Available online at www.sciencedirect.com
SCIENCE DIRECT
Toxicology and Applied Pharmacology 214 (2006) 1–7
Toxicology and Applied Pharmacology
www.elsevier.com/locate/ytaap

Comparison of effects of estradiol with those of octylmethoxycinnamate and 4-methylbenzylidene camphor on fat tissue, lipids and pituitary hormones

Dana Seidlová-Wuttke, Julie Christoffel, Guillermo Rimoldi, Hubertus Jarry, Wolfgang Wuttke *

Department of Clinical and Experimental Endocrinology, University of Göttingen, Germany

Received 11 July 2005; revised 3 November 2005; accepted 3 November 2005
Available online 20 December 2005

Abstract

Octylmethoxycinnamate (OMC) and 4-methylbenzylidene camphor (4MBC) are commercially used absorbers of ultraviolet (UV) light. In rats, they were shown to exert endocrine disrupting including uterotrophic, i.e. estrogenic effects. Estrogens have also metabolic effects, therefore the impact of oral application of the two UV absorbers at 2 doses for 3 months on lipids and hormones were compared with those of estradiol-17β (E2). E2, OMC and 4MBC reduced weight gain, the size of fat depots and serum leptin, a lipocyte-derived hormone, when compared to the ovariectomized control animals. Serum triglycerides were also reduced by the UV screens but not by E2. On the other hand, E2 and OMC reduced serum cholesterol, low density lipoproteins and high density lipoproteins; this effect was not shared by 4MBC. While E2 inhibited, OMC and 4MBC stimulated serum LH levels. In the uterus, both UV filters had mild stimulatory effects. 4MBC inhibited serum T4 resulting in increased serum TSH levels.

It is concluded that OMC and 4MBC have effects on several metabolic parameters such as fat and lipid homeostasis as well as on thyroid hormone production. Many of these effects are not shared by E2. Hence, other than estrogen-receptive mechanisms may be responsible for these effects.

Keywords: Estradiol; OMC; 4MBC; Lipids; Pituitary hormones

Introduction

A number of absorbers of ultraviolet (UV) light, particularly those of the camphor and cinnamate line, are suspected to be endocrine disrupters (EDs) (Holbech et al., 2002; Inui et al., 2003; Ma et al., 2003; Mueller et al., 2003; Schlumpf et al., 2004; Tinwell et al., 2002). EDs are chemicals which are wantedly or unwantedly incorporated and which interfere with endogenous hormone production or action. Since octylmethoxycinnamate (OMC) and 4-methylbenzylidene camphor (4MBC) are major UV absorbers used in sunscreens and since they are transcutaneously absorbed (Janjua et al., 2004), a thorough investigation of their putative ED properties seems advisable. This can be done by studying their binding properties to estrogen receptors of which two have been cloned (estrogen receptor (ER) α and β) (Albertazzi and Purdie, 2001; Katzenellenbogen et al., 2001; Nilsson et al., 2001) and of which recombinant proteins are available. Cytosolic extracts of uteri express ERα and ERβ (Korach et al., 2003) but in addition there are estrogen-binding sites which are not ERs but of which the structure has not been identified (Caltagirone et al., 1997; Jarry et al., 2003; Markaverich et al., 1988). Hence, a tool to study estrogenic effects of putative endocrine disrupters is to study their binding properties to these recombinant receptors or to a cytosolic extract of uterine tissue.

Estradiol 17β (E2) has effects on many organs of the body. Our increasing knowledge about tissue-specific expression of the 2 ERs and of the coactivators and corepressors begins to make it understandable that substances which bind to ERs may have very different effects in different organs. This is the basis for clinically used selective estrogen receptor modulators (SERMs) such as tamoxifene or raloxifene which have negligible estrogenic effects in the uterus, antiestrogenic effects in the mammary gland and the brain but estrogenic effects in the bone. Therefore, substances with an estrogenic potential should be studied in organs known to

* Corresponding author. Klin. und Exp. Endokrinologie, Universitäts-Frauenklinik Göttingen, Robert-Koch-Str. 40, 37075 Göttingen, Germany. Fax: +49 551 396518.
E-mail address: ufkendo@med.uni-goettingen.de (W. Wuttke).

0041-008X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2005.11.002

be regulated by E2. For example, the effects of the sunscreen on pituitary hormones, particularly on the release of LH where E2 exerts a negative feedback in ovariectomized (ovx) rats (Evans et al., 2002; Jarry et al., 2003) should be determined. The effects of E2 on hormones of the thyroid axis are controversially discussed (Boado et al., 1983; Franklyn et al., 1987; Lisboa et al., 1997; Thomas et al., 1986). Serum cholesterol, high density and low density lipoproteins (HDL and LDL) in the rat are known to be suppressed by E2 whereas serum triglycerides stored in very low density lipoproteins (VLDL) (Ginci et al., 1997; Parini et al., 2000) are increased. Hence, estrogenicity of putative EDs could also be exerted on serum lipids. Lipocytes express primarily ERα (Anwar et al., 2001; Joyner et al., 2001) and E2 exerts lipolysis in these cells (Misso et al., 2003; Palin et al., 2003). Since chronically E2-treated animals accumulate little fat depots, their fat tissue-derived leptin levels in the blood are low in comparison to fat accumulating ovx animals (Seidlova-Wuttke et al., 2003b) Hence, determination of fat depots, triglycerides and leptin gives also indications of estrogenic activities of EDs. In a recent publication, it was shown that both OMC and 4 MBC are absorbed through the skin such that serum concentrations were clearly measurable, but more importantly unwilling oral ingestion may be of major importance. Therefore, we studied the effects of a 3-month-lasting oral-treatment with OMC and 4MBC and compared them with the effects of E2 as positive and with these chronically ovx rats which served as negative controls.

Material and methods

In vitro experiments. The tracer estradiol 17β-J^{125} was purchased from NEN (Dreieich, Germany). Recombinant human ERα and ERβ were obtained from PanVera (Madison, USA). All other chemicals were purchased from Sigma (Deisenhofen, Germany). Subtype-specific ERα and ERβ ligand-binding assays were performed according to the method described by Kuiper et al. (1997) with the exception that samples were incubated for 18–20 h at 6 °C in triplicates and that bound and free tracer was separated by adsorption on dextran coated charcoal. In previous experiments, we demonstrated that substances which did not bind to recombinant ERα or ERβ protein might well bind to yet unidentified estradiol-binding proteins present in cytosolic extracts of uteri. Therefore, a cytosolic extract of porcine uteri (Jarry et al., 2003). was also tested for its binding properties to OMC and 4MBC.

In vivo experiments. Female Sprague–Dawley rats weighing 250 ± 5 g were used for the present experiments. Allowance to perform these experiments was obtained from the Bezirksregierung Braunschweig (permission No. Az. 509.42502/01–13.00 dated July 17, 2000). Upon arrival from the supplier (Winkelmann, Borchen, Germany), they were fed with pelleted chow in which soy proteins were replaced by potato proteins (ssniff, Soest, Germany). One week after arrival, they were ovariectomized (ovx). Animals had food and water ad libitum. The animal quarters were illuminated from 06.00 a.m. until 06.00 p.m. Room temperature was 25 °C at a relative humidity of 55%.

Experiments were performed in which animals remained either unsubstituted (negative controls) or were substituted with E2 (positive controls) or treated with OMC or 4MBC immediately after ovx. From experience with the animals' food intake in the last 2 weeks prior to the beginning of the experiments, it was known that the mean food intake of each animal was approximately 20 ± 2 g. On this basis, E2 and the EDs were added such that 20 g food contained 0.325 mg of E2 (as E2-benzoate (EB) 0.5 mg); 50 or 250 mg per 20 g food of OMC or 4MBC, respectively. On the basis of twice weekly measurements of food intake, the exact amount of food intake could be calculated; these and the respective intake of EDs are shown in Table 1.

Animals were weighed and vaginal smears taken twice weekly. Prior to experimentation and 12 weeks after onset of E2 or ED-supplemented food, animals were anesthetized (inhalation anesthesia with isoflurane) and the size of a paratibial fat depot recorded by computer tomography (XCT Research SA + STRATEC Medizintechnik GmbH, Pforzheim). This fat depot is located in the lower hindleg and reacts extremely sensitive to withdrawal of endogenous estrogens with an increase in size and to substitution with estrogenic substances (Seidlova-Wuttke et al., 2003b) and this can perimetrically be quantified. The percentage of this fat depot surface in relation to the total surface of the CT plane at the height of the metaphysis of the tibia can be calculated as an exact measure of the size of this fat depot.

Serum analysis. The blood samples collected from all animals were centrifuged (3000×*g*, 10 min) and the serum used for measurement of luteinizing hormone (LH) and thyroid-stimulating hormone (TSH) by a specific radioimmunoassay (RIA) supplied by the NHPP (Dr. A.F. Parlow, Harbor-UCLA Medical Center, Torrance, CA). Serum thyroxin (T4) and triiodothyronine (T3) and Estradiol 17β were determined by RIA specifically designed for rat serum (DSL, Sinsheim, Germany), serum leptin by a specific RIA (Linco Research, St. Charles, MO, USA) and cholesterol, HDL, LDL and triglycerides by enzymatic assays (Hitachi, Roche, Mannheim, Germany).

Statistical evaluation. Data were expressed as means ± SEM. Significant differences between the control and treatment groups were analyzed by one-way ANOVA followed by Dunnett's post hoc test for multiple comparisons (Prism™, Graph Pad, San Diego, USA). P values < 0.05 were considered significant.

Table 1
Average daily intake of food and test substances

Substance	Daily food intake (g)	Daily intake of test substance (mg)
Negative control	21.12	0.0
E2	17.82	0.445
OMC low (Eusolex 2292)	20.99	52.47
OMC high	22.31	278.87
4MBC low (Eusolex 6300)	23.71	59.27
4MBC high	22.83	285.37

Each group of animals consisted of 12 rats. OMC, 4MBC and E2 were purchased from Merck (Darmstadt, Germany) and Sigma (Taufkirchen, Germany).

Table 2
Affinity (EC50) of E2, OMC and 4MBC to bind to ERα, ERβ and uterine cytosolic E2-binding sites (M)

In vitro competition	E2	4MBC	OMC
ERα	7×10^{-9}	$>10^{-3}$	$>10^{-3}$
ERβ	2×10^{-9}	3.5×10^{-5}	$>10^{-3}$
Cytosolic E2 binding sites	4×10^{-10}	1×10^{-4}	$>10^{-3}$

Results

Table 2 details binding properties (i.e. the EC50 values) of E2, OMC and 4MBC to ERα and to ERβ recombinant protein preparations and to a cytosolic extract of porcine uteri. OMC neither bound to the 2 recombinant ER protein preparations nor to the cytosolic preparation of porcine uterine tissue. 4MBC, on the other hand, bound to the recombinant ERβ preparation as well as to the cytosolic preparation of the uterus but not to the ERα protein. In each case, 4MBC was 4 decades less potent than E2 to displace the radioactive tracer from the recombinant ERβ and 6 decades less potent with respect to the cytosolic-binding assays.