Cellular and Molecular Neurobiology (2019) 39:169–180
https://doi.org/10.1007/s10571-019-00652-0

REVIEW PAPER

Brain Control of Sexually Dimorphic Liver Function and Disease: The Endocrine Connection

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Received: 3 October 2018 / Accepted: 10 January 2019 / Published online: 17 January 2019

Abstract
A multistep signaling cascade originates in brain centers that regulate hypothalamic growth hormone-releasing hormone (*Ghrh*) and somatostatin expression levels and release to control the pattern of GH secretion. This process is sexually fine-tuned, and relays important information to the liver where GH receptors can be found. The temporal pattern of pituitary GH secretion, which is sex-specific in many species (episodic in males and more stable in females), represents a major component in establishing and maintaining the sexual dimorphism of hepatic gene transcription. The liver is sexually dimorphic exhibiting major differences in the profile of more than 1000 liver genes related to steroid, lipid, and foreign compound metabolism. Approximately, 90% of these sex-specific liver genes were shown to be primarily dependent on sexually dimorphic GH secretory patterns. This proposes an interesting scenario in which the central nervous system, indirectly setting GH profiles through GHRH and somatostatin control, regulates sexual dimorphism of liver activity in accordance with the need for sex-specific steroid metabolism and performance. We describe the influence of the loss of sexual dimorphism in liver gene expression due to altered brain function. Among other many factors, abnormal brain sexual differentiation, xenoestrogen exposure and D2R ablation from neurons dysregulate the GHRH–GH axis, and ultimately modify the liver capacity for adaptive mechanisms. We, therefore, propose that an inefficient brain control of the endocrine growth axis may underlie alterations in several metabolic processes through an indirect influence of sexual dimorphism of liver genes.

Keywords GH · GHRH · Dopamine receptor; Hypothalamus · Xenoestrogens · Liver · Gene dimorphism · Growth hormone

The GHRH–GH-Liver Axis

Adaptation to limited resources, such as food, sexual mates and valuable territories is a primary driving force in evolution. To this end, a complex array of brain and peripheral processes has been selected for millions of years in mammals. One main player is the brain-pituitary growth hormone (GH) axis which establishes social rank dominance by determining body size, muscle strength, and aggression. It also initiates a neuroendocrine/exocrine cascade which dictates optimum liver performance to assure metabolic and behavioral advantages in a sexually dimorphic manner.

The brain orchestrates a multistep signaling cascade that regulates hypothalamic growth hormone-releasing hormone (*Ghrh*) and somatostatin expression and release, to control the pattern of GH secretion. This process is sexually fine-tuned, and relays important information to the liver where GH receptors can be found. The temporal pattern of pituitary GH secretion, which is sex-specific in many species (episodic in males and more stable in females), is a key component in establishing and maintaining the sexual dimorphism of hepatic gene transcription (Jaffe et al. 2002; Waxman and O'Connor 2006). Major differences in steroid, lipid, and foreign compound metabolism are found in the liver (Roy and Chatterjee 1983; Vijayakumar et al. 2011) linked to the sexual dimorphic gene expression for more

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than 1000 liver genes, 90% of which were shown to be under pituitary control, primarily dependent on sexually dimorphic GH secretory patterns (Fig. 1).

This sexual dimorphic expression of liver genes agrees with the sex-dependent physiological requirements for steroid metabolism (Jaffe et al. 2002; Roy and Chatterjee 1983; Waxman and O'Connor 2006). For example, during pregnancy or the estral cycle in females the liver is exposed to changing levels of steroid hormones, and therefore, sex-specific hepatic steroid metabolism is needed (Waxman and O'Connor 2006). Accordingly, many enzymes such as steroid hydroxylases belonging to the cytochrome P450 superfamily (CYP), are expressed in the liver in unique, sexually biased patterns (Thangavel et al. 2004; Waxman and Holloway 2009). However, the physiological significance of GH-dependent liver genes goes far beyond the need for a differential steroid metabolism. They participate in sexually dimorphic behaviors such as social dominance and aggression, in glucose metabolism, alcohol preference, and may even be involved in the differential susceptibility to some liver diseases. For example, chronic hepatitis, primary sclerosing cholangitis and hepatocellular carcinoma are predominant in males, while primary biliary cirrhosis, autoimmune hepatitis or alcoholic liver disease in females (Durazzo et al. 2014); Rogers et al. 2007). Furthermore, sex differences in drug pharmacokinetics have been repeatedly described in humans (Anderson 2005; Jaffe et al. 2002; Meibohm et al. 2002).

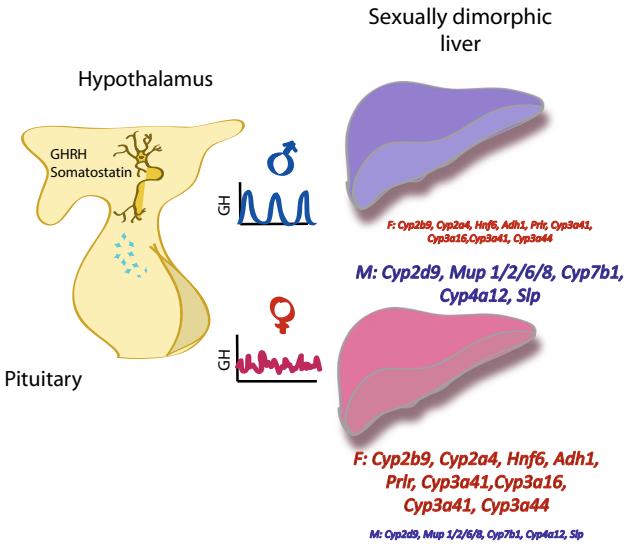

Fig. 1 Sexual dimorphism of the GHRH–GH-liver axis. A multistep signaling cascade originates in brain centers that regulate hypothalamic growth hormone-releasing hormone (GHRH) and somatostatin expression levels and release, which then relay in the pituitary to control GH pulsatile secretion. The temporal pattern of pituitary GH secretion is sex-specific in many species (episodic in males and more stable in females) and represents a major component in establishing and maintaining the sexual dimorphism of hepatic gene transcription. GH impacts on the liver and establishes a dimorphic expression of female predominant (F) or male predominant (M) genes which convey to male and female livers a sex-related GH-dependent signature. Livers from males (upper) have higher levels of M genes, while female livers have enhanced F genes

This proposes an interesting scenario in which the central nervous system, indirectly setting GH profiles through GHRH and somatostatin control, regulates sex-dependent liver activity in accordance with brain sex differences and the need for sex-specific steroid metabolism and performance. Sex-specific gene expression in the liver extends to large numbers of transcription factors, receptors, signaling molecules, and enzymes which may contribute as mediators of a wide range of biological processes including lipid metabolism, behavior, and inflammation (Noain et al. 2013; Waxman and Holloway 2009; Waxman and O'Connor 2006).

Where do these sexual differences originate? It all begins in the brain, where hypothalamic GHRH and somatostatin neurons are regulated in a sex-specific dynamic process.

Brain Control of GHRH and Somatostatin Neurons

Many hormones, neuropeptides, growth factors, and cytokines regulate the somatotrope axis acting at hypothalamic, pituitary or peripheral levels.

A sexually differentiated secretory pattern is the cornerstone of GH secretion in many species including rats, mice and humans (Muller et al. 1999; Wehrenberg and Giustina 2000). In males, GH secretion is characterized by high-amplitude and low-frequency pulses occurring between long nadir periods, which allow the resensitization of GH receptor signaling. These long interpulse nadirs of GH levels in males, induce high transcriptional rates of genes that promote body growth (Low et al. 2001). In females, on the other hand frequent and overlapping plasma GH peaks occur and GH levels are more constant in circulation (MacLeod et al. 1991; Steyn et al. 2016; Wehrenberg and Giustina 1992).

Pulsatile GH secretion is primarily regulated by synchronic but not overlapping secretion of two neuroendocrine hormones from the hypothalamus, GHRH and somatostatin that stimulate and repress GH secretion, respectively (Steyn et al. 2016). Such exquisite and timely regulation is the result of a complex and dynamic network of neurotransmitters and peptides which control GHRH and somatostatin synthesis and release, including GHRH and somatostatin themselves. GHRH is mainly expressed in the arcuate nucleus of the hypothalamus, as well as in anterior hypothalamic region and dorsomedial and ventromedial nuclei (Wehrenberg and Giustina 2000). GHRH neurons receive afferents from somatostatin neurons

which participate in the GHRH–somatostatin interplay that closely directs GH pulsatility. GH autofeedback is involved partly in suppression of GHRH expression indirectly through an increase in somatostatin. A greater complexity arises since hypothalamic GHRH and somatostatin can each regulate its own secretion and reciprocally control the secretion of its counterpart (Giustina and Veldhuis 1998) (Fig. 2).

GHRH and somatostatin receive afferents and are neuromodulated by a vast array of neurotransmitters (Fig. 2).

Dopamine

The role of dopamine on GH regulation is still on debate. Inhibitory as well as stimulatory effects on plasma levels of GH have been reported in vivo depending on experimental conditions (Kitajima et al. 1989; Muller et al. 1999). Dopamine receptors can stimulate GH, if other neural inhibitory inputs to the pituitary are removed. In vitro, dopamine stimulation and inhibition of GH have been described at pituitary and hypothalamic levels (Bluet-Pajot et al. 1990; Jin and Hashizume 2015; Muller et al. 1999).

Nevertheless, consistent with a mandatory role of dopamine on GHRH release dopamine receptor type 2 (D2R) knockout mice ($Drd2^{-/-}$) are growth retarded evidencing an alteration in the GHRH–GH–IGF–I axis (Diaz-Torga et al. 2002).

GH-deficient children undergo an increase in their growth rate after L-DOPA treatment (Huseman and Hassing 1984). And remarkably, high frequencies of the A1 allele of the D2R were found in a group of children with idiopathic short stature. This polymorphism of the D2R was associated to impaired growth (Miyake et al. 1999), mild GH deficiency, decreased nocturnal GH secretion, slightly retarded bone maturation, and low IGF-I levels.

Noradrenalin/Adrenalin

Alpha adrenergic neurons stimulate GH secretion by increasing GHRH release and inhibiting the somatostatin tone, while beta-adrenergic neurons increase somatostatin and have the opposite effect, (Chihara et al. 1984; Lim and Khoo 2000; Massara and Camanni 1972). Clonidin, and alpha2-adrenergic agent has been, therefore, used as a provocative GH-releasing test.

Acetyl Choline

Cholinergic muscarinic pathways stimulate GH secretion, probably interacting with somatostatin. In this regard, pyridostigmine, an indirect agonist which blocks acetyl cholinesterase action, increases GH secretion (Ghigo et al. 1998), while muscarinic antagonists prevent sleep or GHRH-induced GH release.

Opioids

Both endorphins and enkephalins stimulate GH in humans, while opiate antagonists may attenuate exercise-induced GH response. Their effect was attributed to GHRH release, and a possible interaction with the GH secretagogue receptor. (Muller et al. 1999).

Serotonin

Brain serotonin pathways facilitate GH release in rodents, probably inducing GHRH release (Giustina and Veldhuis 1998).

GABA

GABA-A and GABA-B agonists also facilitate GH secretion in rats, sheep and humans, by interacting with somatostatin,

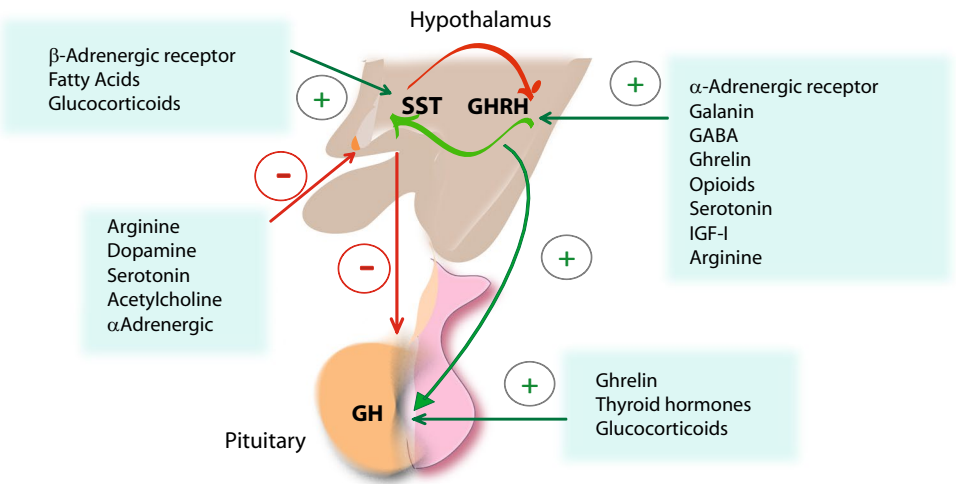

Fig. 2 Brain regulation of hypothalamic somatostatin (SST) and GHRH expression. Green lines (+) indicate a positive action and red lines (−) indicate an inhibitory action

galanin, serotonin and/or benzodiazepine receptors. Several clinical conditions associated to excessive (acromegaly or diabetes) or decreased (old age) GH secretion present a low GH response to GABAergic agonists. Nevertheless, an inhibitory action at the pituitary has also been forwarded (Powers 2012).

Other Neurotransmitters, Steroids and Peptides

The participation of several other neurotransmitters, steroids and peptides modifying GHRH–somatostatin release has been proposed (Fig. 2).

Galanin causes stimulation of GH secretion when infused alone and also enhances GHRH-stimulated GH secretion. L-arginine stimulates GH and can be used as a provocative test for GH secretion. Glucocorticoid treatment has a biphasic effect on GH secretion: an initial acute stimulation, followed by suppression. The latter is the clinically important effect, as excess endogenous and exogenous glucocorticoids are well known to suppress growth in children. Glucocorticoid inhibition of GH involves modulation of hypothalamic somatostatin, and a stimulatory effect has been proposed by direct actions on the pituitary (Giustina and Wehrenberg 1992). Ghrelin is an orexigenic peptide, initially isolated from stomach that can stimulate the release of GH (Ghigo et al. 2005). IGF-1 which is stimulated by GH can impact hypothalamic neurons during development regulating axonal outgrowth of GHRH neurons, and is therefore involved in adequate growth program (Decourtye et al. 2017).

Impact of Neonatal Testosterone Treatment on Sexual Differentiation of the GHRH–GH Axis

Sexually dimorphic plasma GH profiles that originate in the brain, are first evidenced at puberty, and are generated by neonatal gonadal steroids which imprint brain centers controlling the GH axis (Jansson and Frohman 1987). The effects of abnormal neonatal exposure to androgens, estrogens or endocrine disruptors on reproduction and sexual behavior have been extensively studied (Becu-Villalobos et al. 1997; Becu-Villalobos and Libertun 1995; Colciago et al. 2006; Dorner 1981; de Mengido et al. 1987; Monje et al. 2007; Wilson and Davies 2007; Zama and Uzumcu 2010). However, the impact of neonatal steroid exposure on the GH axis, as well as the underlying sexual differences in hepatic enzymes, have not received similar attention (Ramirez et al. 2010, 2012, 2014). Different brain perturbations during fetal and postnatal development may unleash endocrine adaptations that permanently alter metabolism, and ultimately increase the susceptibility to develop a metabolic disease; and GH action at the liver may be involved.

Sex-specific organization of the developing central nervous system occurs in restricted or critical periods of neural differentiation (Arnold and Gorski 1984; Becu-Villalobos et al. 1997). In newborn males, testosterone is aromatized to estradiol, which ultimately masculinizes the developing central nervous system, whereas a female brain develops largely in the absence of estradiol (Becu-Villalobos et al. 1997; Dorner 1981). In this process, minimum estrogen levels are also needed for female brain imprinting, and the testosterone product, dihydroxytestosterone (DHT) may also impact in male differentiation (Negri-Cesi et al. 2008). As a result, a broad spectrum of brain functions develops in accordance with sex physiology, assuring behavioral and neuroendocrine responses in males and females and ultimately reproductive success. In this respect, female rodents treated neonatally with testosterone propionate (TP) have impaired cyclic gonadotropin release, inappropriate gonadotropin response to the removal or imposition of negative feedback signals, and altered gonadotropin secretion during development (Becu-Villalobos et al. 1997). Therefore, abnormal developmental exposure to steroid hormones or endocrine disrupting chemicals during this critical period may result in anomalies in fitness, metabolism and reproduction (Fernandez et al. 2009; Gore 2008).

Neonatal steroids also impact on the sexually differentiated GHRH–GH axis. Administration of testosterone to female newborn mice (TP females) has an organizational effect on hypothalamic *Ghrh* and not on somatostatin mRNA levels. Increased *Ghrh* mRNA expression in TP females may bring about the higher somatotrope population found in this group compared to control females, and ultimately, the increase in serum and liver IGF-I and body weight (Ramirez et al. 2010). Similar observations were made in rats. Testosterone administered to neonatal female rats increased GHRH neurons in adulthood independently of mean *Ghrh* mRNA levels in those neurons. Female TP rats had also increased *Ghrh* mRNA sensitivity to testosterone during the pubertal spurt; and furthermore neonatal testosterone treatment resulted in significantly higher levels of Somatostatin mRNA in the adult animal (Chowen et al. 2004; Wehrenberg and Giustina 2000). Hence, neonatal sex steroid administration may partially determine the ability of the hypothalamus and pituitary gland to secrete GHRH and GH throughout life.

This altered GH secretion has profound effects on liver gene dimorphism. In TP females there were long lasting and divergent effects for different GH-dependent and sex-specific liver genes. Female-predominant genes (*Cyp2b9, Cyp2a4, Adh1,Hnf6, Prlr, and Cyp3a41*) were downregulated, while no masculinization of male-predominant genes (*Cyp2d9, Mup 1/2/6/8, Cyp7b1, Cyp4a12, Slp*) or protein expression were found (Ramirez et al. 2010) (Fig. 3). These results indicate an impact of early brain organization on sexual

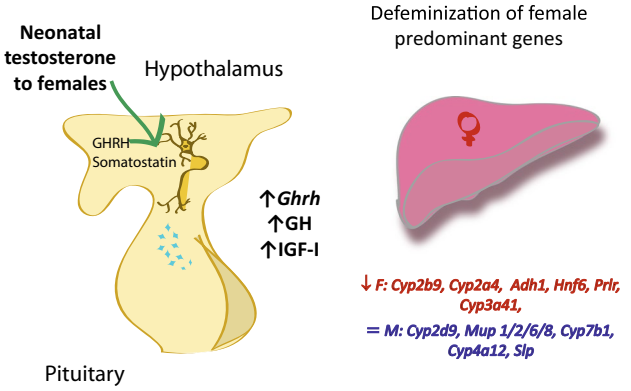

Fig. 3 Neonatal testosterone administration to females defeminizes liver gene expression in adult mice, and consequently sexual dimorphism is lost. ↑arrows indicate increase in expression or protein levels, ↓arrow indicates decrease, and =symbol indicates no change

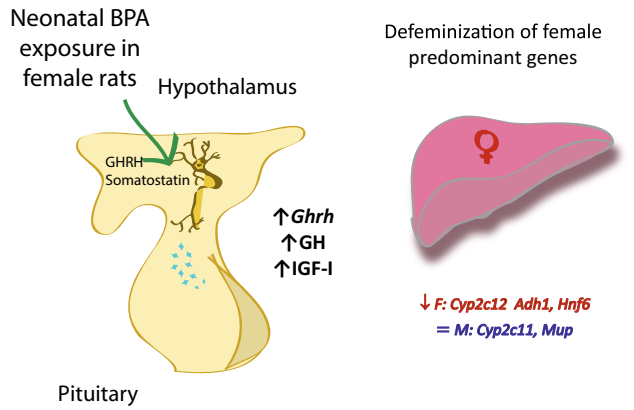

Fig. 4 Neonatal bisphenol A (BPA) exposure in females defeminizes liver gene expression. Liver sexual dimorphism is lost. ↑ arrows indicate increase in expression or protein levels, ↓arrow indicates decrease, and = symbol indicates no change

dimorphism of liver genes which should be considered when analyzing deleterious effects of endocrine disruptors.

To this respect, a great public concern is that certain industrial chemical substances released into our environment may impact on the developing brain (Diamanti-Kandarakis et al. 2009). For example, bisphenol A (BPA), a constituent of polycarbonate plastics and epoxy resins used in the food industry and in dentistry is a weak estrogen, and could potentially impinge on the sex-specific organization of endocrine sensitive tissues and circuits. It has been shown that neonatal BPA exposure interferes with the hypothalamic imprinting of endogenous hormones, permanently altering the reproductive axis in female rats (Fernandez et al. 2009, 2010). However, it is important to bear in mind that the effects of neonatal BPA exposure go beyond reproductive function, and evoke an increase in pituitary GH content and liver IGF-I concentration supporting partial masculinization of the GH axis (Ramirez et al. 2012) in females. These GH changes produced a defeminization in mRNA expression of GH-dependent female predominant liver genes (*Cyp2c12, Adh1, and Hnf6*) and no changes in male predominant liver genes (*Cyp2c11*) or proteins (MUP) (Ramirez et al. 2012) (Fig. 4), in concordance with liver gene defeminization obtained by neonatal testosterone administration in mice (Fig. 3). Therefore, early exposure of females to endocrine-disrupting chemicals may impact on drug and foreign compound metabolism.

Epigenetic Regulation of Sexual Dimorphism of Liver Genes

Epigenetics is an emerging area which explains changes in gene expression or function which are not related to DNA base mutations. Permanent changes such as those established by hormones in the developing brain may involve epigenetic modification of DNA, such as DNA methylation, histone modifications, or microRNA (miRNA) abundance. These epigenetic marks may modify permanently sex differences in hepatic gene expression (Lenz et al. 2012; Nugent and McCarthy 2011).

In this context, it can be suggested that lack of masculinization of male genes in females treated with testosterone at birth may be related to an incomplete induction of male episodic GH secretion, or to epigenetic factors, such as methylation of gene promoters, chromatin accessibility, or modifications of histones.

Changes in chromatin structure and accessibility are a hallmark of epigenetic regulation and can be probed by global analysis of open chromatin sites by DNase I digestion followed by high-throughput sequencing (DNase sequencing). The liver epigenome is characterized by GH-dependent sex differences in open chromatin regions (DHS) and states (Ling et al. 2010), as well as sex differences in local chromatin marks at some sex-biased genes (Sugathan and Waxman 2013). Accessibility of the chromatin may be important in dictating sex differences of liver gene expression. Importantly, chromatin accessibility is a plastic process that can be modified by GH secretory patterns so that liver genes are expressed as needed by sex.

For example, continuous GH infusion resembling a feminine pattern of GH secretion closes many male-biased DHS and opens female-biased DHS in male liver (Ling et al. 2010). As a result, several male-biased genes are downregulated while female-biased genes are upregulated (Holloway et al. 2006). Concurrently, histone modifications accompany chromatin accessibility in response to continuous GH, with a decrease in histone repressing marks in female genes, and an increase in repressing marks in male genes (Lau-Corona et al. 2017).

On the other side, pulsatile GH administration to hypophysectomized males produces a rapid increase in liver chromatin accessibility at discrete localized regulatory sites, and transcriptional activity for some, but not all, liver STAT5-dependent male-biased genes, indicating a complex and gene-specific epigenetic regulatory process (Connerney et al. 2017).

Compelling evidence also suggests that miRNAs participate in sex-biased expression of liver genes. miRNAs mostly repress translation by targeting mRNAs for degradation or by silencing translation. Even though they often impart modest effects on target mRNAs, they can simultaneously regulate a subset of genes, conferring an integrated response in sex-biased transcriptional networks. The participation of miRNAs in TP-treated females has not been documented yet but RNA sequencing revealed 24 sex-biased mouse liver miRNAs. Two of them, miR-1948 and miR-802, were validated as functional components of the GH regulatory network that shapes sex-differential gene expression in mouse liver (Hao and Waxman 2018).

Methylation of CpG sites in DNA sequences, and especially in CpG-rich areas (termed CpG islands), is a modification that can be duplicated across cellular division, and therefore, maintained throughout a lifespan. DNA methylation may evoke a decrease in gene expression by several mechanisms which involve a blockade of the transcriptional machinery from accessing start sites on a gene's promoter (Garcia-Carpizo et al. 2011). In general, methylation of a promoter is inversely associated with gene expression.

Chromatin accessibility, or MiRNA abundance has not been studied in TP females, but a role for methylation in the regulation of sex-specific liver gene expression was suggested. Promoter methylation of *Cyp7b1* and *Hnf6* liver genes in adult mice predicted sexual differences of their mRNA expression: in females higher methylation of the *Cyp7b1* promoter was found, and lower methylation of the *Hnf6* promoter compared to males, consistent with male and female predominance previously described for these genes (Ramirez et al. 2014). Neonatal imprinting of the brain by testosterone increased *Hnf6* promoter methylation in females, and therefore, in the adult liver this transcription factor was defeminized or decreased (Ramirez et al. 2014). On the other hand, high methylation of the *Cyp7b1* promoter remained unchanged in TP females, and, in accordance gene expression did not increase in this group. These results point to DNA methylation participation in the imprinting by neonatal steroids of sex-dependent liver gene expression.

Dopaminergic Regulation of GHRH–GH Liver Axis

An illustrative example of the integrated axis which starts in the brain and impacts in liver gene expression is the dopaminergic regulation of GHRH–GH liver axis.

The D2R orchestrates key roles in adaptive functions that improve fitness, reproduction, and survival. D2Rs in the nucleus accumbens and striatum participate in neural circuits related to food intake and reproduction (Berridge and Robinson 1998; Kelley and Berridge, 2002; Palmiter 2008). D2Rs are also fundamental in motor coordination, locomotion, executive planning, in the establishment of social dominance in males, and hormone regulation (Morgan et al. 2002).

In this context, D2R regulation of GHRH and its indirect impact on sexually dimorphic liver signature propose two additional adaptive properties of central D2Rs: (a) D2R regulation of high-pulsatile levels of GH in adult male mice reinforces increased body growth by differential regulation of liver proteins involved in the process and, in parallel, (b) D2Rs indirectly modify the excretion of GH-dependent liver major urinary proteins (MUPs) in male urine modifying a behavioral cue to territory protection.

Several studies demonstrate that pharmacological or surgical intervention of central dopaminergic pathways may impact on liver gene expresion. For example, after lesioning the tuberoinfundibular dopaminergic pathway in male rats a decrease in the activity and protein levels of liver CYP2B, CYP2C11 and an increase in CYP1A were found, and after lesioning the tuberoinfundibular or mesolimbic dopaminergic pathways an increase in liver CYP3A was evidenced (Wojcikowski et al. 2007). Furthermore, neuroleptics, such as sulpiride or remoxipride, which are D2R antagonists, downregulated liver CYP2C11 and CYP3A protein or mRNA expression (Rane et al. 1996), as well as constitutive and benzopyrene-induced *CYP1A1, CYP1A2* and *CYP1B1* expression in male rats (Harkitis et al. 2015). Because these enzymes play a crucial role in the metabolism and toxicity of environmental toxicants, dopamine antagonists can be envisioned as beneficial strategies in reducing the risk of toxicity to envorinomental polutants or pre-carcinogens (Harkitis et al. 2015). Nevertheless, many of these enzymes are not sexually dimorphic (Wauthier and Waxman 2008), and a hallmark of liver gene expression is its sexual dimorphism. To this regard, experiments with male and female mice with global or selective neuron disruption of D2Rs unequivocally demonstrated a role for sexually dimorphic impact of central D2Rs on liver gene expression.

Evidence that D2Rs regulate a neuroendocrine/exocrine cascade, which controls body growth, liver gene

expression, and male-to-male territorial behavior was obtained by analysis of *Drd2*$^{-/-}$ mice and complemented by specific ablation of D2Rs in neurons. *Drd2*$^{-/-}$ mice revealed the unexpected importance of D2Rs in the regulation of the GHRH–GH axis and body size in males (Diaz-Torga et al. 2002). *Drd2*$^{-/-}$ mice had lactotrope hyperplasia, chronically elevated prolactin levels and impaired reproductive capacity, and strikingly, they had a lower number of pituitary somatotropes, lower response to GHRH (Garcia-Tornadu et al. 2006), reduced GH and IGF-I serum levels, and were dwarfs (Diaz-Torga et al. 2002). In addition, transgenic mice expressing *Cre* from a rat nestin promoter, Tg(Nes-cre)1Kln/J (Tronche et al. 1999; Zimmerman et al. 1994) were used to eliminate D2Rs selectively from cells of neural origin (neuroDrd2KO), and these mice showed that lack of central D2Rs impaired growth hormone-releasing hormone (GHRH) expression, which ultimately accounted for a somatotrope shortfall, decreased IGF-I levels, and dwarfism (Noain et al. 2013). On the other hand, prolactin secretion and breeding issues were normal in neuroDrd2KO mice (Ramirez et al. 2015).

D2Rs mainly couple to inhibitory signals, therefore, inhibition of periventricular somatostatin neurons by D2Rs would modify GHRH expression and release in the arcuate nucleus (McCarthy et al. 1992; Muller et al. 1999). These neurons are involved in the rhythmic GHRH neuronal firing that establish the male pattern of GH release (Low et al. 2001; Muller et al. 1999), and influence serum levels of IGF-I that determine body size and weight, indirectly impacting on reproductive fitness and competitiveness among males.

Deficits in the male pattern of GH release are associated with feminization of sexually dimorphic liver proteins (Oshida et al. 2016b; Wauthier et al. 2010; Wauthier and Waxman 2008), and therefore, the study of several liver genes in this model were illustrative to explain mechanisms of masculine adaptation which involve D2Rs. GH-dependent sexually dimorphic liver proteins are manifold and include MUPs (McIntosh and Bishop 1989;Udy et al. 1997), a group of pheromones excreted at high levels in the urine and which are used in male-to-male social interactions (Chamero et al. 2007; Hurst and Beynon 2004). Male mice use MUPs to scent-mark and countermark territories (Hurst and Beynon 2004), thus generating a chemical barrier for territorial defense and protection to gain access to receptive females.

Liver *Mup* genes were drastically downregulated in neuroDrd2KO males, and sexual dimorphism was lost for this gene. As a consequence, their urine showed a lower content of MUP and, therefore, males lost a signaling cue related to territorial defense (Hnasko et al. 2006; Noain et al. 2013). These findings revealed a connection between central D2Rs and the neuroendocrine–exocrine cascade that impacts on social dominance and male to male competition.

Disruption of neuronal D2Rs also impaired liver sexual dimorphism for several other genes (Ramirez et al. 2015). Male-predominant genes mostly decreased in global *Drd2*$^{-/-}$ and in neuroDrd2KO mice. *Cyp2d9* was decreased in male and *Cyp7b1* mRNA in both sexes in neuroDrd2KO mice, and as a result, sexual dimorphism of the male predominant liver genes was lost (*Cyp7b1, Mup1/2/6/8* and *Mup1*) or greatly decreased (*Cyp2d9*). mRNA expression levels of three female predominant genes, *Cyp3a16, Cyp3a44 and Cyp3a41*, decreased in females and was unaltered in males; and mRNA expression of other four female predominant genes, *Cyp2a4, Cyp2b9, Adh1 and Prlr*, increased in males, and was unaltered in females; with the consequent loss of sexual dimorphism for the seven genes (Fig. 5).

Therefore, drugs affecting brain D2Rs might modify sexually dimorphic gene expression and CYP enzyme activity in the liver, establishing a little-explored link between the central nervous system and liver gene expression.

Many psychotropic drugs prescribed in a variety of diseases such as Parkinson's disease, bipolar disorder, schizophrenia and depression, act on D2Rs and their signaling pathways (Bonci and Hopf 2005). As described, D2Rs regulate the GHRH–GH axis, therefore, these drugs may eventually modify the liver expression and sexual dimorphism of various drug-metabolizing CYP enzymes, as well as of other liver genes, compromising the clearance and/or toxicity of drugs and xenobiotics.

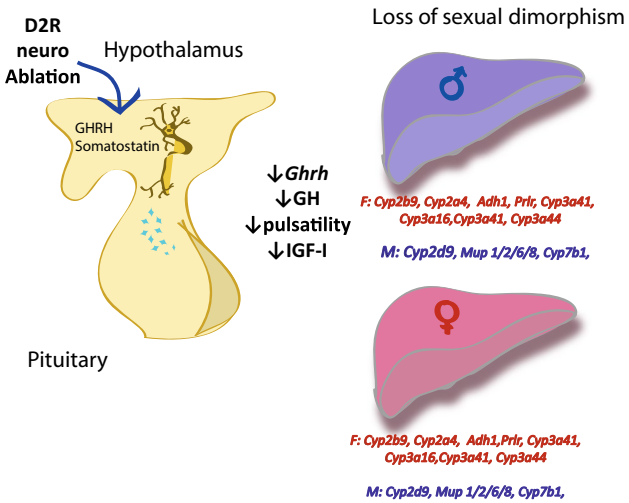

Fig. 5 Disruption of dopamine D2 receptor (D2R) from neurons produces dwarf mice. Hypothalamic *Ghrh* mRNA levels decrease, GH pulsatility is lost in males and lower levels of serum IGF-I are found. Most male predominant genes (M) decrease in male livers, while female predominant (F) genes decrease in female livers. As a consequence liver sexual dimorphism is lost

Other Factors that Perturb the Hypothalamo-Pituitary-Liver GH Axis and Feminize Gene Expression in Male Livers

Even though our first examples dealt with the defeminization of female predominant genes, livers of male mice appear to be more susceptible to feminization. Computational methods using microarray-derived gene lists were developed to evaluate the effects of diverse interventions, mutations, chemicals and hormones on GH signaling in mouse liver (Oshida et al. 2016b, a).

Compounds such as glucocorticoids, FGF15, xenobiotics, and angiotensin II; metabolic conditions such as diabetes, obesity, high-fat diet; hypophysectomy, and old age feminize male livers. Furthermore, mutants in which GH secretion or action is impaired undergo liver feminization (*Ghr−/−*, *Ghrhr−/−*, *Prop1−/−*, *Ar−/−*, *Stat5b−/−*, *Hnf4a−/−* (Amador-Noguez et al. 2005; Clodfelter et al. 2006; Holloway et al. 2006, 2008; Oshida et al. 2016b, a)).

In summary, GH-activated signaling in mouse liver may be altered by diverse chemicals, hormones or stressors (Fig. 6). This evokes dysregulated gene expression and loss of sexual dimorphism, which may be ultimately associated with adverse effects on the liver and metabolism as we expand in the next section.

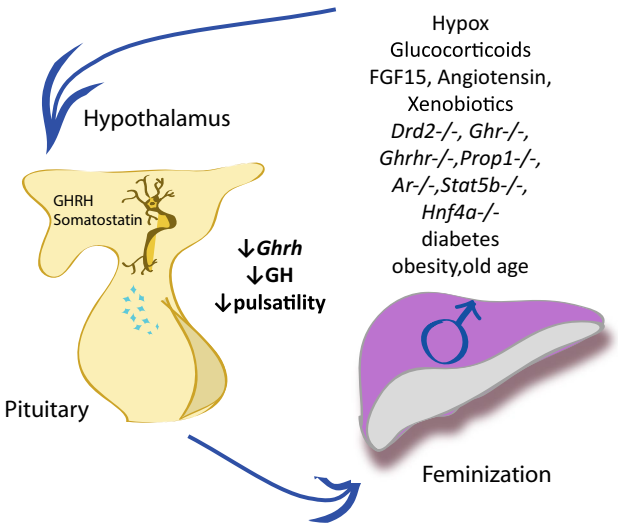

Fig. 6 Effects of gene mutations, chemicals, physiological and pathological states and hormones on feminization of male predominant genes. Compiled from (Amador-Noguez et al. 2005; Holloway et al. 2008, 2006; Oshida et al. 2016b, a; Ramirez et al. 2015; Waalkes et al. 2004; Clodfelter et al. 2006)

Defective Sexual Dimorphism of GHRH–GH Function and Impairment of Liver Performance

Sexually dimorphic GH release patterns are dependent on interactions between somatostatin and GHRH input. While GHRH stimulates GH secretion, somatostatin has the opposite effect, but diverse feedback mechanisms can modulate the action of the hypothalamic factors (Steyn et al. 2016). There is an ultrashort feedback by which somatostatin and GH can modulate their own release, and a short feedback by which GH can stimulate somatostatin and inhibit GHRH release in the median eminence, Finally several peripheral and neuronal inputs can impinge on GHRH and somatostatin release (Fig. 2) (Steyn et al. 2016). Alterations of this axis at diverse levels have profound effects on sexual liver dimorphism. Loss of sexual dimorphism in liver gene expression in general (Oshida et al. 2016b), and as described in altered brain sexual differentiation, xenoestrogen exposure or D2R ablation from neurons, may underlie alterations in several metabolic processes. These are very prominent in rodents but have also been demonstrated in humans, in whom they may be overlooked by the heterogeneous backgrounds of human population.

Thrombotic Processes

For example, male sex is an independent risk factor for thrombotic processes such as myocardial infarction, venous thromboembolism, or thrombotic stroke (Kyrle et al. 2004; McRae et al. 2006). Studies using the GH-deficient *little* mice suggest that GH secretory patterns participate in establishing this sexual difference. The *little* male mouse is protected from thrombosis, while administration of pulsatile GH worsened the clotting phenotype in male *little* mice and in control female mice. These changes were associated to alterations of liver sexual dimorphic expression of coagulation inhibitors *Proc, Serpinc1, Serpind1 and Serpin5*, which were accordingly modulated by sex-specific GH patterns (Wong et al. 2008). Therefore, susceptibility to clotting-related diseases may depend on sexual dimorphic gene expression dictated by GH secretory patterns.

Liver Cancer

Hepatocellular carcinoma is a male-predominant cancer, associated with chronic hepatitis. In male mice, hepatocellular carcinoma and tumorigenic hepatitis were strongly associated with liver-sex disruption, defined as the loss of a gender-identifying hepatic molecular signature (Rogers et al. 2007). Deranged hepatocellular metabolism evoked by loss

of liver gene sexual dimorphism may underlie the development of hepatocellular carcinoma. Liver-sex disruption was described by microarray in mice with induced tumorigenic hepatitis, and most of altered genes were GH-dependent. A diseased male liver may accumulate altered pools of cholesterol, bile acids, and long chain fatty acids, resulting in the use of alternative pathways, oxidative damage, and inflammation (Anderson et al. 2004). Therefore, it is plausible that maintenance of sexual dimorphic liver gene expression is needed for the normal sex-dependent physiological requirements of a healthy liver.

Steatosis and Obesity

GH deficiency is clinically associated with a high incidence of non-alcoholic steatohepatitis (NASH), which can be reversed by GH administration (Takahashi et al. 2007). Furthermore, a mutation in the *STAT5B* gene in a male patient was associated with striking obesity (Vidarsdottir et al. 2006). Thus, the functional impairment of GH signaling in the liver may derange lipid homeostasis, and be a molecular event that leads to several metabolic diseases including obesity and fatty liver.

Disruption of liver sexual dimorphism has been recorded in the prophet of Pit1 mutated (*prop1*^{df}) male and female dwarf Ames mice. In this model, an almost complete loss of sexual dimorphism was found in the liver. Anomalous GH secretion had profound effects on liver genes involved in fatty acid and steroid hormone metabolism (Amador-Noguez et al. 2005). High-fat diets, obesity, metabolic syndrome, and diabetes were consistently associated with feminization of liver STAT5b function in response to altered GH pulses (Oshida et al. 2016b). Similarly, neonatal exposure to endocrine disruptors (Ramirez et al. 2012) or hazardous and chemical substances (Oshida et al. 2016b; Waalkes et al. 2004) impact on sexual dimorphism of liver gene expression, and may sustain a vulnerable liver.

Alcohol Metabolism

Finally, the loss of sexual dimorphism of liver enzymes and proteins may be relevant in the ability of the liver to respond to diverse challenges. GH sexual dimorphism may participate in altered ethanol metabolism. The liver enzyme alcohol dehydrogenase (ADH) metabolizes the majority of ethanol through ethanol oxidation (Simon et al. 2002). It is a sexually dimorphic GH-dependent enzyme (Potter and Mezey, 2001;Simon et al. 2002), with higher levels of expression and activity in female compared to male rodents (Quintanilla et al. 2007; Simon et al. 2002). Neonatal brain imprinting by testosterone defeminizes this enzyme (Ramirez et al. 2010), and exacerbates alcohol induced liver damage (Ellefson et al. 2011). These changes may also become relevant when considering the predisposition of toxic effects exerted by alcohol which is sex-dependent and related to the activity and expression of ethanol metabolizing enzymes (Gramenzi et al. 2006; Quintanilla et al. 2007). Therefore, altered imprinting and loss of sexual dimorphism of the GHRH–GH axis may compromise the metabolizing capacity of the adult liver.

Summary: A Brain Connection Dictating Liver Sexual Dimorphism

This novel central-endocrine-liver pathway is viewed timely from a wide spectrum of fields, from Neuroscience to Endocrinology and Metabolism. The brain is the hierarchical master of a pathway which establishes sex-specific temporal patterns of pituitary GH secretion to ultimately determine the sex-biased transcription of hundreds of genes in the liver, and impart sex differences in liver physiology, metabolism, and disease. In this way the brain dictates adaptive functions modifying liver physiology.

The correct balance of GHRH and somatostatin is translated into sex specific GH secretory patterns needed not only for differential growth rate, but for adequate steroid metabolism and sex related behaviors. Underlying these phenotypic distinctions are sex differences in the expression of an unexpectedly large number of liver genes, as shown in mouse, rat, and human livers. Sex-specific gene expression in the liver extends beyond the genes involved in steroid hormone metabolism or reproductive behavior, to include large numbers of nuclear factors, receptors, signaling molecules, secretory products and enzymes. These may contribute as mediators of a wide range of biological processes.

In this regard, a loss of sexually dimorphic gene signature in the liver is related to liver disease, as demonstrated in metabolic alterations, hepatocarcinoma, or thrombosis. Furthermore, this signature is paramount in steroid, and drug metabolism, and finds an origin in brain control of GH secretion, unveiling a strong interaction between the brain, pituitary and liver.

Finally, exogenous exposure to natural hormones, hazardous substances and environmental endocrine disruptors during early life can induce permanent changes in brain control of the GHRH–GH axis and increase liver disease risk during adulthood. These disruptors may have negative effects not only on the reproductive tract, but also target the GHRH–GH-liver axis altering sexual dimorphism of liver gene expression, and predisposing to liver vulnerability.

Acknowledgements This work was supported by Grants from Agencia Nacional de Promoción Científica y Tecnológica, Argentina: PICT 330-2013; PICT 1343-2015; PICT 526-2016, Fundación Rene Barón, Fundación Williams, Consejo Nacional de Investigaciones Científicas y Técnicas; Pfizer 2015 Latin America ASPIRE Endo.

Author Contributions DB was the main contributor (writing, searching, editing). BB, MCR, LV, PC, AMO, E S, and FLV, discussed ideas, helped in the outline of the review, and corrected the text.

Compliance with Ethical Standards

Conflict of interest Paolo Catalano was a recipient of Pfizer 2015 Latin America ASPIRE Endo for young investigators. The rest of the authors have nothing to disclose.

Research Involving Human Participants and/or Animals This is a review and does not involve human participants and/or animals.

Informed Consent No informed consent is need.

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