Ornithine decarboxylase inhibition and the uterotrophic response to oestrogen in rats

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Summary. Pretreatment of mature ovariectomized rats with DL-5 α -difluoromethylornithine (DFMO) inhibits the 6-h uterine blood flow response to oestradiol and also depresses the stimulatory effect of oestradiol on wet and dry uterine weight. Results from this study indicate that polyamine synthesis is implicated in the uterine response to oestrogen.

Introduction

Ornithine decarboxylase (EC 4.1.1.17) catalyses the first, and possibly rate-limiting, reaction in the biosynthesis of putrescine and the polyamines spermidine and spermine. Interest in the polyamines has grown because of increasing recognition of their essential roles in cellular growth, multiplication and differentiation (Tabor & Tabor, 1976; Fozard *et al.*, 1980a). Direct proof of the functional role of ornithine decarboxylase has been difficult to obtain, mainly because of the lack of tools with which to manipulate selectively the polyamine biosynthetic pathway. However, a potent and selective inhibitor of ornithine decarboxylase that acts through the mechanism of substrate-induced irreversible inhibition has been developed. This compound DL- α -difluoromethylornithine (DFMO) has been shown to be an effective and selective inhibitor of ornithine decarboxylase *in vivo* (Prakash, Schecter, Grove & Koch-Weser, 1978; Danzin, Jung, Grove & Bey, 1979; Manni & Wright, 1983).

As oestrogen selectively stimulates new RNA synthesis and thereafter increases protein synthesis, a useful system for studying polyamine synthesis and accumulation is the oestrogen-stimulated uterus of the ovariectomized rat (Russell & Taylor, 1971).

In the present study DFMO was used to inhibit ornithine decarboxylase and so investigate the possible role of the polyamines in the uterotrophic response to oestrogen.

Materials and Methods

Mature virgin female rats of a CD-derived Sprague–Dawley random-bred strain from the Animal House, University of Bradford, were used throughout and were housed in light (07:00–19:00 h) and temperature (18°C) controlled rooms. Food and water were available *ad libitum*. All the animals used in this study were bilaterally ovariectomized at least 14 days before further experimentation and were randomly assigned to groups.

Measurement of blood flow. The microsphere technique (previously described by Phaily & Senior, 1978) was used to determine blood flow in the rat anaesthetized with sodium pentobarbitone (60 mg kg⁻¹). The microspheres used were 15 μ m in diameter (NEN-Trac, New England Nuclear, Boston, MA, U.S.A.) uniformly labelled with ⁴⁶Sc and suspended in 10% (w/v) dextran containing 0.01% (w/v) Tween 80. Uterine blood flows were calculated using wet weights of tissues. When dry weights are quoted the tissues were dried until their weights remained constant.

Drug administration. Oestradiol (oestra-1,3,5(10)-triene-3,17 β -diol) (BDH, Poole, U.K.) was dissolved in a 10% (v/v) solution of propylene glycol and was administered intravenously (i.v.) at a dose level of 0.5 $\mu\text{g kg}^{-1}$ into the tail vein of the conscious rat. DFMO was dissolved in 0.9% (w/v) sodium chloride solution and given by the intraperitoneal (i.p.) route, at a dose level of 200 mg kg^{-1} . The solvents used did not have any effect on uterine blood flow or weight and these results have been grouped as control values.

Statistical analysis. The results (expressed as mean $\pm$ s.e.m.) were compared using Student's *t* test (two tailed) Snedecor & Cochran, 1979).

Results

From the results of preliminary studies, involving doses of DFMO ranging from 25 to 400 mg kg^{-1} , it was decided to use a dose of 200 mg kg^{-1} for further work. Fozard, Prakash & Grove (1980b) have shown that this dosage is sufficient to suppress ornithine decarboxylase activity. None of the doses of DFMO used had any effect on organ blood flow or the appearance of the animals in general.

Effect of oestradiol and DFMO on uterine blood flow

Uterine blood flow increased rapidly after the injection of oestradiol. The marked elevation seen 3 h after oestradiol treatment was beginning to decline by 6 h after oestradiol injection (Table 1).

Table 1. The effect of 0.5 μg oestradiol kg^{-1} and 200 mg DFMO kg^{-1} used separately and together on uterine blood flow and weight in rats

Treatment	No. of rats	Body wt (g)	Cardiac output (ml min ⁻¹)	Uterine blood flow		% Cardiac output to uterus	Uterine weight (mg)		Water content of uterus (mg)
				ml min ⁻¹	ml min ⁻¹ 100 g ⁻¹		Wet	Dry	
(a) None (control)	9	304	93	0.065	66	0.067	107	23	85
		± 7	± 6	± 0.01	± 12		± 8	± 3	
DFMO	6	323	118	0.089	78	0.076	123	25	99
		± 6	± 10	± 0.01	± 14		± 15	± 3	
Oestradiol	8	321	104	0.94	735	0.96	128	35	93
		± 7	± 11	$\pm 0.10^{**}$	$\pm 66^{**}$		± 7	$\pm 2^{*}$	
DFMO + oestradiol	7	311	108	0.96	910	0.89	107	23	84
		± 9	± 3	$\pm 0.11^{**\ddagger}$	$\pm 103^{**\ddagger}$		$\pm 55^{\dagger}$	$\pm 1^{\ddagger}$	
(b) None (control)	9	304	93	0.065	66	0.067	107	23	85
		± 7	± 6	± 0.01	± 12		± 8	± 3	
DFMO	6	315	104	0.080	87	0.080	97	20	76
		± 4	± 4	± 0.03	± 28		± 7	± 2	
Oestradiol	8	306	90	0.56	394	0.63	144	27	117
		± 5	± 5	$\pm 0.07^{**}$	$\pm 46^{**}$		$\pm 8^{*}$	± 1	
DFMO + oestradiol	6	328	115	0.25	233	0.24	114	20	94
		± 8	± 15	$\pm 0.04^{*\P}$	$\pm 40^{\dagger*}$		$\pm 9^{\dagger}$	$\pm 2^{\P}$	

* Values are mean $\pm$ s.e.m.

$\dagger$ DFMO was given 1 h before a single injection of oestradiol. Measurements were made (a) 3 h and (b) 6 h after oestradiol injection.

$\ddagger$ Values are significantly different from those of control group: $^{*}P < 0.05$; $^{**}P < 0.001$.

$\P$ Values are significantly different from those of oestradiol alone group: $^{\dagger}P < 0.05$; $^{\ddagger}P < 0.001$; $^{\P}P < 0.005$.

DFMO alone had no significant effect on blood flow, but when given in conjunction with oestradiol a reduction in uterine blood flow was seen 6 h after oestradiol administration (Table 1). None of the treatments had a significant effect on the blood flow through the other organs investigated (kidney, stomach, duodenum, adrenal and spleen) and cardiac output was also unaffected by the treatments. In the non-oestrogen-stimulated uterus the number of microspheres in the organ was inadequate for accurate blood flow determination but in these animals there was a balance between right and left kidney flow and also right and left adrenal flow, indicating that the distribution of microspheres is uniform. If the number of microspheres injected is significantly higher than 100 000 haemodynamic effects may occur in the rat (Tsuchiya, Walsh & Frohlich, 1977).

Effect of oestradiol and DFMO on uterine weight and water content

When given alone, DFMO had no effect on uterine weights. After oestradiol injection uterine wet weight was increased, and this increase was significant 6 h after injection. The increase in uterine water content was reflected in the wet weight values. The initial increase in uterine dry weight had declined 6 h after oestradiol injection.

DFMO pretreatment suppressed the increase in wet and dry weight in response to oestradiol both at 3 and 6 h after the oestrogen treatment. Uterine water content was correspondingly decreased.

Discussion

The early stimulation of uterine blood flow by oestradiol was unaffected by pretreatment with DFMO, but blood flow values 6 h after oestradiol administration were below the control value. Oestradiol is known to cause a sustained elevation of uterine blood flow (up to 72 h after a single i.v. injection $0.5 \mu\text{g kg}^{-1}$; K. Marshall & J. Senior, unpublished observation) and the suppression of this increase in blood flow by DFMO pretreatment, with the subsequent inhibition of polyamine synthesis, may indicate that this response to oestradiol is partly dependent on protein synthesis. The protein synthesis inhibitor, cycloheximide (used at a dose which should not affect prostaglandin synthesis; Castracane & Jordan, 1976), has also been shown to inhibit the early hyperaemic response to oestradiol (Majid, 1981).

It is possible that oestradiol exerts its action on ornithine decarboxylase via the products of the cyclo-oxygenase and/or lipoxygenase pathways. Wing & Rillema (1983) suggest that prostaglandins may modulate the action of prolactin on ornithine decarboxylase activity via a cAMP-dependent mechanism. Nakadate, Yamamoto, Ishii & Kato (1982) have published data which suggest that lipoxygenase products are also involved in induction of ornithine decarboxylase. Inhibition of prostaglandin synthesis also reduces the hyperaemic response to oestradiol by up to 50% (Phaily & Senior, 1978).

The DFMO suppression of true uterine growth, indicated by dry weight in this study, is more marked 3 h after oestradiol administration. At this time, ornithine decarboxylase activity is increasing steeply and reaches a peak about 4 h after injection (Bulger & Kupfer, 1976; Levy, Mester & Baulieu, 1981; Lavia, Stohs & Lemon, 1983; Webster, Zaloudek & Inman, 1984). In fact, the increase in dry weight usually seen 3 h after oestradiol administration has been abolished (dry weight remaining at control levels). However, 6 h after oestrogen injection, dry weight remains depressed. These results appear to be in contradiction to those obtained by Rorke & Katzenellenbogen (1984) who found that although DFMO did inhibit ornithine decarboxylase activity and the subsequent increase in spermidine concentration, uterine weight was unaffected by DFMO pretreatment, but these authors did not measure uterine dry weight and the rats used were sexually immature. Levels of ornithine decarboxylase do differ; there is evidence that activity of the enzyme declines with age (Das & Kanungo, 1982), activity being greatest during periods of rapid growth or regeneration.

Moreover, the results presented here refer to an acute oestradiol effect. Alternatively, these differences could indicate that enzyme inhibition may have switched the emphasis from the pathway of polyamine biosynthesis catalysed by ornithine decarboxylase to an alternate route of polyamine regulation. Slotkin *et al.* (1982) also found differences between the responses of mature and immature animals to DFMO and have postulated that pathway divergences may occur in different tissues at different developmental stages. These authors also found that the concentration of spermine, unlike putresine and spermidine, was refractive to acute change.

Pretreatment of rats with DFMO also decreased uterine water content in response to oestradiol, thus indicating that the decrease in wet weight could be attributed to a reduced water intake. This may be suggestive of a connection between protein synthesis and uterine wet weight. If such a link does exist, then uterine oedema may also be mediated by the receptor-genome interaction. Indeed, the induction of ornithine decarboxylase by oestradiol can be blocked by protein synthesis inhibitors, such as actinomycin D (Russell & Taylor, 1971), which have been shown to suppress selectively the genomic response to oestrogen (Tchernitchin & Galand, 1982). There are also results which suggest that ornithine decarboxylase activity reflects changes in nuclear oestrogen receptor levels. With respect to water imbibition, Chen, Kyriakidis & Canellakis (1982) found that low concentrations of colchicine (which disrupts the microtubule-microfilament cytoskeleton of the cell) inhibits enhancement of ornithine decarboxylase. Colchicine can, therefore, block eosinophil migration through the endothelial lining of the extravascular space. Colchicine also prevents the uterine oedematous response to oestrogen (Soto & Tchernitchin, 1979). Eosinophil migration could, therefore, provide a common denominator between water imbibition and protein synthesis via the polyamine synthetic pathway. The results presented in this study do not preclude the possibility of such an event.

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