European Journal of Pharmacology 715 (2013) 256–261

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Endocrine pharmacology

Growth hormone-independent suppression of growth hormone-dependent female isoforms of cytochrome P450 by the somatostatin analog octreotide ☆

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ARTICLE INFO

Available online 21 May 2013

Keywords:
Cytochrome P450
CYP2A1
CYP2C12
CYP2C7
Growth hormone
Octreotide
Ubiquitination

ABSTRACT

Octreotide is a potent somatostatin analog therapeutically used to treat several conditions including hyper growth hormone secretion in patients with acromegaly. We infused octreotide into female Sprague Dawley rats every 12 h for 6 days at levels considerably greater than typical human therapeutic doses. Resulting circulating growth hormone profiles were characterized by ~25% reduction in plasma levels, including both pulse and interpulse components, but still contained in an otherwise female-like “continuous” secretory profile. The normally elevated feminine expression levels (protein and/or mRNA) of CYP2C12, CYP2A1, CYP2C7 and insulin-like growth factor-1 (IGF-1), all dependent on the continuous feminine growth hormone profile, were dramatically down-regulated. Octreotide suppression of the female-dependent levels of CYPs (cytochromes P450) and IGF-1 could not be explained by the apparently inconsequential alterations in the feminine circulating growth hormone profile. In this regard, somatostatin and its analogs are known to have a myriad of extra-pituitary actions effecting nearly all tissues in the body. Focusing our attention on CYP2C12, accounting for $>40\%$ of the total hepatic cytochrome P450 content in the female rat liver, we found a ~4-fold increase in hepatic ubiquitin-CYP2C12 levels in octreotide treated rats suggesting a possible contributing factor for the $>60\%$ suppression of CYP2C12 protein concentrations.

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1. Introduction

Octreotide is a potent somatostatin analog most commonly used to reduce blood levels of growth hormone and insulin-like growth factor-1 (IGF-1), also known as somatomedin C, in acromegaly patients (Lamberts et al., 1993; Yang and Keating, 2010). Whereas a single dose of the drug to humans (Marbach et al., 1985) or rats (Turner and Tannenbaum, 1995) can profoundly reduce plasma growth hormone concentrations for many hours, the therapeutic goal, however, is to achieve normalization of growth hormone and IGF-1 levels in patients (Lamberts et al., 1993; Yang and Keating, 2010).

In addition to regulating IGF-1 expression, growth hormone regulates expression of both constituent and inducible cytochromes P450 (CYP) and its own growth hormone receptor in rat liver, as well as in every other species examined (Jansson et al., 1985; Shapiro et al., 1995). In fact, the sex differences in CYP expression exhibited in rats, mice and humans appear to be solely regulated by sex differences in secretory growth hormone profiles (Shapiro et al., 1995; Dhir et al., 2006). Whereas males and females secrete a similar daily amount of growth hormone, females secrete a so-called “continuous” growth hormone profile comprised of numerous daily pulses interrupted by short-lived interpulses of usually low or barely detectable hormone concentrations. In contrast, the “episodic” masculine growth hormone profile is characterized by significantly fewer but larger secretory bursts of the hormone separated by lengthy interpulses that are invariably devoid of growth hormone. In fact, it is the difference between the continuous (female) and episodic (male) circulating growth hormone profiles, and not plasma hormone levels per se that are responsible for phenotypic sexual dimorphisms ranging from growth patterns to expression levels of hepatic enzymes (Jansson et al., 1985; Shapiro et al., 1995). Moreover, the sex-dependent growth hormone profiles are composed of various “signaling elements” to which each hepatic CYP isoform is independently responsive.

These signals may be recognized by the hepatocyte in the frequencies, concentrations and/or durations of the pulse and interpulse periods. Alternatively, the hepatocyte can monitor the mean

Abbreviations: CYP, cytochrome P450; IGF-1, insulin-like growth factor-1; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

☆This work was previously presented, in part, at the 18th North American ISSX Meeting, Dallas, TX October 14–18, 2012.

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0014-2999/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2013.05.013

plasma concentration of the hormone (Pampori and Shapiro, 1996, 1999; Agrawal and Shapiro, 2000). Since the therapeutic intent of octreotide administration is to simply reduce elevated plasma concentrations of growth hormone to near normal without altering the characteristic profile, we measured expression levels of key CYP isoforms as well as IGF-1 and growth hormone receptor as indicators or markers of alterations, possibly subtle, but physiologically significant, in the feminine growth hormone profile resulting from octreotide infusion.

2. Materials and methods

2.1. Animals

Female Sprague-Dawley CD rats obtained from Charles River Laboratories (Wilmington, MA) were housed in the University of Pennsylvania Laboratory Animal Resources facility under the supervision of certified Laboratory Animal Medicine veterinarians, and were treated according to a research protocol approved by the University's Institutional Animal Care and Use Committee. Rats were housed under conditions of regulated temperature (20–23 °C) and photoperiod (12 h light-dark cycle; lights on at 7:00 AM).

2.2. Catheter implantation and octreotide treatment

Eight female rats at 10 weeks of age were implanted with our patented indwelling right atrial catheters as described previously (Pampori et al., 1991). After 4–5 days, serial blood samples were collected from each rat (40 μl every 15 min for 8 h). Another 4–5 days later, the unrestrained and unstressed catheterized rats were infused, iv, with 25 μg octreotide (Sandostatin)/kg body weight (Bioniche Pharma, Rosemont, IL) over 30 s and the catheter flushed with diluent. Five minutes later, serial blood samples were again collected from each rat. Twelve hours after the first injection of octreotide, a second 25 μg/kg dose was infused. During the next 5 days rats were treated with octreotide at the same dose every 12 h. Five minutes following the 11th dose of octreotide, serial blood samples were again collected. Twelve hours after the previous dose, the rats received their final evening infusion of octreotide. On the following morning (10 h post infusion) the rats were quickly decapitated, the livers quickly excised, weighed, infused with ice cold physiologic saline and minced; a portion reserved for mRNA determinations was plunged into liquid nitrogen and subsequently stored at −80 °C. The remaining minced liver was used for microsome preparation. In an additional experimental group, 7 females (10 weeks of age) were implanted with indwelling right atrial catheters and treated with an equivalent amount of diluent (95 μl/kg body weight of physiological saline/infusion) in the same manner as octreotide for 12 infusions. These rats were decapitated 10 h after the last treatment to serve as controls for the biochemical analyses.

2.3. Growth hormone

With the exceptions of using plasma instead of whole blood and including plasma from long-term hypophysectomized female rats as controls, circulating growth hormone was measured by a sensitive sandwich ELISA using the same materials and methods as reported by Steyn et al., 2011 and modified by us (Das et al., 2013).

2.4. RNA isolation

Total RNA from liver tissue was isolated using Trizol® reagent (Life Technologies, Carlsbad, CA) purified with the Qiagen RNeasy mini kit and treated with DNase I in order to remove any trace of genomic DNA using RNase-Free DNase Set (Qiagen, Valencia, CA) according to the manufacturer's protocol. RNA concentrations and purity were determined by UV spectrophotometry (A260/280 > 1.8 & A260/230 > 1.7) and integrity was verified by the intensities of 28S and 18S rRNA bands on a denaturing agarose gel visualized on a FluorChem IS-8800 Imager (Alpha Innotech, San Leandro, CA).

2.5. Northern blots

Northern blots were probed with a $\gamma^{[32P]}$ labeled oligonucleotide probe for CYP2C11 and CYP2C12 mRNA (Waxman, 1991) using generally followed procedures including hybridization and high stringency washing conditions as we described previously (Pampori and Shapiro, 1996). The consistency of RNA loading between samples was confirmed by ethidium bromide staining of 18S and 28S ribosomal RNAs and were verified and normalized with an 18S oligonucleotide probe (Li et al., 2000). The hybridized mRNA signals were quantified by scanning the autoradiographs with a FluorChem IS-8800 Imager (Alpha Innotech).

2.6. Quantitative reverse transcription-PCR (qRT-PCR)

CYP2C12, CYP2C11, CYP2A1, CYP3A2, CYP2C6, CYP2C7 and rat growth hormone receptor gene expressions were determined by qRT-PCR using SYBR green on an Applied Biosystem 7500 Fast Real-Time PCR System (Life Technologies). RNA isolation, concentration and purity determination were performed as mentioned above. cDNA synthesis was completed using the High Capacity RNA-to-cDNA kit (Life Technologies) as per instructions with appropriate no-reverse transcription (−RT) and non-template controls. PCR primers for CYP2C12 and CYP2C11 (Ahluwalia et al., 2004), CYP3A2 (Kisanga et al., 2005), CYP2A1 (He et al., 2007), CYP2C6 (F:5′-TCAGCAGGAAAACGGATGTG-3′, R:5′-AATCGTGGTCAGGAATAAAAATAACTC-3′), CYP2C7 (Choi et al., 2011) and β-actin (F:5′-CACGGCATTGTCACCAACTG-3′, R:5′-CTGGGTCATCTTTTCACGGT-3′) were synthesized by Integrated DNA Technologies (Coralville, IA) whereas primers for growth hormone receptor (F:5′-TGATGCGGATGAGAAGACTG-3′, R:5′-AAGTACCATCGCACATGTCA-3′) were synthesized by Real Time Primers (Elkins Park, PA). To analyze IGF-1 mRNA expression, an IGF-1 TaqMan® assay (Rn00710306_m1) was performed using β-actin (Rn00667869_m1) as the housekeeping gene on an Applied Biosystem step-one plus q-PCR instrument as per the manufacturer's recommended protocol (Life Technologies).

2.7. Western blotting

As previously described, hepatic microsomes were prepared from freshly isolated rat livers (Shapiro et al., 1989) and assayed for individual CYP isoforms by western blotting (Pampori et al., 1995). The blots were probed with monoclonal anti-rat CYP2C11 and anti-rat CYP3A2 (Detroit R&D, Inc., Franklin, MI), anti-rat CYP2C12 (a gift from Dr. Marika Rönnholm, Huddinge University Hospital, Huddinge, Sweden), monoclonal anti-rat CYP2C6 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and anti-growth hormone receptor (a gift from Dr. G. Peter Frick, University of Massachusetts Medical School, Worchester, MA) and detected with an enhanced chemiluminescence kit (Amersham Biosciences Corp, Piscataway, NJ). Signals were normalized to a control sample repeatedly run on all blots and/or to the expression of β-actin (Sigma Chemical Co., St. Louis, MO). The protein signals were scanned and the densitometric units were obtained as integrated density values quantitated by using FluorChem IS-8800 Imager (Alpha Innotech) software supplied with the gel documentation system.

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