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MORPHOLOGICAL EFFECTS OF THE CATECHOL ESTROGENS ON RAT EPIDIDYMAL EPITHELIA

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Summary—The 2-hydroxy and 4-hydroxyestradiols (2-/4-OHE_2) caused marked cytotoxic effects, including vacuolation and nuclear changes, in rat epididymal epithelia, after exposure to very low levels (40 ng/rat/week) for 20 weeks. The effects of the 2-/4-OHE_2 metabolites were more pronounced than that of estradiol-17β(E_2).

INTRODUCTION

The presence of an estrogen-binding protein has been demonstrated in the epididymis of several mammalian species including the rat [1–3]. The presence of estrogen receptors (ER) in the epididymis suggests a biological function for estradiol-17β(E_2) in this male accessory genital organ. It is known that the 2-hydroxyestradiol-17β(2-OHE_2) and the 4-hydroxyestradiol-17β-(4-OHE_2) metabolites of E_2, also known as the catechol estrogens, have similar or higher affinities for ER than E_2 itself [4].

In prostatic tissues, which contain ER, Nieuwoudt *et al.* [5] showed that increases in the levels of E_2 and 2-/4-OHE_2 caused corresponding increases in protein and DNA content. The maximum stimulation was obtained with steroid concentrations lower than 40 ng/rat [5].

We have previously shown that 2-/4-OHE_2 are more potent cell proliferators than E_2 in MCF-7 and HeLa cells [6, 7]. The fact that the catechol estrogens stimulate DNA synthesis [5] and cell proliferation [6, 7] in susceptible cells prompted us to investigate the possible cell proliferating effects of these compounds on the rat epididymis.

There are reports describing the degenerating effects of "high" levels of E_2 (50 μg/rat/day) on epididymis development and structure [8, 9] but no reports on the effects of the catechol estrogens could be found.

In this paper we will show the morphological effects on epididymal epithelia of rats exposed to very low levels of E_2, 2-OHE_2 and 4-OHE_2 (40 ng/rat/week) for periods of 5, 20 and 23 weeks.

EXPERIMENTAL

Materials and methods

Twenty-two-day-old Sprague–Dawley rats were used in the experiments. The rats were supplied by and maintained at the H. A. Grové Research Centre (Pretoria). The animals were housed in individual cages in a temperature-controlled room in which a constant 12 h light-12 h dark cycle was maintained. The rats were fed on an Epol laboratory chow diet and water was available *ad libitum*.

E_2, 2-OHE_2 and 4-OHE_2 were supplied by Sigma Chemical Co. (St Louis, MO). The steroids were dissolved in ethanol and diluted with propane-1,2-diol containing 0.33 mM L-ascorbic acid [10]. The ratio of ethanol–propane-1,2-diol was 1.25:198.75 ml. The rats were divided into three groups of 8 rats each. The groups were weekly injected i.p. with 0.2 ml of the ethanol–propane-1,2-diol mixture (vehicle) which contained doses of 40 ng of E_2, 2-OHE_2 or 4-OHE_2, respectively, for periods of 5, 20 and 23 weeks. Control rats received 0.2 ml saline or vehicle for the same time periods.

The rats were sacrificed by decapitation. The testis and epididymis (caput and corpus regions) were removed and fixed in Bouin's fixative and processed for paraffin sections. Some sections were cross-sections and some longitudinal. The sections were stained with haematoxylin and eosin (H and E) for light-microscopic studies.

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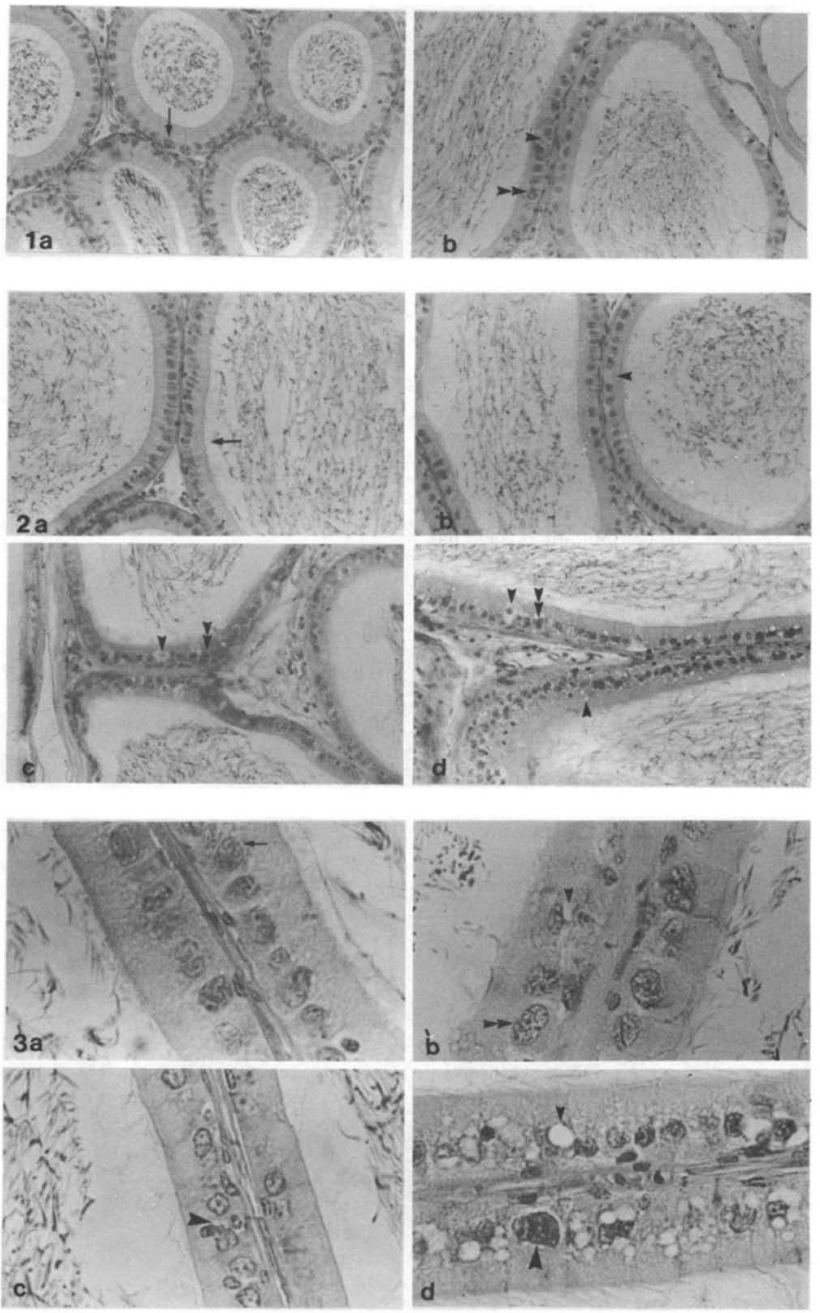

Figs 1–3. Cytotoxic effects of E_2 and 2-/4-OHE_2, on rat epididymal epithelia, depicted in the photographs, are indicated by the following: (➤) cytoplasmic vacuolation; (➤➤) nuclear effects including binucleated and displaced nuclei; (▶▶) giant and multinucleated cells. (←) indicate normal epithelium. Magnification of Figs 1 and 2, ×150; Fig. 3, ×600. (1a) Control epididymis after 5 weeks exposure to vehicle alone, (1b) epididymis exposed to 2-OHE_2 for 5 weeks. (2a) Control after 20 weeks; (2b–d) effects after exposure to E_2, 2-OHE_2 and 4-OHE_2, respectively, for 20 weeks. (3a) 23-week control, (3b–d) effect of E_2, 2-OHE_2 and 4-OHE_2, respectively, after 23 weeks treatment.

RESULTS

Testes

At these very low dosages of the estrogens no noticeable effects on the morphology of the testes were seen.

Control epididymis

No morphological lesions were present in the epididymal cells of rats exposed to saline or vehicle only (Figs 1a, 2a and 3a).

Estrogen-exposed epididymis

In the 5-week experiments only slight toxic effects including cytoplasmic vacuolation and slight nuclear changes were seen in epididymal epithelia exposed to the catechol estrogens (Fig. 1b). The effects of 2-OHE_2 and 4-OHE_2 appeared to be similar at this stage. After 20 weeks E_2 caused only slight effects (Fig. 2b). Increased cytotoxic effects were present in the epithelia exposed to 2-OHE_2 (Fig. 2c) and marked cytoplasmic vacuolation and nuclear changes were seen in the epithelia of rats exposed to 4-OHE_2 (Fig. 2d). In the 23-week experiments binucleated and multinucleated epithelium cells were observed after exposure to E_2 (Fig. 3b), 2-OHE_2 (Fig. 3c) and 4-OHE_2 (Fig. 3d). The effects of catechol estrogens were more prominent than those caused by E_2 (Figs 3b–d).

DISCUSSION

Our results indicate that the epithelium is very susceptible to the cytotoxic effects of 2-/4-OHE_2. These cytotoxic effects of 4-OHE_2 > 2-OHE_2 > E_2 were unexpected findings since we anticipated cell proliferating effects because previous studies in ER-containing cells showed cell growth when exposed to low levels of the estrogens [5–7]. The degenerative changes seen in the epididymal epithelia of rats treated with especially 2-/4-OHE_2 were similar to the atrophy of the epididymis described by Rao and Chinoy [9] when high levels of E_2 (50 μg/rat/day) were used. It is known that up to 30% of orally administered E_2 is hydroxylated in the liver to form 2-OHE_2 and approx. 5% is converted to 4-OHE_2 [11]. The 2-/4-OHE_2 metabolites are again rapidly methoxylated by the ubiquitously-present-enzyme catechol *O*-methyltransferase (COMT) to their corresponding 2-/4-methoxyestradiols (2-/4-$MeOE_2$) [11].

In a previous study we showed that the cytotoxic effects on dividing MCF-7 cells, when exposed to high levels of E_2, 2-/4-OHE_2 and 2-$MeOE_2$ were exclusively caused by the 2-$MeOE_2$ metabolite [6]. Administration of high levels of E_2 to male rats as reported by others [8, 9] may lead to the formation of substantial amounts of 2-/4-OHE_2 and subsequently to equally high amounts of the 2-/4-$MeOE_2$ metabolites. Because of the rapid methoxylation of 2-/4-OHE_2 in mammals [11] it is also possible that 2-/4-$MeOE_2$ may exert the toxic effects observed in the epididymis.

We suggest that if sufficient quantities of these E_2-metabolites are formed after E_2 treatment, that they, rather than E_2, are responsible for the toxic lesions in the epididymal epithelium.

The affected epithelium may not be able to produce or secrete the compounds necessary for sperm maturation [12, 13]. Insufficient sperm maturation when spermatozoa transversed the epididymis of estrogenized rats have been observed by others [12, 13]. In view of the marked toxic effects caused by the catechol estrogens and especially the 4-OHE_2 metabolite, we suggest that the male infertility, observed when males are exposed to high levels of E_2, may be attributed to the toxic effects of the E_2-metabolites on the epididymis.

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