2-Fluoro-oestradiol does not cause uterine refractoriness but inhibits oestradiol-induced implantation in rats*

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Summary. An intravenous injection of 2-fluoro-oestradiol simultaneously with an implantation-inducing dose of oestradiol reduced the number of implantation sites in delayed implanting hypophysectomized rats maintained with progesterone. Administration of 2-fluoro-oestradiol 1 h before or after oestradiol had no effect. Furthermore, injection of as much as 500 ng 2-fluoro-oestradiol 48 h before administration of oestradiol failed to have any effect upon implantation, i.e. failure to block implantation was correlated with failure to induce the uterine refractory state. These results suggest that conversion of primary oestrogens to catechol oestrogens could be important for implantation as well as for the induction of the oestrogen refractory state in the uterus.

Introduction

In the rat implantation of the blastocyst into a progesterone-primed uterus requires the action of oestrogen (Psychoyos, 1973, 1974). The nature and mechanism of this oestrogenic action are undefined, but the oestrogenic action in implantation could be mediated via catechol oestrogens (Dey & Johnson, 1986; Dey *et al.*, 1986), which are a major group of active metabolites formed by aromatic hydroxylation of phenolic oestrogens at the carbon 2 or 4 position. 2-Fluoro-oestradiol, which is a potent oestrogen in terms of many of the receptor-mediated actions of oestrogen, is a poor substrate for catechol oestrogen formation (Krey *et al.*, 1980) and does not induce implantation in the delayed implanting rat even when used in high dosage (Dey *et al.*, 1986). Furthermore, 2-fluoro-oestradiol acts as an inhibitor of oestrogen-2-hydroxylase (50% inhibitory concentration = 10 μ M) in pig blastocysts when tested *in vitro* (unpublished data). In the present study the effect of 2-fluoro-oestradiol on implantation and the uterine refractory state that are usually induced by oestradiol was determined in delayed implanting rats.

Materials and Methods

Adult (250–275 g) rats (Holtzman Company, Madison, WI) were hypophysectomized, under ether anaesthesia, on Day 3 of pregnancy (morning of the day of finding vaginal spermatozoa is Day 1), using the parapharyngeal approach. A 5% solution of glucose was provided for drinking water after this operation. At the time of the operation and daily thereafter the animals received (subcutaneously) 2 mg progesterone (Sigma Chemical Co., St Louis, MO) dissolved in 0.1 ml sesame seed oil. On the 5th day of treatment (Day 8 of pregnancy) the animals were lightly anaesthetized with ether before being injected (intravenously; i.v.) with oestradiol (Sigma) and/or 2-fluoro-oestradiol. 2-Fluoro-oestradiol was prepared and generously provided by Dr J. G. Liehr (University of Texas Health Science Center, Galveston, TX). Analyses by direct inlet mass spectrometry and gas chromatography-mass spectrometry indicated that the compound was free of oestradiol but it contained about 2% of another fluorinated oestradiol, possibly 4-fluoro-oestradiol. The steroids were dissolved in ethanol and diluted with saline just before use. One group of 6 animals received oestradiol 1 h before and another group of 5 rats received the oestradiol 1 h after a dose of 300 ng 2-fluoro-oestradiol.

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In a separate experiment, animals received (i.v.) various doses of 2-fluoro-oestradiol 2 days before being injected (i.v.) with oestradiol. At 24 h after the administration of oestradiol all animals were injected (i.v.) with 0.5 ml of a 1% solution of Chicago Blue B (Sigma) in 0.15 M-NaCl 15 min before being killed by an overdose of ether. The implantation sites were delineated by the extravasation of the blue dye secondary to increased capillary permeability (Psychoyos, 1973). If no implantation sites were found the uterus was flushed with saline to recover the blastocysts. Animals without implantation sites or blastocysts were excluded from the study, as were those with incomplete hypophysectomy: the latter failed to show delay of implantation because of endogenous oestrogen.

Results

Extensive experience with the hypophysectomy model of delayed implantation has demonstrated that 20–30 ng oestradiol consistently induce a full complement of implantation sites in all animals (Dey *et al.*, 1986). In the present study, 15 females treated with 30 ng oestradiol each had about 11 implantation sites (Table 1). When 300 ng 2-fluoro-oestradiol were administered just before the dose of 30 ng oestradiol, none of the 7 females exhibited implantation sites but they did have a normal number of blastocysts in their uteri. Increasing the dose of oestradiol to 40 ng while keeping the dose of 2-fluoro-oestradiol at 300 ng resulted in implantation in 33% of the rats. The percentage dropped to 14% when the dose of 2-fluoro-oestradiol was raised to 500 ng and given just before 40 ng oestradiol. When the 300 ng 2-fluoro-oestradiol was given 1 h before or after the oestradiol, all animals had sites.

In the second experiment, all animals were given 50 ng oestradiol 2 days after the administration of 2-fluoro-oestradiol (Table 1). Doses of the latter compound as high as 500 ng had no effect upon the implantation-inducing action of oestradiol.

Table 1. The effect of 2-fluoro-oestradiol (2-FI-E₂) on implantation-induction activity of oestradiol in hypophysectomized delayed-implanting rats

Treatment	No. of animals	No. with implantation sites	No. of implantation sites*	No. of blastocysts†
<i>Exp. 1: interference with oestradiol-induced implantation by 2-fluoro-oestradiol</i>				
30 ng oestradiol	15	15	10.9 ± 0.8	—
30 ng oestradiol plus 300 ng 2-FI-E ₂ ‡	7	0	—	9.3 ± 0.8
40 ng oestradiol plus 300 ng 2-FI-E ₂ ‡	6	2	11.4	5.0 ± 1.7
40 ng oestradiol 1 h after 300 ng 2-FI-E ₂	5	5	8.4 ± 1.7	—
40 ng oestradiol 1 h before 300 ng 2-FI-E ₂	6	6	10.0 ± 1.1	—
40 ng oestradiol plus 500 ng 2-FI-E ₂ ‡	7	1	7	7.6 ± 0.9
<i>Exp. 2: effect of 2-fluoro-oestradiol on oestrogen-induced uterine receptivity</i>				
50 ng oestradiol 48 h after 300 ng 2-FI-E ₂	10	9	10.8 ± 1.1	10
50 ng oestradiol 48 h after 400 ng 2-FI-E ₂	8	7	11.0 ± 0.8	5
50 ng oestradiol 48 h after 500 ng 2-FI-E ₂	5	5	10.6 ± 0.7	—

*Includes only animals with sites.

†Includes only animals without sites.

‡Compounds administered essentially simultaneously.

Discussion

Substitution of fluorine on carbon 2 of the oestradiol molecule produces a compound that can mimic oestradiol in many actions except in the initiation of implantation. We previously postulated (Dey & Johnson, 1986) that conversion of the oestrogen to a catechol was necessary for induction of implantation, but 2-fluoro-oestradiol is a poor substrate for oestrogen-2/4-hydroxylase and it is doubtful that significant amounts of 2-hydroxyoestradiol can be produced (Krey *et al.*, 1980). The question is whether 2-fluoro-oestradiol interferes with aromatic hydroxylation of oestradiol. Our observations on pig blastocysts and those of others upon rat liver indicate that 2-halo-oestrogens are potent inhibitors of oestrogen-2-hydroxylase (Brueggemier & Kimball, 1983). These observations are consistent with our present finding that the implantation-inducing effect of oestradiol was compromised by the simultaneous presence of 2-fluoro-oestradiol. However, interference of oestradiol action by 2-fluoro-oestradiol in implantation is short lived, because treatment with the latter compound 1 h before or after oestradiol was completely ineffective in altering implantation. There is no doubt that 2-fluoro-oestradiol binds to the cytosolic-nuclear receptor and exhibits many receptor-mediated oestrogenic actions (Krey *et al.*, 1980; Katzenellenbogen *et al.*, 1980; Liehr *et al.*, 1986). Because this compound fails to induce implantation (Dey *et al.*, 1986) it is suggested that oestrogen interaction with the classical receptor is not the sole mediator of oestrogen action for implantation.

Not only does 2-fluoro-oestradiol not induce implantation, it does not induce the uterine refractory state. However, it is not surprising that an oestrogen that cannot induce uterine receptivity also fails to induce the post-receptive refractoriness. Uterine receptivity for blastocyst implantation is time- and hormone-dependent (Psychoyos, 1974; Yoshinaga, 1980), i.e. uterine receptivity occurs only for a limited period in a progesterone-primed uterus. After oestrogen injection the progesterone-primed uterus becomes refractory to a second injection of oestrogen after a period of 24 h. This induction of refractoriness by oestrogens also does not appear to involve the classical cytosolic-nuclear oestrogen receptor because uterine receptivity to implantation as well as induction of the refractory state are not inhibited by actinomycin D; i.e. transcription does not appear to be required (Finn & Martin, 1974; Leroy *et al.*, 1976). We do not know whether conversion to a catechol oestrogen is necessary for an oestrogen to induce the refractory state. The present study clearly shows that an oestrogen that is converted to 2-hydroxyoestradiol to a very limited extent does not induce the refractory state when administered 2 days before an implantation-inducing dose of oestradiol; the latter was given at a dose twice that necessary to induce implantation. At present our information regarding induction of the refractory state is too limited to make any prediction about the mechanism of action of oestrogens or catechol oestrogens in this important physiological function.

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