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Effects of a 5-day treatment with the UV-filter octyl-methoxycinnamate (OMC) on the function of the hypothalamo-pituitary–thyroid function in rats

Holger Klammer [a], Christiane Schlecht [a], Wolfgang Wuttke [a], Cornelia Schmutzler [b], Inka Gotthardt [b], Josef Köhrle [b], Hubertus Jarry [a,*]

[a] *Department of Clinical and Experimental Endocrinology, University of Goettingen, Robert-Koch-Str. 40, 37075 Goettingen, Germany*
[b] *Institute of Experimental Endocrinology, Charité-Universitätsmedizin Berlin, Schumannstr. 20/21, 10117 Berlin, Germany*

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Abstract

Octyl-methoxycinnamate (OMC) is one of the most frequently used UV-filters in sunscreens to protect the skin against the noxious influence of UV radiation. Recently, OMC was suspected to act as an "endocrine active chemical" (EAC) with estrogenic actions. While EACs have been investigated thoroughly for interference with reproductive function in mammalians, surprisingly little efforts have been made to investigate an interference of EACs with the hypothalamo-pituitary–thyroid (HPT) axis despite the expression of estrogen receptors in all parts of this axis. Therefore, we conducted an *in vivo* study with ovariectomised rats treated for 5 days with different doses of OMC or 17β-estradiol (E2) as a control. Determined parameters comprised serum levels of TSH, T4 and T3, hypothalamic TRH mRNA expression, protein-expression of the sodium–iodide-symporter (NIS) and the TSH receptor and the activities of thyroid peroxidase (TPO) in the thyroid and the T3-responsive hepatic type I 5′deiodinase (Dio1) in the liver.

While E2 did not affect TSH-, T4- or T3-levels, OMC caused a dose-dependent decrease of serum concentrations of all of these hormones. TRH expression remained unaffected, while in the thyroid, expression of the TSH receptor but not of NIS was stimulated by OMC. TPO activity was unaltered but Dio1 activity was reduced by OMC. Thus, our results demonstrate a non-estrogenic interference of OMC within the rodent HPT axis with inadequate feedback response to impaired thyroid hormone status, indicated by decreased serum thyroid hormone and hepatic Dio1 levels.

Keywords: OMC; Rat; Thyroid; Estrogen; TRH; TSH; T3; T4

1. Introduction

Ethylhexyl-methoxycinnamate (formerly known under the trivial name octyl-methoxycinnamate, OMC), is one of the most frequently used chemical UV-filters worldwide. It is contained in high amounts (up to 1 of 10 parts per weight) in sunscreens to protect the skin against the noxious influence of UV radiation. Recent studies indicated, that OMC may have significant undesirable effect as an "endocrine active chemical" (EAC) (Schlumpf et al., 2001; Schlumpf et al., 2004) and regulatory authorities like the Scientific Committee of Cosmetic Products and Non-Food Products (SCCNFP) of the European Union have

* Corresponding author at: Universitätsfrauenklinik Klinische und Experimentelle Endokrinologie Robert-Koch-Strasse 40, D-37075 Goettingen, Germany. Tel.: +49 551 396522; fax: +49 551 396518.
E-mail address: hubjarry@med.uni-goettingen.de (H. Jarry).

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released statements of concern on use of OMC. The term EAC addresses those chemicals, which possess the ability to interfere with endocrine systems, like the hypothalamo-pituitary–gonadal axis which might be of major concern during perinatal development and differentiation of vertebrate organisms. In the last decades research on adverse effects of EAC focussed on putative estrogenic and anti-androgenic affects in wildlife populations and laboratory animals (for review, see (Gelbke et al., 2004; Gray et al., 2006; Safe, 2004). With regard to OMC, an estrogenic multi-organic risk assessment has been reported which revealed that OMC exerts both estrogenic as well as non-estrogenic activities in uterus, vagina and liver (Klammer et al., 2005; Seidlova-Wuttke et al., 2006b).

Similarly to 17β-estradiol (E2) and both its receptor subtypes, the thyroid prohormone T4 and its biological active metabolite T3 affect the function of virtually all organs during development and in adulthood since the various forms of the T3-binding thyroid hormone receptors are broadly expressed throughout the body (O'Shea and Williams, 2002; Shahrara et al., 1999). However, despite the pivotal function of thyroid hormones in the organism, little attention has been paid to interference of EACs with the hypothalamo-pituitary–thyroid axis (HPT) in mammals. Likewise, the literature regarding effects of E2 on the HPT axis has been mainly compiled before the molecular characterisation of T3 receptors and the data reported are contradictory (Boado et al., 1983; Franklyn et al., 1987; Lisboa et al., 1997; Thomas et al., 1986). In a recent study with ovariectomised (ovx) rats which were treated with E2 and various EACs for 3 months via the food, OMC at a dose of approximately 200 mg/kg bodyweight caused a reduction of T4-levels without affecting T3- or TSH-levels (Seidlova-Wuttke et al., 2006a). In contrast, E2 did not affect these parameters, further supporting the assumption that OMC might be an EAC with both, estrogenic but also non-estrogenic properties.

To further investigate the activity of OMC on the HPT axis, dose-response relationships of several parameters of the HPT axis were investigated following a treatment protocol with ovx rats previously used to conduct a multi-organic risk assessment study (Klammer et al., 2005). Following parameters of the HPT axis were analysed: TRH expression in the hypothalamus was measured at mRNA level, TSH-, T4- and T3-serum levels were determined by radioimmunoassay. Expression of two-key factors of thyroid function, the sodium–iodide-symporter (NIS) and the TSH-receptor (TSHR), was analysed on protein-level by western blot. In addition, the enzyme activities of the thyroid peroxidase (TPO) in the thyroid, the key enzyme catalysing iodination of tyrosyl-residues in thyroglobulin and their coupling to iodothyronines and the T3-responsive type I 5′deiodinase (Dio1) in the liver were determined.

2. Methods

2.1. Test substances

OMC (ethylhexyl-methoxycinnamate, EUSOLEX 2292®) was obtained from Merck KG (Frankfurt, Germany) and 17β-estradiol-valerate was purchased from Sigma–Aldrich GmbH (Taufkirchen, Germany). The test substances were dissolved in olive oil, which also served as solvent control.

2.2. Animals and treatment

Female rats, used in this study, were housed and treated in accordance with the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS 123). The experiments were approved by a permit issued by the Landesamt für Verbraucherschutz, Braunschweig, Germany.

For all experiments female Sprague–Dawley rats, purchased from Winkelmann (Borchen, Germany) were used. Animals were kept under standard conditions (soy-free formulation of diet, ssniff SM R/M, 10 mm, supplied by ssniff Spezialdiäten GmbH, Soest, Germany and water *ad libitum*, lights on from 5:30 am to 6:00 pm, room temperature 23 °C and a relative humidity of 55%). At the age of 3 months, all rats were bilaterally ovariectomised (ovx). After a recovery phase of 17 days, the animals were treated via gavage once per day with either 1 ml pure olive oil (Ctl), 600 μg/kg bodyweight 17β-estradiol-valerate (E2) or 10, 33, 100, 333 or 1000 mg/kg OMC (OMC-10 to OMC-1000). The treatment was conducted each day between 5:30 and 6:00 am. The animals were observed for daily clinical signs of impairment of health or systemic toxicity. Bodyweight was determined daily. At day 5 of treatment, the animals were decapitated under deep CO_2 anaesthesia 3–4 h after the last application. Blood was collected from the trunk and serum samples were stored at −20 °C. The collected organs were frozen in liquid nitrogen and stored at −70 °C until further processing. The mediobasal hypothalamus (MBH) was dissected from the frozen brain as described previously (Leonhardt et al., 2000)

2.3. Serum analysis

Thyroid hormones (total T3/T4) were measured using commercially available kits validated for rat serum following the manufacturers' instructions (Active® T3, Active® Thyroxin, DSL, Sinsheim, Germany). The TSH radioimmunoassay was performed, as described previously, with reagents kindly supplied by the Hormone Distribution Program of the NIDDK (Baur et al., 2000). The TSH tracer was labelled with ^{125}I (Hunter and Greenwood, 1962).