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Effect on reproductive organs of three clomiphene analogues

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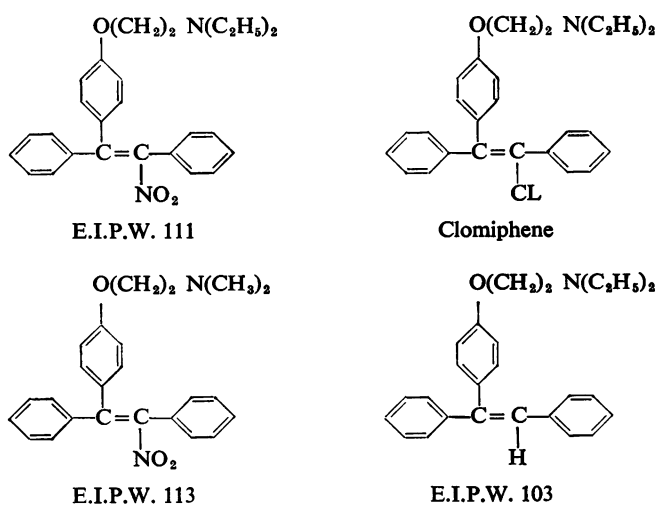
Summary

1. The oestrogenic properties of four triphenylethylene compounds were compared. They can be listed as follows in order of their oestrogenic potency: 1-[*p*-(β-diethylaminoethoxy)-phenyl]-2-nitro-1,2-diphenylethylene (E.I.P.W. 111) > clomiphene > 1-[*p*-(β-dimethylaminoethoxy)-phenyl]-2-nitro-1,2-diphenylethylene (E.I.P.W. 113)>1-[*p*-(β-diethylaminoethoxy)-phenyl]-1,2-diphenylethylene (E.I.P.W. 103). The oestrogenic potency of compound E.I.P.W. 111 was found to be about 1/10th of that of oestradiol.

2. Compound E.I.P.W. 111 is partially antioestrogenic but not progesteronic in nature. It has no androgenic effect.

Introduction

Contradictory observations on the biological properties of several triphenylethylene derivatives have been reported (Robson & Schonberg, 1937; Dodds, Fitzgerald & Lawson, 1937; Grundy, 1957; Holtkamp, Greslin, Root & Lerner, 1960; Harper & Walpole, 1966). Recently, clomiphene, a new triphenylethylene derivative which possesses both oestrogenic and antioestrogenic activities has aroused much interest (Holtkamp *et al.*, 1960; Roy, Mahesh & Greenblatt, 1964). In previous communications the antifertility properties, mode of action and



E.I.P.W. 111 — Clomiphene

E.I.P.W. 113 — E.I.P.W. 103

FIG. 1.

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toxicity of a new clomiphene analogue, 1-[*p*-(β-diethylaminoethoxy)-phenyl]-2-nitro-1,2-diphenylethylene as citrate (E.I.P.W. 111) (Fig. 1) were described (Pakrashi, Basu & Paul, 1969; Basu, 1972). This drug acts as a potent oral contraceptive in mice and was prepared by Dasgupta, Chatterjee & Das (1967). In the present work further biological properties of this compound were investigated and compared with those of clomiphene, 1-[*p*-(β-dimethylaminoethoxy)-phenyl]-2-nitro-1,2-diphenylethylene (E.I.P.W. 113) and 1-[*p*-(β-diethylaminoethoxy)-phenyl]-1,2-diphenylethylene (E.I.P.W. 103) (Fig. 1).

Methods

The oestrogenic and antioestrogenic potencies of the compounds were tested using uterine weights and vaginal smears as described by Burn, Finney & Goodwin (1950). Ten immature Swiss albino mice were used in each experimental group.

The naturally occurring oestrogen, oestradiol (17β-oestradiol benzoate solution, Schering, A.G., Berlin) was used for comparison. The stock solution was diluted

TABLE 1. *Oestrogenic effect of the compounds E.I.P.W.* 111, 113, 103 *and clomiphene in immature female mice*

Treatment	Amount of drug	Mean weight uterus with fluid (mg)
Control	—	9·62±0·606
	μg/mouse:	
Oestradiol	0·25/1 day	11·0 ±0·7 (a)
	0·35/1 day	24·7 ±1·38 (b)
	0·5/1 day	44·5 ±1·69 (b)
	0·05/2 days	59·7 ±1·3 (b)
	mg/kg body weight:	
E.I.P.W. 111	2·5/1 day	20·8 ±0·23 (b)
	3/1 day	22·4 ±0·93 (b)
	5/2 days	23·2 ±0·26 (b)
	7·5/2 days	24·0 ±1·18 (b)
	10/2 days	26·0 ±1·16 (b)
	15/2 days	28·2 ±1·26 (b)
Clomiphene	2·5/1 day	17·77±1·09 (b)
	5/2 days	21·56±0·956 (b)
	7·5/2 days	27·54±0·907 (b)
	10/2 days	39·56±0·969 (b)
	15/2 days	26·34±0·939 (b)
E.I.P.W. 113	2·5/1 day	15·5 ±0·822 (b)
	10/2 days	19·96±0·791 (b)
	15/2 days	20·2 ±0·349 (b)
	2·5/1 day	11·18±1·06 (c)
	10/2 days	12·2 ±0·536 (d)
	15/2 days	13·6 ±0·616 (b)

(a) $P<0·3$, (b) $P<0·001$, (c) $P<0·4$, (d) $P<0·01$. Ten animals in each group.

TABLE 2. *Antioestrogenic effect of compound E.I.P.W.* 111 *in immature female mice*

Group	Amount of oestradiol ((μg/mouse)/day)	Amount of compound E.I.P.W. 111 (mg/kg body weight)	Mean weight uterus with fluid (mg)
Control	0·5	—	38·1 ±1·72
E.I.P.W. 111	0·5	2·5/1 day	31·82±1·79 (a)
		5/2 days	32·56±1·18 (a)
		10/2 days	33·64±1·73 (b)
		20/2 days	34·8 ±1·13 (b)

(a) $P<0·05$, (b) $P<0·2$. Ten animals in each experimental group.

with olive oil and injected intramuscularly into immature mice in doses between 0·25 and 1·0 μg/mouse (see Table 1). The test compounds (E.I.P.W. 111, clomiphene, E.I.P.W. 113 and 103) were given orally in amounts between 2·5 mg/kg and 30 mg/kg. To determine the antioestrogenic potency of compound E.I.P.W. 111 it was fed to four different groups of mice at doses between 2·5 mg/kg and 40 mg/kg (see Table 2). In all experiments 0·5 μg oestradiol/mouse was given either alone (control group) or together with the test compound. All animals were killed 48 h after the last drug administration. The uteri were removed, washed in a 0·9% solution of sodium chloride and weighed on a semi-micro balance. The uteri of the mice receiving oestradiol alone (0·35 and 0·5 μg/day) or 2·5 and 3 mg/kg of the test compounds alone were fixed in Bouin's solution for histological studies.

The progesteronic effect of E.I.P.W. 111 was tested on colony-bred female, immature rabbits weighing about 900 g by the technique of McPhail (1934). Olive oil was used as the vehicle for progesterone. At least five animals were used in each group. The intramuscular injections were made alternately into the left or right hind leg. The animals were oestrogen-primed by the injection of 5 μg oestradiol/rabbit for 3 consecutive days. Two control groups of oestrogen-primed rabbits were used. One group was injected with 0·2 ml of olive oil only for 3 days. Each rabbit of the second group received 0·25 mg progesterone/kg (progesterone, Schering, A.G., Berlin) for 3 days. To detect a positive progesteronic effect of the test compound 4 (mg/kg)/day was given orally for 3 days to oestrogen-primed rabbits.

To study a possible androgenic effect of compound E.I.P.W. 111, a single dose of 3 mg/kg was given for 3 days to ten immature male mice, weighing 7–9 g. Ten untreated animals were used as controls. All mice were killed 48 h after the last dose was given to the experimental animals. The testes, seminal vesicles and ventral prostates were removed, weighed and fixed in Bouin's solution for histological examinations.

Results

The effects of oestradiol and the triphenylethylene compounds on uterine weight are listed in Table 1. The smallest dose of oestradiol to cause a slightly pinkish appearance of the vagina, to open it and to produce a few cornified cells in the smear even after 24 h was 0·35 (μg/animal)/day.

Although treatment with a single dose of 0·25 μg of oestradiol/mouse caused only a slight increase in uterine weight, a significant increase occurred after 0·35 μg/mouse (Table 1). These doses also caused an increase in uterine diameter, thickened the luminal and glandular epithelia, and caused the appearance of a large number of glands.

Compounds E.I.P.W. 113 and 103 did not induce any significant changes in the vagina with a single dose of 2·5 mg/kg; 10 and 15 mg/kg given on 2 days caused opening of the vagina and the occasional presence of cornified cells in the smear within 48 h after the last dose given. On the other hand, opening of the vagina and production of some cornified cells were effected even with a single dose of 2·5 mg/kg E.I.P.W. 111 and of clomiphene citrate usually 48 h after the drug had been fed to the mice.

The effects of the test compounds on the uterine weights are listed in Table 1. Significant changes were produced with a single dose of 2·5 mg E.I.P.W. 113 and E.I.P.W. 111, the response with E.I.P.W. 111 being greater. The uterine weight response increased with increasing doses of E.I.P.W. 111. In contrast, E.I.P.W. 103 was only effective at doses from 10 mg/kg for 2 days onwards. Clomiphene citrate in doses from 2·5 mg/kg for 1 day to 10 mg/kg for 2 days induced a sharp increase in uterine weight. However, the response to 15 mg/kg for 2 days was similar to that of 7·5 mg/kg for 2 days. At a single dose of 2·5 mg/kg compound E.I.P.W. 113 caused proliferation of the luminal and glandular epithelia in the endometrium, whereas E.I.P.W. 103 caused only an increase in the diameter of the lumen. High proliferation of glandular and luminal epithelia was also observed in the uteri of animals treated with E.I.P.W. 111 and clomiphene at the same doses, the changes being less in clomiphene-treated than in the E.I.P.W. 111-treated mice. Thus, based on vaginal cytology, changes of uterine weight and of uterine histology, the order of oestrogenic potency of the compounds tested was: E.I.P.W. 111>clomiphene>E.I.P.W. 113>E.I.P.W. 103. E.I.P.W. 111 possesses about 1/10th of the oestrogenic potency of oestradiol as similar effects were obtained in the mouse with a dose of 3 mg/kg of E.I.P.W. 111 and 0·35 μg/mouse (or approximately 300 μg/kg/mouse of oestradiol).

E.I.P.W. 111 was also tested for possible antioestrogenic activity in mice treated with 0·5 μg of oestradiol. As in the case of the control group treated with oestradiol alone, the vagina of the mice which also received E.I.P.W. 111 became pinkish, opened slightly, and a number of cornified cells appeared in the vaginal smear, the changes being more prominent after 48 h of drug treatment. Table 2 shows that the uterine weight of all groups of mice which received E.I.P.W. 111 in addition to oestradiol were lower than those receiving oestradiol alone. However, this difference was statistically significant ($P<0·05$) only in mice receiving E.I.P.W. 111 2·5 mg/kg for one day and 5 mg/kg for two days.

No progesteronic effect, such as enlarged endometrium with villi, was observed in the endometrium of oestrogen-primed rabbits treated with E.I.P.W. 111. The changes in uterine endometrium were similar to those seen in rabbits treated with oestradiol only.

No significant changes were observed in the weights of seminal vesicles, prostates and testes of mice treated with E.I.P.W. 111. Histological examination of these tissues did not reveal any changes which would point towards an androgenic effect of the compound.

Discussion

Compound E.I.P.W. 111, like clomiphene, exerted uterotrophic effects in immature female mice at doses between 2·5 and 15 mg/kg. Even at the dose of 2·5 mg/kg, cornified cells were present in the vaginal smear and the endometrial growth confirmed the oestrogenic properties of these compounds (Hisaw, Meyer & Fevold, 1930; Allen, Smith & Gardner, 1937, 1939; Astwood, 1938; Burrows, 1949; Hisaw, 1950). The same doses of E.I.P.W. 113 produced a smaller oestrogenic response while compound E.I.P.W. 103 was almost devoid of an oestrogenic effect. In order of oestrogenic potency the compounds can be listed as follows:

E.I.P.W. 111>clomiphene>E.I.P.W. 113>E.I.P.W. 103. The oestrogenic potency of 3·0 mg/kg of compound E.I.P.W. 111 was about equal to that of 0·35

μg of oestradiol/10 g mouse. However, when compound E.I.P.W. 111 (2·5–20 mg) was administered to mice simultaneously with oestradiol (0·5 μg/mouse) for a day, it was found to partially antagonize the uterotrophic response to oestradiol (Table 2). However, the antioestrogenic action of compound E.I.P.W. 111, as measured by the vaginal cornification and uterotrophic effect, was much weaker than its oestrogenic one. Clomiphene, a compound of similar structure, also possesses oestrogenic as well as antioestrogenic activity at the same dose levels (Roy *et al.*, 1964). The antioestrogenic activity of clomiphene was also reported by Holtkamp *et al.* (1960). Ethamoxytriphetol (MER-25), another compound of this series, also antagonizes the effects of oestrogens on the uterus, vagina and pituitary glands in mammals and those on the oviduct and plasma phosphorus in chicks (Lerner, Holthaus & Thomson, 1958). ICI 47, 699, the *cis*-isomer of 1-[*p*-(β-dimethylaminoethoxy)-phenyl]-1,2-diphenyl-2-ethylethylene was found to behave in all respects as a conventional 'oestrogen' inducing uterine growth in immature rats and vaginal cornification in spayed rats and mice (Harper & Walpole, 1966). The compound E.I.P.W. 111 has no apparent androgenic or progesteronic effects. It resembles clomiphene in these respects (Holtkamp *et al.*, 1960).

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