Mechanisms of action of the antifertility agents U11,100A and U11,555A in the rat

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Summary. Mature female rats were given U11,555A and U11,100A orally on Day 2 of pregnancy and the number of implantations counted on Day 9. Using doses which reduced the implantation number to approximately 40% that of the controls, it was shown that the reduced number was not due to an effect on egg transport or to a delay of implantation.

Introduction

The chemically similar non-steroidal drugs, U11,555A (2[p-(6-methoxy-2-phenylinden-3-yl)-phenoxy]-triethylamine hydrochloride: Upjohn, Michigan) and U11,100A (1[2-(p-[3:4 dihydro-6-methoxy-2-phenyl-1-naphthyl]-phenoxy)-ethyl]pyrrolidine hydrochloride: Upjohn), have been shown to inhibit implantation when administered after coitus (Duncan, Stucki, Lyster & Lednicer, 1962; Duncan, Lyster, Clark & Lednicer, 1963; Nelson, Davidson & Wada, 1963; Greenwald, 1965; Morris, van Wagenen, McCaan & Jacob, 1967; Emmens, Miller & Owen, 1968). The mechanism of action of these compounds in the rat has been extensively investigated and primary effects involving zygotoxicity (Nelson *et al.*, 1963), accelerated tubal transport (Yoshida & Craig, 1968; Greenwald, 1965), and interference with decidualization (Coppola & Perrine, 1965; Greenwald, 1965) have been proposed. The compounds, a diphenylidene (U11,555A) and a diphenyl dihydronaphthalene (U11,100A), are antioestrogenic (Duncan *et al.*, 1962, 1963). Because the antioestrogen Tamoxifen (ICI 46,474) has been shown to delay implantation in the rat (Major & Heald, 1974) by altering the timing of post-coital oestradiol synthesis and release from the ovary (Watson & Alam, 1975; Watson, Anderson, Alam, O'Grady & Heald, 1975), the present experiments were undertaken to examine whether implantation was similarly delayed by U11,100A and U11,555A.

Materials and Methods

The rats were virgin females of a Sprague–Dawley strain (200–250 g) with a 5-day oestrous cycle. They were housed in windowless rooms at 22°C and with lights on from 06.00 to 20.00 hours. Animals were caged with proven males and the morning that spermatozoa were found in the vaginal smear was designated Day 1 of pregnancy.

In Exp. 1 the animals were divided into 3 groups of 6 and on Day 2 were given an oral dose of the vehicle or of the hydrochloride salt of U11,555A or U11,100A made up in 0.5% (w/v) aqueous Tween 80. Various doses of the drugs were given and the animals were killed on Day 9 to determine the number and weight of the implantation sites (Table 1). In Exp. 2, rats were treated with a single dose of the vehicle, 60 µg U11,100A/kg or 1.0 mg U11,555A/kg on the morning of Day 2 and killed at 10.00 hours on Days 3, 4, 5, 6 or 7 to examine the effect on egg transport. Eggs and blastocysts were recovered by flushing the oviduct and uterus with saline. Each ovary was weighed and the CL were counted. The uterus was weighed wet and its dry matter content was determined after heating for 24 hr at 100°C. To visualize implantation sites (on Day 6 of pregnancy) animals were injected i.v. with 1.0 ml 1% (w/v) diamine blue in 0.9% (w/v) NaCl, 10 min before killing.

Results

As the drug doses increased, the number and weight of implantation sites decreased similarly (Table 1) as reported for Tamoxifen (O'Grady, Heald & O'Hare, 1970).

Table 1. Number and wet weight of implantation sites (means $\pm$ S.E.M.) in rats (no. in parentheses) receiving different oral doses of U11,100A and U11,555A on Day 2 and killed on Day 9

Treatment and dose	No. of implantations	Wet wt of implantation sites (mg)
None (controls)	10.9 $\pm$ 0.42 (10)	70.4 $\pm$ 5.77
U11,555A (mg/kg)		
0.25	10.2 $\pm$ 0.7 (5)	47.1 $\pm$ 1.72
0.50	10.8 $\pm$ 0.8 (6)	48.9 $\pm$ 0.86
1.0	6.1 $\pm$ 1.94 (7)	27.6 $\pm$ 8.7
2.0	1.8 $\pm$ 0.21 (8)	10.8 $\pm$ 4.9
2.5	0	0
U11,100A (μ g/kg)		
15.62	9.28 $\pm$ 0.65 (7)	52.18 $\pm$ 3.12
31.25	9.0 $\pm$ 1.86 (6)	43.4 $\pm$ 9.01
62.5	5.8 $\pm$ 1.78 (6)	29.7 $\pm$ 8.57
105.0	0	0

The dose of Tamoxifen which produced a 50–60% fall in the weight and number of implantation sites also delayed implantation (Major & Heald, 1974), and therefore doses of 60 μ g U11,100A/kg and 1.0 mg U11,555A/kg were used for Exp. 2.

The results of Exp. 2 are shown in Table 2. The effect of the drugs on the dry weight of the uterus was variable and did not follow the changes observed in the control animals. No acceleration of egg transport was detected on Day 3 but by Day 4 the number of blastocysts in the oviduct of the treated groups were lower than in the control group and the number of blastocysts in the uterus was higher. By Day 5 all the blastocysts were in the uterus and there was no detectable difference between the treated and control groups. On Day 6 all the blastocysts shed had implanted in the control animals, but less than 50% of those in the treated animals had done so. The blastocysts recovered from the uterus appeared microscopically normal and the zona pellucida had been shed.

There was no further increase in the number of blastocysts implanted by Day 7; the number of unimplanted blastocysts in rats receiving U11,555A was reduced almost to zero, but blastocysts were still present in the uterus of the animals receiving U11,100A, suggesting an absence of uterine contractility at this time. The survival to term of the blastocysts of the treated animals was checked in two groups of 5 animals dosed on Day 2 of pregnancy; those receiving 60 μ g U11,100A/kg did not produce any litters and only 2 of those receiving 1.0 mg U11,555A/kg gave birth, producing 3 and 4 young.

Discussion

Previous examinations of the mechanisms of action of U11,100A and U11,555A have been generally conducted with doses sufficient to inhibit implantation in the rat when given on any one of Days 1–5 of pregnancy and have been similar to the maximal doses examined in the present study (see Duncan *et al.*, 1962; Greenwald, 1965). At these levels both compounds accelerate tubal transport of eggs (Yoshida & Craig, 1968, 1969; Greenwald, 1965) but are not zygotoxic (Duncan & Lyster, 1963; Duncan & Forbes, 1965). Tubal transport was not affected by the doses used in the present study but a majority of the blastocysts present in the uterus on Days 5, 6 and 7 did not implant, and survival of the embryos that did implant was poor. These effects differ from those of Tamoxifen.

Table 2. Effect of 1.0 mg U11,555A/kg and 60 µg U11,100A/kg given orally on Day 2 of pregnancy on implantation in rats

Treatment	Day of autopsy	No. of rats	No. of eggs recovered		No. of eggs		Implantations (% of no. of eggs recovered)	Mean (±S.E.M.) ovarian wet wt (mg)	Dry wt of uterus as % of wet wt (mean ± S.E.M.)
			(% of no. of CL)		Oviduct	Uterus			
Control	3	6	67 (100%)		67	0	—	60.6 ± 3.53	20.0 ± 0.13
	4	6	60 (81%)		38	22	—	54.0 ± 2.52	18.7 ± 0.34
	5	6	57 (73%)		0	57	—	61.5 ± 3.61	17.8 ± 0.23
	6	6	0		0	0	67 (100%)	50.5 ± 1.70	16.8 ± 0.41
	7	3	0		0	0	30 (100%)	48.3 ± 5.05	18.1 ± 0.26
U11,555A	3	6	61 (90%)		61	0	—	50.1 ± 2.59	19.7 ± 0.11
	4	6	41 (59%)		9	32	—	53.1 ± 2.20	20.5 ± 0.34
	5	6	57 (86%)		0	57	—	46.3 ± 2.70	18.9 ± 0.14
	6	6	29 (41%)		0	29	32 (46%)	47.8 ± 3.63	18.57 ± 0.49
	7	6	3 (4%)		0	3	23 (34%)	45.3 ± 3.59	19.23 ± 0.34
U11,100A	3	6	61 (90%)		61	0	—	47.7 ± 2.32	19.3 ± 0.15
	4	6	51 (74%)		21	30	—	46.1 ± 2.59	19.5 ± 0.35
	5	6	60 (90%)		0	60	—	48.8 ± 4.66	18.9 ± 0.36
	6	8	36 (41%)		0	36	29 (33%)	49.7 ± 3.09	19.9 ± 0.81
	7	10	55 (52%)		0	55	25 (23%)	49.1 ± 2.76	19.6 ± 0.31

This drug causes a delay of implantation of about 20–24 hr (Major & Heald, 1974), paralleled by a similar delay in the release of nidatory oestradiol (Watson *et al.*, 1975) and in the burst of stromal mitosis which precedes the decidual response (O'Grady, Armstrong, Moore & Vass, 1974), but does not affect survival of the embryos to term. It is possible that U11,555A and U11,100A affect the uterus directly, perhaps by modification of the endometrial cell cycles.

However, although experiments have shown that U11,555A is without effect upon deciduoma formation in traumatized uteri and that U11,100A reduces deciduoma formation (Greenwald, 1965), the formation and maintenance of the deciduoma are related to circulating hormone concentrations. The finding that U11,100A antagonizes oestrogen-induced uterine growth in the immature rat, possibly because of failure to stimulate cytoplasmic oestrogen receptors (Clark, Peck & Anderson, 1974), may not apply to the adult animal.

Our results suggest, but do not prove, that U11,100A and U11,555A interfere with the synthesis of progesterone and oestrogen by the ovary.

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