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Rapid Communication

Oestrogenic Activity of an Environmentally Persistent Alkylphenol in the Reproductive Tract but not the Brain of Rodents

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Compounds with oestrogenic actions present in the environment as a result of human activity may represent a threat to health and reproductive efficiency in human and wildlife populations. We show here that parenteral administration of octylphenol, a recently described environmental oestrogen derived from one group of non-ionic surfactants, is active in stimulating oestrogen-dependent uterine growth in prepubertal rats, but has no influence on perinatal sexual differentiation of the rat brain. These results extend previous *in vitro* findings to show that alkylphenols exert weak oestrogenic activity *in vivo* in mammals.

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INTRODUCTION

An increasing concern in human reproductive medicine is the possible adverse effect of xenobiotic oestrogens in the environment or diet. Thus, attention has been drawn to the possible role of exposure to environmental oestrogens in abnormal gonad development, decreased sperm quality and oestrogen-sensitive breast cancer in humans [1–3] and wildlife populations are also believed to be at risk from environmental oestrogenic pollution [4]. A new class of xenobiotic oestrogens, alkylphenolic compounds, derived from degradation of one class of non-ionic surfactants added to detergents, toiletries, herbicides etc (annual global production over 300,000 tons), have recently been described in sewage effluent and are oestrogenic in fish [5]. Alkylphenols such as nonyl- or octylphenol are also oestrogenic in mammalian breast cancer cell proliferation assays [6] and are transcriptionally active *in vitro* through the oestrogen receptor [7].

Since it is important to know the bioavailability and oestrogenicity of this class of compound in the mammalian body to assess their possible impact, we have carried out two preliminary experiments delivering octylphenol (OP) to rats. Firstly, we examined the ability of OP to stimulate uterine growth in prepubertal female rats, a well established oestrogenic bioassay [8]. Specific areas of the rodent and human brain exhibit morphological sex differences [9, 10]. The sexually dimorphic nucleus of the preoptic area (SDN-POA) in the rat is the best characterized of these regions and sex differences in its cytoarchitecture are known to be critically dependent on perinatal oestrogen exposure [9]. Aromatization of testosterone to oestrogen in the brain of the male ensures high local concentrations of oestrogen resulting in an SDN-POA of substantially larger volume and cross sectional area. An SDN-POA of male proportions can similarly be induced in female rats by the perinatal administration of synthetic oestrogens such as diethylstilbestrol (DES) [9]. Thus, in a second experiment we have examined the ability of OP to induce oestrogen-dependent sex differences in the SDN-POA of the rat and compared its actions with DES using the same protocol of perinatal steroid administration as these earlier studies [9] and relative doses based on oestrogenic potency of OP *in vitro* [7].

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Table 1. Uterotropic activities of diethylstilbestrol (DES) and octylphenol (OP) in immature female rats

Group	*n*	Body weight (g)	Uterine wt (mg)
Vehicle	5	56 ± 2	39 ± 4
DES	5	48 ± 1*	141 ± 12**
OP	5	58 ± 1	78 ± 6**

*$P < 0.05$, **$P < 0.01$ vs vehicle group (Wilcoxon signed-rank test).

MATERIALS AND METHODS

Experiment 1

Three groups of 23-day-old female Wistar rats received s.c. injections on 3 consecutive days of 100 μl ethyl oleate vehicle, alone or containing either 5 μg of DES (Sigma, Poole, U.K.) or 10 mg of OP (2000-fold higher mass dose than DES; 4-(*tert*-octyl) phenol; Aldrich, Gillingham, U.K.). 24 hours following the last injection, rats were sacrificed, their uteri excised and weighed and fixed in formaldehyde.

Experiment 2

On each of the last 4 days of gestation, pregnant female rats received s.c. injections of 100 μl ethyl oleate vehicle, alone or containing either 20 μg DES or 40 mg OP (2000 × DES dose). Following delivery of litters, all pups received a daily s.c. injection on postnatal days 1–4 of 50 μl ethyl oleate alone or containing either 1 μg DES or 2 mg OP (2000 × DES dose). Litters were weaned at 21 days and sexes caged separately from 24 days of age. At 60 days of age, a maximum of 6 male and female rats per treatment were deeply anaesthetized with Avertin and transcardially perfused with heparinized saline followed by 10% formaldehyde in phosphate buffered saline. Whole brains were removed and immersion fixed for 10 days prior to embedding, sectioned at 60 μm through the preoptic area and stained with thionin. Coded slides were examined blind and the boundaries of the SDN-POA on each side of the brain drawn using a camera lucida. SDN-POA area was calculated using computer-based image analysis. Testes were dissected and weighed following removal of the epididymis.

RESULTS

DES induced a 260% increase in uterine weight ($P < 0.01$) accompanied by a 15% decrease in body weight. OP was active in inducing a 100% increase in uterine weight ($P < 0.01$) without a change in body weight (Table 1 and Fig. 1).

In confirmation of earlier reports, the SDN-POA area was 115% greater ($P < 0.01$) in vehicle treated males than in females; and DES significantly increased SDN-POA area in females by 46% (Table 2). OP treatment was found to have no effect on SDN-POA morphology. Neither compound influenced SDN-POA area in males. Body weight was slightly increased in females by both DES and OP perinatally. In males,

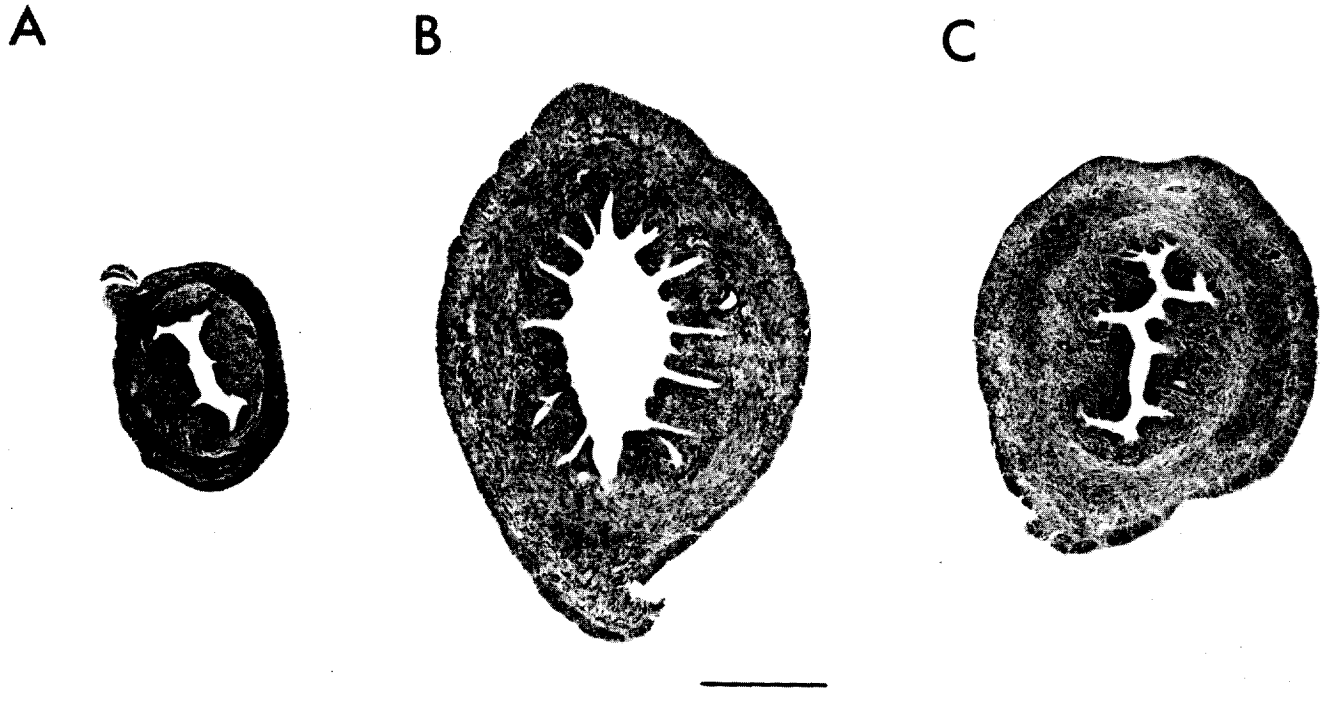

Fig. 1. Histological appearance of rat uterus following treatment with A, vehicle; B, DES and C, OP. Transverse sections stained with haematoxylin and eosin. Scale bar, 0.5 mm.

Table 2. Effects of perinatal exposure to DES and OP on area of the sexually dimorphic nucleus of the preoptic area (SDN-POA) of the brain of rats 60 days postnatally

	Females			Males			
Group	n	Body weight (g)	Area of SDN-POA (mm^2)	n	Body weight (g)	Testis weight/100 g body weight (g)	Area of SDN-POA (mm^2)
Vehicle	6	183 ± 4	0.0325 ± 0.0026	6	298 ± 9	0.716 ± 0.068	0.0699 ± 0.0021**
DES	6	198 ± 3*	0.0475 ± 0.0039*	3	267 ± 18	0.635 ± 0.142	0.0683 ± 0.0056
OP	6	200 ± 3*	0.0362 ± 0.0020	6	314 ± 9	0.874 ± 0.058	0.0590 ± 0.0032

*$P < 0.05$, **$P < 0.01$ vs vehicle female group (Wilcoxon signed rank test).

combined testis weight was 30% greater following OP but this difference was not significant when expressed per unit body weight (Table 2).

DISCUSSION

Alkylphenols such as OP have recently been described to bind directly to oestrogen receptors from trout, to stimulate vitellogenin gene expression in trout hepatocytes, to be mitogenic in human breast cancer cell lines and to stimulate transcription in mammalian and avian cell cultures through the oestrogen receptor [7].

To our knowledge this is the first report on the potential *in vivo* oestrogenicity of alkylphenols in a mammalian species. These early findings lead us to conclude that OP is weakly oestrogenic at peripheral target tissues when administered subcutaneously, but that acute perinatal exposure does not reduce gross testis development. The lack of effect of OP on brain sexual differentiation may be due to insufficient penetration into the brain, to the relative lack of sensitivity of this process or to intrinsic inactivity of OP at neural oestrogen receptors. In contrast, the masculinization of the female SDN-POA by DES shown here is in good agreement with previous reports [9]. Although the nature of gonadal steroid action on the SDN-POA appears relatively well defined, its physiological significance with respect to sexually differentiated neural functioning remains unclear [9]. On a mass basis, OP is more than 2000 times less effective than DES in our study and some 1000-fold less active than 17β-oestradiol *in vitro* [7]. Further information on dose, duration and routes of exposure to alkylphenols will be needed to assess the impact of these degradation products of a widely used group of surfactants on health and reproduction in human and wildlife populations.

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REFERENCES

1. Sharpe R. M. and Skakkebaek N. E.: Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet* **341** (1993) 1392–1395.
2. Ginsburg J.: Environmental oestrogens. *Lancet* **343** (1994) 284–285.
3. Stone R.: Environmental estrogens stir debate. *Science* **265** (1994) 308–310.
4. Colborn T. and Clement C.: Chemically-induced alterations in sexual and functional development: the wildlife/human connection. In *Advances in Modern Environmental Toxicology*. (Edited by T. Colborn and C. Clement). Princeton Scientific Publishing Co., Princeton, NJ (1992) Vol. XXI.
5. Jobling S. and Sumpter J. P.: Detergent components in sewage effluent are weakly oestrogenic to fish: an *in vitro* study using rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Aquatic Toxic.* 27 (1993) 361–372.
6. Soto A. M., Lin T. M., Justicia H., Silvia R. M. and Sonnenschein C.: An 'in culture' bioassay to assess estrogenicity of xenobiotics (E-SCREEN). In *Advances in Modern Environmental Toxicology* (Edited by T. Colborn and C. Clement). Princeton Scientific Publishing Co., Princeton, NJ (1992) Vol. XXI, pp. 295–309.
7. White R., Jobling S., Hoare S. A., Sumpter J. P. and Parker M. G.: Environmentally persistent alkylphenolic compounds are estrogenic. *Endocrinology* **135** (1994) 175–182.
8. Lan N. C. and Katzenellenbogen B. S.: Temporal relationships between hormone receptor binding and biological responses in the uterus: studies with short and long-acting derivatives of estriol. *Endocrinology* **98** (1976) 220–230.
9. Arnold A. P. and Gorski R. A. Gonadal steroid induction of structural sex differences in the central nervous system. *A. Rev. Neurosci.* 7 (1984) 413–442.
10. Swaab D. F. and Hofman M. A.: Sexual differentiation of the human hypothalamus: ontogeny of the sexually dimorphic nucleus of the preoptic area. *Dev. Brain Res.* 44 (1988) 314–318.