of bile acid metabolism have shown that the

hepatic enzymes that catalyze the formation of sulfate esters of bile acids are, at least in part, under hormonal control [3, 4]. Suppression of bile acid sulfotransferase activity by androgens is suggested by the finding that enzyme activity increases significantly following castration of male rats; this increase can be prevented by treatment with 5α-dihydrotestosterone [4]. Sulfation increases the potential for excretion of bile acids [5, 6] and, thus, offers an alternate pathway for removal of these detergent molecules from the liver in the presence of impaired bile flow [7]. We, therefore, studied the effect of treating male rats with 6-methylene-4-pregnene-3,20-dione on hepatic bile acid sulfotransferase activity in order to determine whether this or similar 5α-reductase inhibitors might be useful agents in the experimental study of the metabolic adaptation to cholestasis.

Materials and methods

Random bred, mature male Sprague–Dawley rats (Charles River Laboratories, North Wilmington, MA) were maintained in hanging cages in a well-ventilated room which was lighted for 14 of 24 hr. Rats had free access to water and pelleted commercial chow. Groups of rats were treated with 5α-dihydrotestosterone, 1.6 mg/kg (Sigma Chemical Co., St. Louis, MO); 6-methylene-4-pregnene-3,20-dione [1], 20 mg/kg; or vehicle, 10% (v/v) ethanol in propylene glycol, 0.15 ml. The average starting weight of 238 g was used to calculate the amount of drug given in 0.15 ml vehicle. Animals were treated for 21 days, receiving subcutaneous injections each morning beginning with the day of castration or sham operation. Castration was performed under ether anesthesia by standard surgical techniques; the wound was closed with stainless steel clips. Sham-operated animals were anesthetized, and both testes were identified through an incision which was treated identically to that in the castrated animals. At the end of 21 days, the animals were stunned by a blow at the base of the neck and exsanguinated by cervical laceration; the liver was rapidly removed and placed in 10 mM potassium phosphate, 150 mM NaCl, pH 7.4. Thereafter the preparation of liver tissues conformed to that which has been reported recently [4]. Specific activity of post-ultracentrification supernatant fraction was measured in each animal by the Sep-Pack method exactly as described [4]. Enzyme activities were normalized to protein concentration as estimated by the biuret method [8]. Immediately prior to being killed, each animal was weighed. Following exsanguination and removal of the liver, the ventral prostate, seminal vesicles and, in sham-operated animals, testes were removed, blotted with filter paper, and weighed. The significance of differences between means was assessed by Student's *t*-test [9], using Bonferroni's inequality adjustment for multiple comparisons [10]. Variation about the mean is expressed as ±1 S.E.M.

Results and discussion

Treatment of sham-operated animals with 6-methylene-4-pregnene-3,20-dione resulted in no change in body weight or liver weight (Table 1). Total hepatic cytosol protein per gram wet weight of liver was also the same, 64.4 ± 3.4 mg in controls vs 60.1 ± 4.1 mg in treated animals. There was, however, a marked reduction in the weight of the seminal vesicles and in the ventral prostate. These effects of treatment were not seen in animals receiving simultaneous treatment with 5α-dihydrotestosterone.

Castrated animals showed an even greater reduction in the weight of the seminal vesicles and the ventral prostate, again without a significant change in the liver or body weight. Treatment of castrated animals with 6-methylene-4-pregnene-3,20-dione resulted in no further reduction in prostate or seminal vesicle weight.

The difference between the effect of treatment with 6-methylene-4-pregnene-3,20-dione and castration on the ventral prostate and seminal vesicles is consistent with the proposed k_{cat} mechanism of inhibition of 5α-reductase [1]. As Brooks *et al.* [11] have shown, effective inhibitors of 5α-reductase result in increased tissue levels of testosterone. The potential effect of this would be that the increased concentration of testosterone would compete with 6-methylene-4-pregnene-3,20-dione for initial binding to the 5α-reductaste [1, 11].

In contrast to its effect on seminal vesicles and ventral prostate, treatment of sham-operated animals with 6-methylene-4-pregnene-3,20-dione had an effect on hepatic bile acid sulfotransferase activity which was equivalent to orchiectomy (Table 2). Treatment with the drug resulted in a "chemical castration". This effect also was prevented by simultaneous treatment with 5α-dihydrotestosterone.

It is important to note that the liver does possess 5α-reductase [12, 13]. However, hepatic 5α-reductase differs from that found in prostate and seminal vesicles. Organ specific differences in 5α-reductase may account in part for the differences in the effect of the drug on hepatic and non-hepatic events. Finally, none of the data is inconsistent with an effect on the pituitary-gonadal axis. However, we observed no difference between control and treated animals with respect to the rate of growth or in testicular weight at the time of sacrifice (2.98 ± 0.03 g vs 3.08 ± 0.07 g respectively). These data suggest that testosterone-mediated events proceeded without interference.

Table 1. Effect of treatment on body weight and weight of liver, seminal vesicles and ventral prostate

Treatment	N	Weight*			
		Body weight	Liver	Seminal vesicles	Ventral prostate
Sham-operated					
Vehicle only	6	327 ± 11	11.8 ± 0.6	0.544 ± 0.033	0.408 ± 0.009
6-Methylene-4-pregnene-3,20-dione	6	324 ± 14	11.6 ± 0.7	0.301 ± 0.029†	0.203 ± 0.020†
5α-Dihydrotestosterone	6	322 ± 7	11.3 ± 0.4	0.596 ± 0.105	0.404 ± 0.026
5α-Dihydrotestosterone + 6-Methylene-4-pregnene-3,20-dione	5	328 ± 14	11.4 ± 0.7	0.485 ± 0.055	0.422 ± 0.047
Castrated					
Vehicle only	6	295 ± 8	11.5 ± 0.6	0.071 ± 0.004†	0.021 ± 0.001†
6-Methylene-4-pregnene-3,20-dione	7	290 ± 7	10.5 ± 0.4	0.087 ± 0.005†	0.018 ± 0.003†
5α-Dihydrotestosterone	8	310 ± 12	11.6 ± 0.7	0.712 ± 0.065	0.330 ± 0.041

* Values shown are average weight of N animals in grams ± 1 S.E.M.

† Significantly different, $P < 0.0024$, from sham-operated controls (vehicle only).

Table 2. Effect of 6-methylene-4-pregnene,3,20-dione on hepatic bile acid sulfotransferase specific activity

Treatment	N	Bile acid sulfotransferase activity (pmoles/min/mg protein)
Sham-operated		
Vehicle only	6	22.8 ± 2.2
6-Methylene-4-pregnene-3,20-dione	6	56.1 ± 6.5*
5α-Dihydrotestosterone	6	16.7 ± 3.2
5α-Dihydrotestosterone + 6-methylene-4-pregnene-3,20-dione	5	18.5 ± 1.7
Castrated		
Vehicle only	6	60.0 ± 7.8*
6-Methylene-4-pregnene-3,20-dione	7	82.7 ± 3.5*
5α-Dihydrotestosterone	8	12.1 ± 1.2

* Values shown are means of determinations on N animals ± one S.E.M.
* Significantly different, $P < 0.0024$, from sham-operated controls (vehicle only).

The results of present studies indicate that 6-methylene 4-pregnene-3,20-dione treatment can stimulate intrahepatic bile acid sulfotransferase activity without major constitutional effects. Furthermore, the effect of the drug on this metabolic pathway was equivalent to that which results from surgical castration. Since it has been shown that 5α-reductase deficiency can be consistent with normal sexual activity and muscular growth [14], the use of inhibitors of 5α-reductase has been proposed in the treatment of dihydrotestosterone-responsive conditions (e.g. prostatic hypertrophy and prostatic cancer) [11, 15]. The data presented here suggest that a 5α-reductase inhibitor such as 6-methylene-4-pregnene-3,20-dione also may be useful in the study of the treatment of cholestasis.

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Valproic acid teratogenicity in whole embryo culture is not prevented by zinc supplementation

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It has been suggested that some of the toxicities caused by the anticonvulsant valproic acid (2-propylpentanoic acid) may be mediated through an alteration of trace metal status [1, 2]. Many of the side effects of valproate therapy are similar to the symptoms associated with zinc deficiency [3, 4]. Valproate has been linked with an increased incidence of spina bifida in infants born to mothers taking the drug [5, 6] and a connection between zinc deficiency and spina bifida and anencephaly has been suggested [7]. Animal studies show that either zinc deficiency or valproate exposure can induce malformations of the neural tube [8, 9]. Eckhert and Hurley reported a reduction in the incorporation of [^{3}H]thymidine into the DNA of the head region of embryos (embryonic age 13 days) in zinc-deficient