Reproductive Toxicology 42 (2013) 49–57

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Valproic acid and its derivatives enhanced estrogenic activity but not androgenic activity in a structure dependent manner

Sandra Stempin[a], Susanne Andres[a], Maja Bumke Scheer[a], Ariane Rode[b], Heinz Nau[b], Albrecht Seidel[c], Alfonso Lampen[a,*]

[a] BfR – Federal Institute for Risk Assessment, Department of Food Safety, Berlin, Germany
[b] Institute for Food Toxicology and Analytical Chemistry, University of Veterinary Medicine, Hannover, Germany
[c] Biochemical Institute for Environmental Carcinogens, Prof. Dr. Gernot Grimmer-Foundation, Grosshansdorf, Germany

ARTICLE INFO

Article history:
Received 16 August 2012
Received in revised form 17 June 2013
Accepted 17 July 2013
Available online 26 July 2013

Keywords:
Valproic acid (VPA)
VPA derivatives
Estrogen receptor (ER)
Androgen receptor (AR)
Luciferase reporter gene assay
ERα
ERβ
Teratogenicity

ABSTRACT

Steroid hormones affect metabolic pathways and cellular functions. Valproic acid (VPA), used as antiepileptic drug, inhibits histone deacetylases and interacts with intracellular receptors. We analyzed the impact of VPA and VPA derivatives on activation of estrogen and androgen receptors (ER and AR) using reporter gene assays. VPA and its long-chain derivatives 2-(2-propynyl)-hexanoic acid [butyl-4-yn-VPA], 2-(2-propynyl)-heptanoic acid [S-pentyl-4-yn-VPA] and 2-(2-propynyl)-nonanoic acid [heptyl-4-yn-VPA] enhanced 17β-estradiol-induced ERα and ERβ activation partly synergistically with a structure–activity correlation. The extent of this effect regarding to ERα activation increased with prolongation of the aliphatic side chain. Regarding AR activation, VPA, S-pentyl-4-yn- and heptyl-4-yn-VPA slightly induced AR activity when tested alone. In combination with the AR agonist 5α-dihydrotestosterone, VPA, S-pentyl-4-yn- and heptyl-4-yn-VPA showed anti-androgenic effects without an apparent structural relation. Our results indicate that VPA and its derivatives affect estrogen signaling with a structural specificity, while the (anti-)androgenic effects of these compounds are not structurally correlated.

1. Introduction

Sex steroid hormones are essential for development, growth and differentiation. In addition to their important function in reproduction they can influence other physiological processes and have an impact on many target tissues [1,2]. For instance, estrogens stimulate signal transduction pathways [3] and affect a number of systems, including the reproductive tract, mammary gland, central nervous system, cardiovascular and skeletal system [4]. Estrogens also influence the pathological processes of hormone-dependent diseases, such as breast cancer or osteoporosis [4]. Androgens control a wide range of developmental and physiological responses, e.g. male sexual differentiation, maturation and spermatogenesis [5].

The biological action of estrogens is mediated through estrogen receptors (ER) referring to the nuclear receptor superfamily [6]. There are two ER subtypes, ERα and ERβ, which show differences in function, tissue expression and ligand binding characteristics [7,8]. The biological actions of the principal physiologically relevant androgens (testosterone and 5α-dihydrotestosterone) are mediated through the androgen receptor (AR) [9].

There are many exogenous substances that are able to act hormonally, influencing hormone-dependent metabolic and signaling pathways. Some of these compounds such as polychlorinated biphenyls, dithiothreitol or bisphenol A are associated with a plurality of diseases [10,11]. Small molecules like fatty acids also come into consideration as possible ligands or modulators of steroid hormone receptors [12]. These fatty acids are ubiquitous molecules with important biological functions [13]. To date, potential interferences of such compounds with the endocrine system have been inadequately investigated. It is well known that fatty acids can modulate some members of the nuclear receptor superfamily such as hormonal receptors or peroxisome proliferator-activated receptors [14–17].

As an example, valproic acid (2-propylpentanoic acid; VPA) is a short-chain fatty acid showing dose-dependent estrogenic or anti-estrogenic effects [18–20] as well as anti-androgenic effects [21]. For medical purposes, VPA is used as anticonvulsant for the

Abbreviations: AP-1, activator protein 1; AR, androgen receptor; DHT, 5α-dihydrotestosterone; E_2, 17β-estradiol; ER, estrogen receptor; HDAC, histone deacetylase; HEK293, Human Embryonic Kidney 293 cells; VPA, valproic acid (2-propylpentanoic acid); butyl-4-yn-VPA, 2-(2-propynyl)-hexanoic acid; E-2-en-VPA, 2-*n*-propyl-2-pentenoic acid; ethyl-4-yn-VPA, 2-ethyl-4-pentynoic acid; heptyl-4-yn-VPA, 2-(2-propynyl)-nonanoic acid; S-pentyl-4-yn-VPA, 2-(2-propynyl)-heptanoic acid.

* Corresponding author.
E-mail address: Alfonso.Lampen@bfr.bund.de (A. Lampen).

0890-6238/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.reprotox.2013.07.019

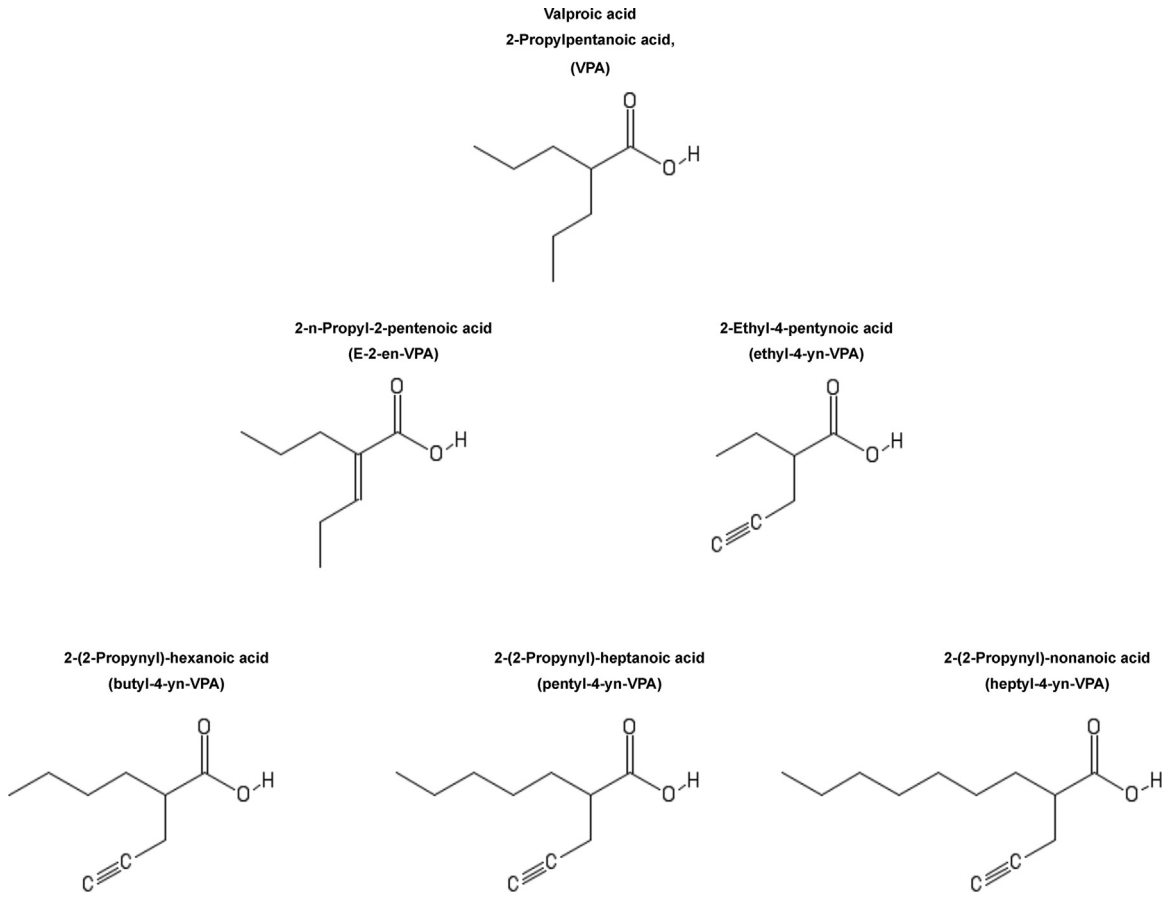

Fig. 1. Structure of valproic acid (VPA) and some of its derivatives.

treatment of several forms of epilepsy [22,23], bipolar disorders [24] and as migraine prophylaxis and therapy [25,26]. VPA is a simple branched-chain fatty acid and represents a good model substance for saturated fatty acids. Many derivatives of VPA are available that cover relevant structural characteristics of fatty acids; e.g. different chain lengths, branched chains or multiple bonds (Fig. 1). Previous studies with VPA and its analogs revealed high structure-specificity for some biological effects like teratogenicity, whereas the anticonvulsant activity and neurotoxicity do not show such a strict structure–activity relationship [27–33].

Although there are already published reports that VPA modulates ER and AR activities, nothing is known about the impact of its derivatives on hormonal receptors. Thus, the aim of our study was to examine the ER and AR activation by the VPA derivatives 2-*n*-propyl-2-pentenoic acid [E-2-en-VPA], 2-(2-propynyl)-hexanoic acid [butyl-4-yn-VPA], the S-enantiomer of 2-(2-propynyl)-heptanoic acid [S-pentyl-4-yn-VPA] and 2-(2-propynyl)-nonanoic acid [heptyl-4-yn-VPA] (for structures see Fig. 1). In addition, we tested the impact of VPA and its derivatives on the agonist-induced ER and AR activation. Due to the observation that VPA and its derivatives show structure–activity relationship for certain biological effects, special attention was drawn on such a possible correlation in relation to the activation potential of these compounds to the tested hormonal receptors.

2. Materials and methods

2.1. Cell culture

Stably transfected Human Embryonic Kidney 293 (HEK293) cells that contain an estrogen-responsive reporter gene construct (*3xERE-tataLUC*) and full-length recombinant human ERα or ERβ (HEK293ERα or HEK293ERβ cells) [34] were kindly provided by Paul van der Saag and Bart van der Burg (Hubrecht Laboratory, Netherlands Institute for Developmental Biology; Utrecht, Netherlands). The cells were routinely cultured in Dulbecco's Modified Eagles Medium (DMEM) Nut Mix F-12 (Gibco, Karlsruhe, Germany) supplemented with 8% fetal calf serum (Biochrom AG, Berlin, Germany), 4 mM L-glutamine, 2% non essential amino acids (NEAA, Gibco) and penicillin-streptomycin (10,000 units/ml, Gibco). For reporter gene assay HEK293ERα or HEK293ERβ cells were plated in a 1:1 mixture of DMEM and Ham's F12 medium without phenol red, supplemented with 4 mM L-glutamine, 2% NEAA (all from Gibco), 0.0625% cysteine and 5% dextran-coated charcoal-stripped fetal calf serum (Biochrom AG).

The human breast cancer cell line MDA-MB-453 that constitutively expresses functional endogenous AR and additionally contains the androgen-dependent reporter gene construct *MMTV.Luciferase.neo* [35], was kindly provided by Kathy Bobseine (U.S. Environmental Protection Agency, Reproductive Toxicology Division, Research Triangle Park; NC, USA). The so called MDA-kb2 cell line was grown in Leibovitz's L-15 medium without phenol red (Gibco) containing 10% fetal calf serum.

Cultures of HEK293ERα and HEK293ERβ cells were maintained in a humidified incubator at 37 °C and 5% CO_2. MDA-kb2 cells were grown without CO_2 in a humidified incubator at 37 °C.

2.2. Reagents

17β-estradiol (E_2), 5α-dihydrotestosterone (DHT), VPA, the AR antagonist flutamide and the histone deacetylase (HDAC) inhibitor