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CYCLOPENTYL ETHERS OF ESTROGENIC STEROIDS PARALLELISM BETWEEN PROLONGED ORAL ACTIVITY AND STORAGE IN BODY FAT OF RATS

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SUMMARY

The 3-cyclopentyl ethers (CPE's) of four estrogens have been compared with the parent steroids in spayed rats for their prolonged vaginotropic activity after a single oral treatment. The parent compounds showed a short lasting vaginal cornification (average: 0·5–2 days). Three of the CPE's induced long-lasting activities as follows: 3-cyclopentyloxy-17α-ethinyl-1, 3,5(10)-estratrien-17-ol (Quinestrol, mean: 9·6 days), 3-cyclopentyloxy-17α-methyl-1,3,5(10)-estratrien-17-ol (ME-CPE: 5·3), and 3-cyclopentyloxy-1,3,5(10)-estratrien-17β-ol (E2-CPE: 3·7), while 3-cyclopentyloxy-1,3,5(10)-estratriene-16α,17β-diol (Quinestradol) showed no prolonged effect. This order of activity is in agreement with the degree of storage of the CPE's in body fat, after a single oral administration.

INTRODUCTION

THE 3-CYCLOPENTYL ethers (CPE's) of estrogenic steroids have been found in mice orally more uterotrophic than parent compounds [1]. One of them, i.e. 3-cyclopentyloxy-17α-ethinyl-1,3,5(10)-estratrien-17-ol (Quinestrol), was demonstrated to be largely stored in body fat of rat [2] and such finding was correlated with both enhancement [3] and prolongation [4, 5] of oral activity.

In a preliminary investigation [6] we have determined that CPE's of other estrogenic steroids, i.e. Estradiol-17β (E2), Estriol (E3), 17α-Methyl-1,3,5(10)-estratriene-3,17β-diol (ME), are stored in body fat at rates not reflecting the enhancement of activity observed in the 3-day Rubin test [1]. The aim of this study was to assess the possible prolongation of the oral activity of these compounds in order to correlate this property with the degree of storage in body fat.

EXPERIMENTAL

(1) *Storage in rat*

This experiment compared the capacity for fat storage following oral administration to rats of Quinestrol, 3-cyclopentyloxy-1,3,5(10)-estratrien-17β-ol (E2-CPE), 3-cyclopentyloxy-1,3,5(10)-estratriene-16α,17β-diol (Quinestradol) and 3-cyclopentyloxy-17α-methyl-1,3,5(10)-estratrien-17-ol (ME-CPE). The rats were Wistar-strain females, 150 g average weight and used in groups of 6–26 depending upon the amount of body fat needed for the test. Each animal received 4 mg of the steroid in 1 ml of sesame oil per os (see Table 1). Thereafter, half of each group was killed at 24 and 72 h. Perirenal fat (approximately 250 mg) was removed, weighed, pooled by time of death and group, and stored at 0°C until extracted.

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Adipose tissue was homogenized in pyrex grinders in 50 ml of 70% ethanol at 0°C. Homogenates were mixed in separatory funnels with equal quantities of methylene chloride, shaken for 3 min. at approximately 60 oscillations per min. Then, phases were allowed to separate, the lower phase was collected and the aqueous phase washed again for 1 min. with methylene chloride. From the assembled organic extracts the solvent was removed in flash-evaporators. Residues were dissolved in sesame oil in concentrations determined by preliminary assays (see Table 1). Extracts were tested in immature albino female mice, 10–12 g, each animals receiving 0·2 ml of the appropriate suspension daily per 3 days after which all were killed and uteri removed and weighed [7].

The four test steroids, dissolved in sesame oil, also were tested at 5 doses levels each on at least 10 mice for comparisons with the uterotrophic effects of the fat extracts. In this comparison 1 μg of steroids corresponded to 100 mg of fat (Fig. 1).

(2) *Oral prolonged vaginotropic activity*

Ovariectomized adult female Sprague–Dawley rats were orally treated, 14 days after surgery, with a single dose (5 μmoles) of E2, E3, ME, 17α-ethinyl-1,

Table 1. Uterotrophic activity in mice of fat obtained from rats treated with four estrogenic cyclopentylethers

Rats (fat donor)			Mice (fat recipient)		
Steroids (single oral dose 4 mg/1 ml sesame oil)	Time from treatment to autopsy (h)	No. of rats	Fat extracts as mg of fresh tissue (daily oral dose 0·2 ml sesame oil)	Uterus weight in mg (mean ± S.E.)	No. of mice
None		7	64	0·4 ± 0·28	8
Quinestrol	24	3	1	27·8 ± 2·4	5
			4	41·7 ± 1·5	5
	72	3	1	29·1 ± 1·6	6
			4	39·3 ± 1·3	6
Quinestradol	24	13	32	6·3 ± 0·6	8
			64	6·1 ± 0·6	8
			128*	8·3 ± 1·17	3
	72	13	32	5·5 ± 0·2	8
			64	5·8 ± 0·3	8
			128*	6·5 ± 0·0	2
E2-CPE	24	11	4	6·0 ± 0·3	6
			16	11·5 ± 1·0	5
			32	37·0 ± 2·0	6
	72	11	4	4·7 ± 0·3	6
			16	6·8 ± 0·2	5
			32	12·0 ± 0·8	6
ME-CPE	24	11	4	8·1 ± 0·9	5
			16	34·7 ± 4·0	6
			32	43·5 ± 2·2	5
	72	11	4	5·4 ± 0·3	6
			16	11·6 ± 1·0	6
			32	13·6 ± 1·3	6

*0·3 ml of vehicle.

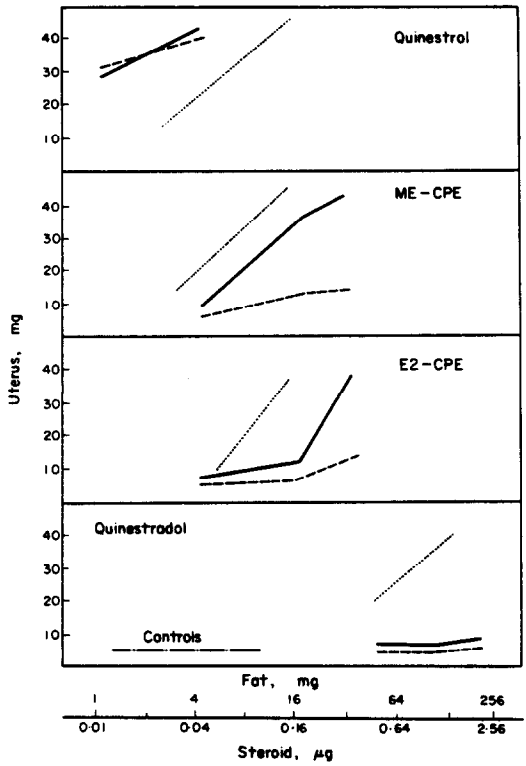

Fig. 1. Uterotrophic activity in the mouse of steroids (· · · · · ·) and of 24-h (●——●) and 72-h (●– – –●) fat sample extracts.

3,5(10)-estratriene-3,17β-diol (EE) and the corresponding 3-cyclopentyl ethers E2-CPE, Quinestradol, ME-CPE, and Quinestrol. After treatment vaginal smears have been daily taken for 14 days. The average duration in days of the vaginal cornification was calculated for each group.

RESULTS AND DISCUSSION

The results of the first phase of this experiment are shown in Table 1, from which it is evident that the equivalent of 1 mg of perirenal fat from rats that received Quinestrol was distinctly more uterotrophic for mice than the equivalent of as much as 128 mg of fat from rats that received Quinestradol. In contrast, extracts of fat from the E2-CPE and ME-CPE groups killed at 24 h caused pronounced uterine hypertrophy when given in amount equivalent to 16 and 32 mg of fat respectively. These effects were reduced by more than half at 72 h. In Fig. 1 the uterotrophic effects of these extracts of fat have been arranged, from bottom to top of the graph, in order of increasing activity. These values are compared with the uterotrophic activity of the stroids. The scale of doses has been arranged for 1 μg of steroids to equal 100 mg of fat. Thus, assuming that the estrogenic material extracted from the fat represent unchanged steroids, these values show that when fat contained more than 1 μg/100 mg the response curve for fat forms to the left of the curve for steroids, e.g. Quinestrol. When, however, fat contained less than 1 μg/100 mg the response curve for fat forms to the right of the standard curve. The concentrations of active steroids in the fat of the rats

Table 2. Concentration of active steroids in perirenal fat (μg/g) 24 and 72 h after oral administration*

Compounds	μg/g	
	24 h	72 h
Quinestrol	35·7	26·6
Quinestradol	0·2	0·2
E2-CPE	4·3	1·8
ME-CPE	5·1	0·1

*Calculated from values shown in Fig. 1.

Table 3. Oral prolonged vaginotropic activity in spayed rats (10 per group)

Treatment	Single dose		Average duration of vaginal cornification in days
	μMoles	mg	
None			0
E2	5	1·36	0·7±0·2
E2-CPE	5	1·70	3·7±0·4
E3	5	1·44	0·8±0·1
Quinestradol	5	1·78	1·3±0·2
EE	5	1·48	1·6±0·3
Quinestrol	5	1·82	9·6±0·6
ME	5	1·43	1·8±0·2
ME-CPE	5	1·77	5·3±0·4

have been calculated from the values shown in Fig. 1, and assembled to form Table 2. These values indicate that 72 h after oral administration only fat from rats that received Quinestrol retained measurable uterotrophic activity.

The data concerning the vaginal cornification is summarized in Table 3. It appears that all 4 tested parent estrogens and Quinestradol lack of prolonged vaginotropic activity while the Quinestrol, ME-CPE and E2-CPE display in decreasing order a prolonged effect.

The results obtained in the two experiments evidentiate a correlation between the storage in body fat and the prolonged oral activity. In both tests the most active compound is Quinestrol, followed by ME-CPE and E2-CPE while Quinestradol is neither stored in body fat, nor endowed with oral prolonged activity. Thus the oral prolonged estrogenic activity of ME-CPE and E2-CPE may be mainly related to their storage in body fat, as already known for Quinestrol.

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