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REVIEW ARTICLE

Developmental and Functional Effects of Steroid Hormones on the Neuroendocrine Axis and Spinal Cord

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This review highlights the principal effects of steroid hormones at central and peripheral levels in the neuroendocrine axis. The data discussed highlight the principal role of oestrogens and testosterone in hormonal programming in relation to sexual orientation, reproductive and metabolic programming, and the neuroendocrine mechanism involved in the development of polycystic ovary syndrome phenotype. Moreover, consistent with the wide range of processes in which steroid hormones take part, we discuss the protective effects of progesterone on neurodegenerative disease and the signalling mechanism involved in the genesis of oestrogen-induced pituitary prolactinomas.

Key words: hormonal programming, oestrogens, androgens, steroid hormones, prolactin

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Introduction

Hormones influence many biological processes throughout the lifespan, and have the potential to cause permanent tissue-specific alterations in anatomy and physiology during important developmental periods. Hormones exert transient effects in adult animals that activate or facilitate physiological processes or behaviours when the hormone is present, as well as permanent effects that act during development to program the pattern and extent of adult responses and contribute to sex differences. The role of steroid hormones in organisational and activational processes participating in reproductive anatomy is well described. These processes affect sexual differences relating to the neuroendocrine system and behaviour. Organisation refers to the actions of hormones in critical periods in early development with respect to the permanent establishment of sexual phenotypes, sexual genitalia, and future potential for masculine and feminine mating behaviour (1–5). Activation is related to the high levels of hormones in puberty or adulthood, linked with changes in masculine and feminine anatomy, as well as breeding behaviour dependent on the season of the year (1–5).

The developmental and programming effects of steroid hormones on the brain only occur during a sensitive period in early life, commonly referred to as the critical period. This period is in close relation to the theory of organisational effects of steroid hormones, comprising an empirical concept that differs among species with regard to timing and brain functions involved (6–11). These early effects are permanent and act to hardwire the brain.

Steroid hormones are lipophilic molecules utilised as chemical messengers by organisms, in which they act on a wide range of tissues and biological functions (6,12). A number of pathological states arise because of problems related to steroid hormone action. These include cancer, steroid insensitivity, abnormal fertility and endocrine alterations. Steroid hormones derive from a common cholesterol precursor (Cholestane C27). There are four major types of steroids: progestins, androgens, oestrogens and corticoids. The rate-limiting step in the synthesis of steroid hormones is the transport of free cholesterol (C27) from the cytoplasm into mitochondria, which is controlled by steroidogenic acute regulatory protein. The enzymatic step from cholesterol (C27) to pregnenolone (C21) (the common branch point for synthesis of progestins, corticoids, androgens and therefore oestrogens) is the limiting step for steroidogenesis once cholesterol is inside the mitochondria. Pregnenolone is subsequently converted to progesterone by 3β-hydroxysteroid dehydrogenase. Progesterone is generated in the ovary, adrenal gland and placenta during pregnancy, as well as the nervous system, in which it plays an important role as a neurosteroid.

This steroid is the principal intermediate for circulating androgens and oestrogens (13–17).

Leydig cells of the testes, thecal cells of the ovary and cells in the reticularis region of the adrenals are responsible for androgen synthesis and secretion (7,11,18). Luteinising hormone (LH) enhances the synthesis in the testes of testosterone. Testosterone can be reduced by 5α-reductase to yield a more active metabolite: 5α-dihydrotestosterone (DHT). This process takes part mainly in the target tissues. In some target tissues, testosterone and androstenedione can also be transformed into the oestrogens, such as 17β-oestradiol (E_2) and oestrone by the cytochrome P450 enzyme aromatase (19).

In females, oestrogens and progestins are synthesised and secreted principally by maturing ovarian follicles, corpora lutea and, during pregnancy, the placenta. The dominant oestrogen secreted is E_2. The follicle is composed of the primary oocyte surrounded by granulosa cells and theca cells. The granulosa cells are responsible for producing oestrogens from androgen precursors synthesised from the theca cells; this hormone activates oestrogen receptors (ERs) in target cells exerting their effects slowly (i.e. as a typical steroid receptor). Although most characterised effects are mediated via nuclear receptors and genomic pathways, there are many examples of very rapid, nongenomic effects of steroids (20,21). Classical ERs present in the cell nucleus (i.e. ERα and ERβ) act as ligand-activated transcriptional regulators, whereas ERs present in the cell membrane and cytoplasm regulate various intracellular signalling pathways and can converge with nuclear ERs to exert genotropic effects. Putative membrane ERs (mERs) include membrane-associated splice variants of ERα (mERα) and ERβ (mERβ), as well as G-protein receptors such as GPR30 and G_q-mER (20,21).

The mechanisms of action for different steroid hormones are relatively similar in the different target tissues. In the physiological situation of low amounts of hormone, classical ERs, androgen receptors (ARs) and progesterone receptors (PRs) are principally localised in the nucleus (22), whereas glucocorticoid receptors are located in the cytoplasm. Steroid hormones move passively from the circulation and interstitial spaces across cell membranes, and bind to and activate nuclear steroid receptor proteins. Then, the hormone-receptor binds to specific short DNA sequences in the promoter region of genes (i.e. hormone response elements) to enhance or repress the transcription of genes.

Steroid hormones are implicated in many biological processes, including development, hypothalamic programming, sexual differentiation, reproductive physiology, behaviour, osmoregulation, metabolism, regulation of the hypothalamic-pituitary-gonadal axis and hypothalamic-pituitary-adrenal (HPA) axis (6,16,23–26). In this review, the programming effect of steroid hormones during prenatal development is discussed. In particular, we analyse the effects of prenatal steroids on epigenetic programming, their impact in pathologies such as polycystic ovarian disease, and their role in behavioural processes such as sexual partner preferences. Furthermore, we discuss the protective effects of progesterone in the neurodegenerative disease amyotrophic lateral sclerosis (ALS) and consider how the Notch signalling pathway may mediate the formation of oestrogen-induced prolactinomas.

Steroid hormones and programming

Oestrogen

Developmental and programming effects of steroids during rat ovary development

Exposure to high levels of steroidal hormones disrupts normal endocrine function and decreases fertility in mammals, including humans, especially when the exposure occurs during critical periods of vulnerability during development.

The established view holds that reproductive function is regulated through the integration of information that comes from the hypothalamus, hypophysis and ovaries, and that gonadotrophins modulate folliculogenesis and steroidogenesis in the ovary (27). Numerous studies performed with the aim of understanding the neural circuits and molecular mechanisms that regulate gonadotrophin-releasing hormone (GnRH) release and steroid feedback demonstrate important roles for classical steroid receptors, membrane steroid receptors and neurosteroids in the hypothalamus (28–30).

Parallel to the endocrine control of reproductive function, experimental evidence indicates that there is complementary regulation through the hypothalamus-celiac ganglion-ovary axis (31). The primary neurotransmitter acting in the ovary is noradrenaline, which is released from neurone terminals originating in the celiac ganglia and acting on the thecal layer of ovarian follicles (32).

In mammals, ovarian folliculogenesis starts with the formation of primordial follicles, a process known as nest breakdown, which allows the oocytes to be surrounded by a layer of somatic cells, thus forming the primordial follicles in a process known as follicular assembly (33). In humans, this process occurs during the third trimester of gestation, whereas, in rats, it occurs between 24 and 72 h after birth (25,34–36). Once follicular development begins, it continues throughout postnatal life in both species. During this time, the oocyte enlarges, whereas the granulosa and theca cells proliferate, increasing the layers of cells surrounding the oocyte. This proliferative phase ends as follicular fluid begins to accumulate and the antral cavity forms (33,34). Each of the different steps of follicular development is controlled by different endocrine and paracrine factors (gonadotrophins, growth factors and steroidal hormones),; making this process vulnerable to hormonal changes induced by external factors (37).

The growing incidence of infertility and reproductive disorders in humans and wildlife has alerted many researchers to the influence on reproductive function of products with oestrogenic activity, that are produced and released into the environment (38). The molecules that mimic or block hormonal activity are known as endocrine disruptors (EDs). They may be synthetic or natural in origin and can alter homeostasis and the hormonal system, either by environmental exposure or by inappropriate exposure during development (35). Exposure to EDs during the sensitive periods can alter the normal development of the ovary, causing alterations in morphological and follicular development and malfunctions during the adult period (39–43). These alterations in the rat ovary can be inherited by the next generation through changes in the pattern of DNA methylation because cellular differentiation of the rat ovary begins around the time of birth. The germ cell re-methylation is initiated during the postnatal period and continues throughout the oocyte growth period until the preantral follicle stage (34,43,44). Regardless of the source of hormones or EDs during this period, they would alter the normal development of the offspring as a result of a reprogramming of the genes.

There are several pathological conditions in which the hormonal environment is altered during development, such as adrenal hyperplasia, obesity and polycystic ovary syndrome (PCOS) (45,46). PCOS is a complex endocrine disorder characterised by hyperandrogenism, ovulatory/menstrual irregularity and polycystic ovaries, which affects 5–10% of women of reproductive age (47). Women with PCOS exhibit a significant increase in androgen concentrations during pregnancy (48). An significant proportion of the first-degree female relatives of women with PCOS have been shown to be at risk for developing PCOS (49). Indeed, in comparison with control girls, PCOS girls exhibit higher levels of anti-Müllerian hormone (AMH), a marker of growing follicles, beginning at the peripubertal stage (50,51). It has been proposed that this inheritance is not the result of a genetic condition but, instead, foetal programming (47–52). Supporting this, experimental treatment of PCOS gestating mothers with the insulin sensitiser drug metformin improved the altered endocrine-metabolic environment of the PCOS mothers, as well as the AMH levels in their daughters, suggesting the follicular alterations described in adult PCOS women may appear early during development (53). This is supported by several studies in animal models that have demonstrated a relationship between programmed polycystic ovary (PCO) morphology during adulthood and prenatal or neonatal exposure to endocrine-disrupting compounds such as oestrogens or aromatisable androgens (41,54–56).

The administration of a single dose of oestradiol valerate to neonatal rats (12 h postnatally) induces early vaginal opening, disrupted cyclicity, appearance of a PCO phenotype, absence of corpus luteum and infertility (41). In addition, this exposure decreases the total number of ovarian follicles mainly as a result of a reduced number of primordial follicles, suggesting that oestradiol acts in the first stages of folliculogenesis when primordial follicles are organising and reprogramming the genes that control ovarian function (42,57). At the molecular level, AMH expression is increased in the ovary of these rats when they are adults. By contrast to AMH expression, AR expression in granulosa cells decreased at the same stage of development, suggesting that the regulatory region of AR and AMH genes could be involved. These results have been confirmed by protein expression data obtained by immunohistochemistry. In summary, these data suggest that oestradiol exposure during the neonatal critical period reprograms AR and AMH expression in the ovary possibly via epigenetic mechanisms that become evident in the adult period, when the full PCO phenotype is acquired (57).

Testosterone

Prenatal programming of sexual partner preferences: the ram model

Mammals are exposed to gonadal, placental and maternal hormones during early development. Hormones must be maintained within an appropriate range over time to program the proper development of the reproductive axis and adult behavioural responses (58). Males develop in an environment of elevated testosterone secreted by the foetal testes that acts to masculinise and defeminise brain structures, physiological processes and behaviours. Differences in testosterone concentrations and sensitivity occur naturally between individuals and can result from environmental challenges during pregnancy. The question of whether the occurrence of same sex partner preferences originates from variations in the prenatal hormonal environment has been studied in using a unique ram model (59).

Domestic rams display variations in sexual partner preferences. Domestic sheep are one of the few mammals apart from humans that exhibit exclusive and durable same sex partner preferences. Approximately 8–10% of domestic rams exhibit a sexual preference for other rams (i.e. male-oriented rams) (60–63). These rams not only mount, but also direct all courtship activities (anal–genital smelling, kicks, nibbles) to other rams, whereas their sexual interest and activity towards females is extremely low or nonexistent. Several hypotheses have been proposed to explain the development of same-sex preferences in rams. These include effects attributed to same-sex rearing, genes, olfactory responsiveness and brain differences (59). Although none of these mechanisms has been investigated extensively, the most compelling evidence supports the idea that this behaviour is partially attributable to brain differences.

Medial preoptic area. The medial preoptic area is essential for mating behaviour in vertebrates (64). Thus, this structure has been of great interest in searching for anatomic differences that could be causally related to sexual preferences. In rats, there is a cluster of neurones in the medial preoptic area, called the sexually dimorphic nucleus of the preoptic area (SDN-POA), which is five- to seven-fold larger in males than in females (65). The SDN-POA is part of a forebrain circuit that integrates sensory cues with hormonal status to modulate sexual behaviour. The larger volume of the male SDN-POA is correlated with the higher concentration of foetal and neonatal testosterone levels in males than in females. Males castrated at birth have much smaller SDN-POAs in adulthood, whereas females treated with testosterone perinatally have larger male-like SDN-POAs as adults. Also, there is evidence that the conversion of testosterone to oestradiol by the aromatase enzyme is required to masculinise the SDN-POA (66). Much like in rats, sheep have a homologue of the SDN-POA, called the ovine SDN (oSDN) that is twice as large in rams than in ewes (62). The oSDN comprises a dense cluster of cells comprising the central component of the medial preoptic nucleus that can be identified by Nissl staining and by abundant expression of aromatase mRNA. Moreover, it is larger in female-oriented rams than in male-oriented rams and does not differ between male-oriented rams and ewes. The differences in volume persist even after adults are gonadectomised and treated with testosterone, demonstrating they are most likely the result of the organisational actions of gonadal steroids occurring during foetal development in sheep (67).

Brains of straight and gay men differ. The observation that male-oriented rams have a smaller oSDN than female-oriented rams is reminiscent of the anatomical difference observed between the brains of gay and straight men (68). The sexually dimorphic nucleus identified in the hypothalamus of humans is called the thirs interstitial nucleus of the anterior hypothalamus (INAH3). The INAH3 is twice as large in straight men than in gay men and women. These cross-species results are some of the strongest evidence that sexual partner preferences and sexual orientation are regulated at the level of the hypothalamus-preoptic area. However, neither study can address the question of whether the difference is the cause or consequence of the behaviour. Indeed, for obvious ethical reasons, this question can only be experimentally tested using an animal model.

Which comes first?. If the smaller size of the oSDN causes males to be attracted to other males, the difference in size should be present prior to expression of the behaviour. Ideally, the volume of the oSDN should be measured over time as the animals become more sexually experienced to determine whether size differences emerge before sexual preferences are expressed. In lieu of this approach, which is not technically feasible, the question of whether the oSDN develops before animals have social experiences (i.e. prenatally) should be considered. Masculinisation of sexual behaviour in sheep occurs during a critical period that begins shortly after the testes differentiate at gestational day (GD)30 and persists until around GD90 (term pregnancy in sheep is approximately 150 days) (69). The oSDN is clearly apparent after this critical period in lamb foetuses (GD135) and is twice as large in males as in females (70). The larger oSDN in males correlates with higher levels of testosterone during the critical period. Thus, development of the oSDN occurs independently from sexual experiences and prior to expression of sexual preferences.

Experiments were performed to directly test whether testosterone exposure determines oSDN volume in late gestation foetuses (70). Biweekly maternal treatment with testosterone from GD30 to 90 significantly enlarged the foetal oSDN in females but had no effect in males. Coincident with this, testosterone-exposed females exhibited masculinised genitalia consisting of a pseudopenis and empty scrotum. These results show that, in sheep, and similar to rats, testosterone acting during the critical period for sexual differentiation masculinises both the brain and external genitalia. Typically, the sex of the brain matches the genitals. However, male-oriented rams have male genitals and a female-typical oSDN, suggesting that separate critical periods for the genitals and the brain might exist within the broad period of sexual differentiation. To test this possibility, biweekly maternal treatments with testosterone were administered from GD30 to 60 (early testosterone) and GD60 to 90 (late testosterone) (71). Early testosterone masculinised the genitalia of genetic females but had no effect on the foetal oSDN. Conversely, late testosterone masculinised the foetal oSDN of females and had no effect on the genitalia. Neither maternal treatment significantly affected male foetuses. These results demonstrate that testosterone affects differentiation of the brain and genitals in different timeframes. Individual critical periods exist for other sexually dimorphic traits in sheep, such as urination posture, the LH surge mechanism and the timing of puberty. Distinct temporal requirements for the action of testosterone or different sensitivities to testosterone metabolites could explain how hormone variations during gestation can produce rams that prefer to mate with other rams but still possess normal masculine genitals and other typical male neuroendocrine traits.

Is aromatisation of testosterone to E_2 needed for masculinisation of the SDN?. The foetal oSDN is characterised by an abundant expression of aromatase and Erα, suggesting that, similar to that of rodents, sheep brain masculinisation and defeminisation may require conversion of testosterone to oestradiol. However, daily treatment of mothers with the aromatase inhibitor androstatrienedione (ATD) had no effects on the sexual preferences and oSDN volumes of adult offspring (72,73), nor did ATD-exposed rams show LH surge responses or behavioural receptivity in response to oestradiol injections. The only difference was that ATD-exposed rams showed lower mounting activity than controls when they reached 18 months of age. Thus, it appears that oestrogens are not essential for masculinisation and defeminisation of sheep brain. These results raise the question of whether masculinisation of mate preferences and oSDN volume are controlled entirely through an AR mechanism or through the combined effects of both androgens and oestrogens.

Is androgen activity responsible for differentiation of male oSDN?. Androgen receptors are also expressed in the developing foetal oSDN (74). Experiments were performed to test the involvement of ARs in the sexual differentiation of sheep brain (75). If ARs mediate masculinisation, foetal exposure to the anti-androgen flutamide should reduce the size of the oSDN in males but not in females. On the other hand, exposure to the androgen agonist DHT should increase the size of the oSDN in females but not in males. Maternal flutamide treatment from GD60 to 90 significantly reduced mean oSDN volumes in GD135 male foetuses. Paradoxically, maternal DHT treatment during the same period also significantly reduced the oSDN volume of males. Neither treatment affected females. In a second experiment, foetuses were delivered during the final maternal treatments (GD85) to analyse whether these treatments elicited compensatory hormonal responses from the hypothalamic-pituitary-gonadal axis. Exposure to flutamide blocked negative-feedback in males, resulting in elevated serum levels of LH and testosterone, but had no effect in females. Thus, flutamide exerts sufficient AR antagonism to reduce oSDN volume even when testosterone is elevated. Exposure to DHT significantly suppressed LH concentrations in males and females and reduced testosterone concentrations in males. These results demonstrate that DHT exerts negative-feedback on the pituitary and consequently inhibits testosterone secretion, which can explain why the oSDN volume was reduced in males and unaffected in females. Apparently, the levels of DHT achieved in the foetal circulation were insufficient to support masculinisation of the oSDN. These results provide convincing evidence to suggest that the prenatal program masculinising the oSDN in eugonadal male foetuses acts through the AR. However, it is also apparent that the hypothalamic and pituitary regions are tonically suppressed in males during the gestational critical period for oSDN masculinisation and respond to disruptions in androgen action with an opposing hormone response intended to stabilise the endocrine milieu (Fig. 1). It is not yet known whether flutamide exposure exerts sufficient AR antagonism to alter male-typical sexual partner preference despite the compensatory increase in circulating testosterone.

Foetal reprogramming by testosterone of reproductive and metabolic parameters in male sheep

Inappropriate exposure to androgens during gestation can alter the trajectory of lamb development and alter adult physiology. For example, an excess of androgen exposure during gestation induces PCOS-like and metabolic traits in the female offspring in mammals, suggesting that the intrauterine environment may play a role in the aetiology of PCOS (76–78). Studies from several laboratories demonstrate that pregnant sheep injected with testosterone during part of their gestation give birth to female offspring, which have several reproductive and metabolic features resembling those observed in PCOS women. In the sheep model, testosterone-treated pregnant ewes exhibit high plasma levels of testosterone resembling those of adult males and plasma concentrations of insulin are similar to control pregnant sheep. The hyperandrogenic sheep model differs from human PCOS mothers, which are both hyperandrogenic and hyperinsulinaemic. Thus, the sheep model isolates the effect of androgens from other stimuli such as insulin, and provides tissue samples for studies in the offspring.

In addition to the results obtained in the female offspring, recent studies have demonstrated that male sheep born to testosterone-

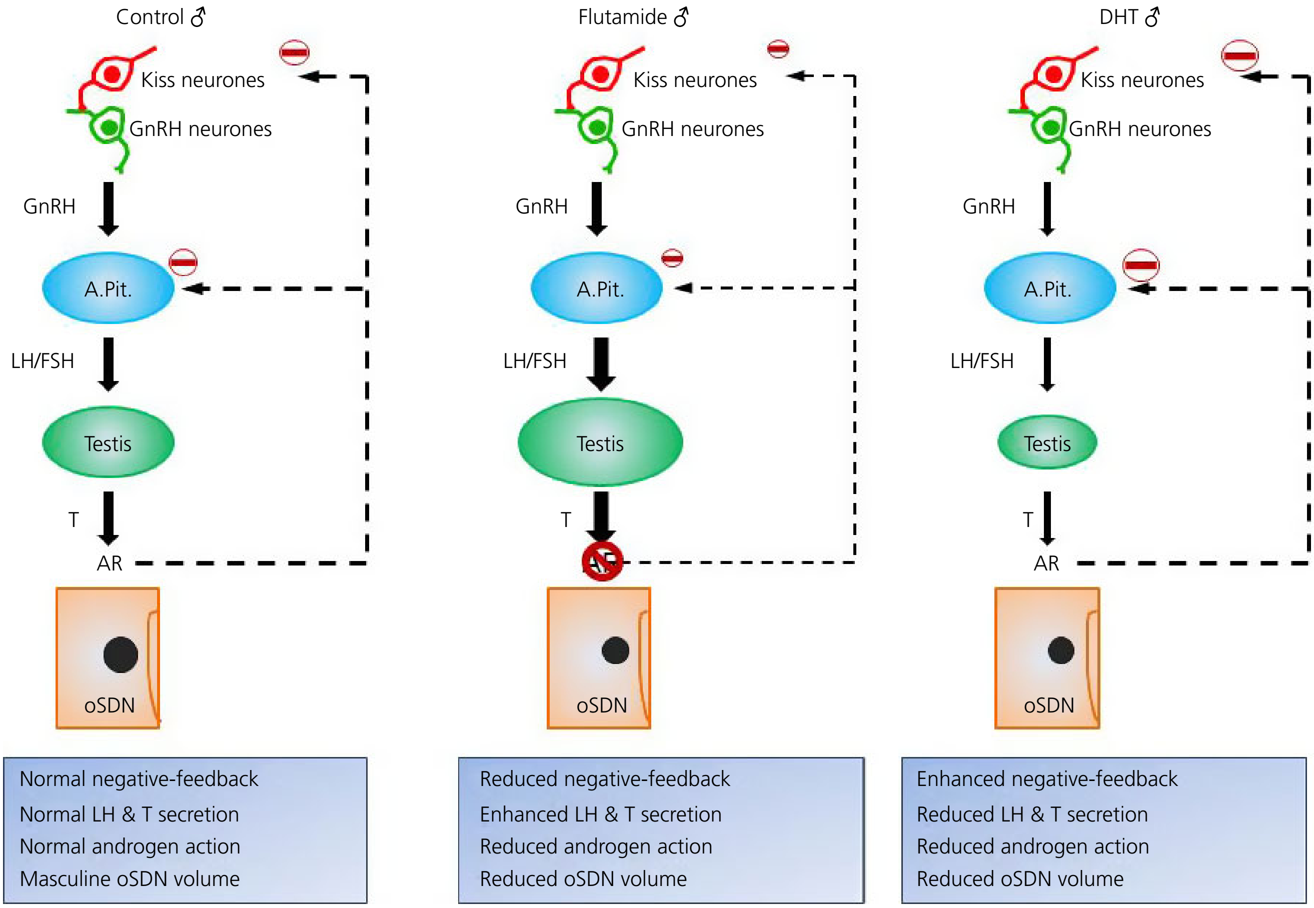

Fig. 1. Response of male foetal hypothalamic-pituitary-testis axis to vehicle (Control), androgen antagonist flutamide and androgen agonist dihydrotestosterone (DHT). Pregnant ewes received injections of vehicle, flutamide and DHT from gestation day (GD)60 to 84 and foetuses were delivered on GD85 for evaluation. In control eugonadal male foetuses, luteinsing hormone (LH) secretion is tonically suppressed by androgens at this age to regulate normal testosterone (T) secretion driving normal androgen action which, in turn, masculinises the ovine sexually dimorphic nucleus (oSDN). Exposure to flutamide blocks negative-feedback, resulting in elevated LH concentrations, enlarged testis and increased testicular testosterone secretion. The increased testosterone competes with the competitive antagonist flutamide for the androgen receptor (AR) and leads to partial, instead of full, inhibition of oSDN masculinisation. Exogenous DHT exposure enhances negative-feedback, further suppressing LH secretion and leading to smaller testis and reduced levels of testicular testosterone. The result of DHT treatment is reduced exposure of the developing SDN to endogenous testosterone and incomplete masculinisation. Both results are consistent with a role for androgen receptor activation in the masculinisation of the ovine SDN. A.Pit, anterior pituitary; FSH, follicle-stimulating hormone; GnRH, gonadotrophin-releasing hormone.

exposed mothers exhibit an altered reproductive and metabolic phenotype. Biometric analysis of testis of adult males showed that Sertoli cell number was higher in males born to hyperandrogenic mothers (T-males) than in control males (C-males) whereas spermatogonia, spermatocytes and spermatids were lower (79,80). Higher numbers of Sertoli cells in the seminiferous tubules was found to be a common feature in testis from T-males from infancy through puberty (81) but not during foetal life (SE Recabarren, V Padmanabhan, A Carrasco, R Fornes, MP Recabarren, R Rey, H Richter, D Sandoval, C Perez-Marín, T Sir-Petermann, PP Rojas-García, unpublished results). Paradoxically, the number of sperm cells was lower in the ejaculate of postpubertal and adult T-males (82). It is widely accepted that there is a strong correlation between number of Sertoli cells and the sperm production in animals and humans (83,84). Consequently, a functional discrepancy exists in these T-males because there is no correlation between the number of sperm cells and the number of Sertoli cells. The ontogeny of this discrepancy is unknown. It may be initiated during foetal life and completed before or after puberty (81,85). In this regard, the mRNA expression of the Sertoli cell marker AMH was higher in foetal and in peripubertal T-males testis (86,87).

The hypothalamic-pituitary-gonadal axis is also altered in T-males. This is apparent in the characteristics of the LH pulsatility, as well as the response of the pituitary gland and testis to a GnRH stimulus. Features of LH pulsatility such as LH pulse amplitude and LH pulse nadir were higher in T-males than in C-males, suggesting a reprogramming of GnRH secretion or a higher pituitary responsiveness to GnRH because the LH pulse frequency was not modified (88). The latter explanation was supported by another study reporting that pituitary gland responsiveness to a GnRH challenge was higher in T-males of 30 weeks of age than in T-males of 20 weeks of age. However, the release of testosterone stimulated by the endogenous LH in T-males of both ages was similar to that of C-males, suggesting that the responsiveness of the Leydig cells to the endogenous LH release was lower in T-males than in C-males (85). This may be a result of modifications in the bioactivity of the LH released by the GnRH challenge because the testosterone response to human chorionic gonadotrophin in adult T-males was similar to that of C-males (82). This reasoning was supported by the finding that mRNA expression of steroidogenic testicular enzymes in T-males was comparable to that of C-males (81), suggesting that the testicular steroidogenesis was not altered. However, it is also possible that lower testosterone secretion could be related to a low number of LH receptors because the expression of LH receptors mRNA was lower in T-males than in C-males (81,89). In summary, the exposure to testosterone during foetal development may be followed by: hypothalamic dysregulation of GnRH secretion, disturbances in the processing of LH secretion after a GnRH stimulus and probable modification in the isoforms of LH released. On the other hand, testosterone secretion after LH stimulation may be also affected as a result of LH receptor availability. As a whole, the fertility of these males may be impaired.

The intravenous glucose tolerance test (IVGTT) has been employed to evaluate various indices of insulin sensitivity in T- and C-males, including the basal glucose/insulin ratio, the area under the insulin curve or mean insulin secretion, and the insulin sensitivity index-composite (ISI-C) (90). Studies in human PCOS mothers show that their sons have an altered metabolic profile from infancy to adulthood, and they develop insulin resistance independent of body mass index (82). In the sheep model, the ISI-C and all other indices were similar between T-males and C-males during infancy, as well as during the prepubertal or postpubertal periods. It could be inferred from these results that an excess of testosterone during foetal development has no impact on the insulin sensitivity during postnatal development. To further analyse the foetal reprogramming of the insulin-glucose homeostasis in males and the contribution of testosterone, the insulin sensitivity was assessed in orchidectomised postpubertal C-males and T-males before and 48 h after an acute testosterone challenge. Basal levels of insulin and glucagon were not different between groups before and after the testosterone challenge. However, T-males released higher insulin compared to C-males during the first 20 min of the test after the testosterone challenge. Plasma levels of glucose were not different between groups during the IVGTT, suggesting that a testosterone challenge was more effective in the release of insulin in T-males under the glucose stimulation. However, the ISI-C was lower in T-males after the testosterone administration, suggesting a decrease in the insulin sensitivity in peripheral tissues. These findings are in contrast to those found in studies in human males born to PCOS mothers, where males exhibited metabolic disarrangements from infancy to adulthood, including the lipid profile and the insulin sensitivity, whereas there was no alteration in sperm cell concentrations or GnRH responsiveness (91,92). However, plasma AMH concentrations were higher in children born to PCOS mothers than in those born to control mothers, suggesting that the Sertoli cell numbers were increased in the male offspring of PCOS-mothers and also indicating that a similar reproductive phenotype may exist in hyperandrogenic male offspring of humans and sheep (91). Further research is needed to explore this possibility.

In conclusion, the sheep model has advantages and disadvantages for studying the programming effect of testosterone during foetal life on reproductive and metabolic parameters in offspring. Despite its limitations (93), it could help clarify the sequelae associated with inappropriate androgen exposure during foetal development and uncover the cause of the neuroendocrine disturbances observed in the affected offspring. It is now apparent that both females and males are susceptible to the reprogramming effects of a hyperandrogenic intrauterine environment.

Protective effects of steroid hormones

Progesterone

Protective effects of progesterone in the degenerative spinal cord

Under physiological conditions, progesterone is traditionally associated with female reproductive functions and pregnancy. Additionally, this steroid exerts neuroprotective and pro-myelinating effects in the central and peripheral nervous system in acute and chronic diseases such as traumatic brain injury, stroke, ischaemia, peripheral neuropathy of traumatic or diabetic origin, Alzheimer's dementia and ALS (94–99). At the cellular and molecular levels, progesterone modulates neuronal survival and plasticity, increases adult neurogenesis, favours the myelination process, inhibits lipid peroxidation, exerts anti-inflammatory properties and regulates astroglial plasticity (99,100). The central nervous system expresses several specific progesterone receptors, such as: (i) the classical intracellular PR; (ii) several isoforms of the membrane PR (mPRα, β and γ); (iii) the progesterone receptor membrane component type 1 (abbreviated PGRMC1 and formerly known as 25DX); and (iv) sigma 1 receptors (99,101,102). Once progesterone reaches the nervous system, either from systemic circulation or produced locally in the brain, it can be metabolised into 5α-dihydroprogesterone (DHP), which is further converted into 3α,5α-tetrahydroprogesterone or allopregnanolone (103). Thus, the metabolism of progesterone inside the nervous system has a profound impact on its mechanism of action: progesterone and DHP interact with the classical intracellular PR, whereas allopregnanolone is a potent allosteric modulator of GABAa receptors.

The Wobbler mouse is an animal model of ALS, the most common motoneurone disease. Wobblers develop a chronic, progressive motoneurone degeneration with selective involvement of brain stem and cervical motoneurones (104). By contrast to ALS patients who show a sex difference in disease incidence, with a higher frequency in men than in women (105), the onset or the progression of the Wobbler disease did not correlate with sex (106). Histologically, ventral horn motoneurones of the cervical spinal cord experience a dramatic cytoplasmic vacuolar degeneration (107), associated with astrocytosis (108–110) and microglial activation (111). Oxidative stress events participate in this mechanism, a finding supported by abnormalities of mitochondrial function in Wobbler mice. In this regard, mitochondria contribute to the production of certain free radicals; namely, superoxide anion and nitric oxide (NO), with the latter caused by the elevated activity of a mitochondrial nitric oxide synthase (NOSmt) (112). Excess levels of NO in association with increased generation of superoxide anions produce the formation of peroxynitrite (ONOO-) leading to oxidative damage. This situation leads to mitochondrial swelling and inhibition of the electron transport chain (113). At the ultrastructural level, vacuolated motoneurones from Wobbler mice present cristolysis and disruption of outer and inner mitochondrial membranes (114).

Progesterone administration to Wobblers exerts neuroprotective and anti-inflammatory effects, such as: (i) lower number of damaged/vacuolated motoneurones; (ii) increased expression of brain derived neurotrophic factor in motoneurones and oligodendrocytes; (iii) restoration of cholinergic neurotransmission and of axonal transport; and (iv) inhibitory effects on astrocytosis (94,114). Recent work suggests that the motoneurone protective effects of progesterone may also depend on the regulation of mitochondrial function.

Progesterone prevents mitochondrial dysfunction in the degenerative spinal cord of wobbler mice. Mitochondria from early stage Wobblers show a higher content of nNOS (NOSmt) in the cervical but not lumbar spinal cord compared to controls. By contrast, this region presents unchanged levels of cytosolic nNOS in the same groups. These events are associated with increased staining of NADPH-diaphorase/NOS in Wobbler's motoneurones. The changes in intramitochondrial nNOS in Wobblers have deleterious consequences for the activity of the electron transport chain. In this regard, the cervical cord of Wobblers shows compromised activities of complexes I and II–III in contrast to normal activities of cytochrome oxidase. Progesterone treatment decreases the level of mitochondrial nNOS in the cervical region and prevents the fall in the activity of complex I (106). These progesterone effects are associated with a reduction of the percentage of vacuolated/damaged motoneurones in Wobbler's cervical region, indicating that mitochondrial abnormalities are linked to motoneurone degeneration (Fig. 2). Additionally, decreased activity and immunostaining of the intramitochondrial enzyme manganese superoxide dismutase (MnSOD), as well as an accumulation of the amyloid precursor protein (APP), are also present in Wobblers motoneurones. The increase of APP at the level of the soma suggests impaired anterograde transport of APP to the terminal. Similarly, transport impairment may facilitate accumulation of nNOS in the cell body and enhanced access of this enzyme to the mitochondria. Exogenous administration of progesterone prevents these abnormalities (106). Hence, the administration of progesterone to clinically afflicted Wobblers: (i) blocks the abnormal increase of mitochondrial nNOS and normalises respiratory complex I; (ii) decreases APP accumulation, a signal of axonal degeneration; and (iii) enhances superoxide dismutation. Therefore, progesterone neuroprotection reduces mitochondriopathy of the cervical spinal cord of Wobbler mice. The enhancement of MnSOD activity and immunostaining by progesterone in the Wobbler supports the concept that this steroid may act as an antioxidant molecule arresting neurodegeneration.

Steroid hormones and human ALS. ALS is an adult progressive neurodegenerative disorder affecting the upper and lower motor neurones (115). Respiratory failure is the most frequent cause of death in these patients (116). Worse prognostic factors in ALS are: (i) bulbar onset; (ii) advanced age; and (iii) a short time between onset and diagnosis (117). Epidemiological studies show a higher frequency of ALS in men than women until menopause, when it becomes the same for both sexes. Thus, the suggestion has been made that sex steroid hormones may play a role in the disease susceptibility (105,118). Recently, ALS has been considered as a hormonal disorder involving changes of circulating gonadal steroids (118). This neurodegenerative disease also represents a stressful condition, in which changes in the HPA axis have been reported sporadically in ALS patients. Thus, loss of circadian rhythm of cortisol in ALS was first reported by Patacchioli *et al.* (119), whereas Monachelli *et al.* has recently found serum cortisol levels increases in ALS patients, further suggesting HPA axis dysfunction (120,121).

Circulating levels of progesterone are increased in male ALS patients compared to male control subjects. Such levels correlate positively with survival time and factors predicting better prognosis. In this regard, endogenous progesterone levels are elevated in benign forms of the disease such as in patients aged younger than 55 years and presenting spinal onset. In the elderly, peripheral

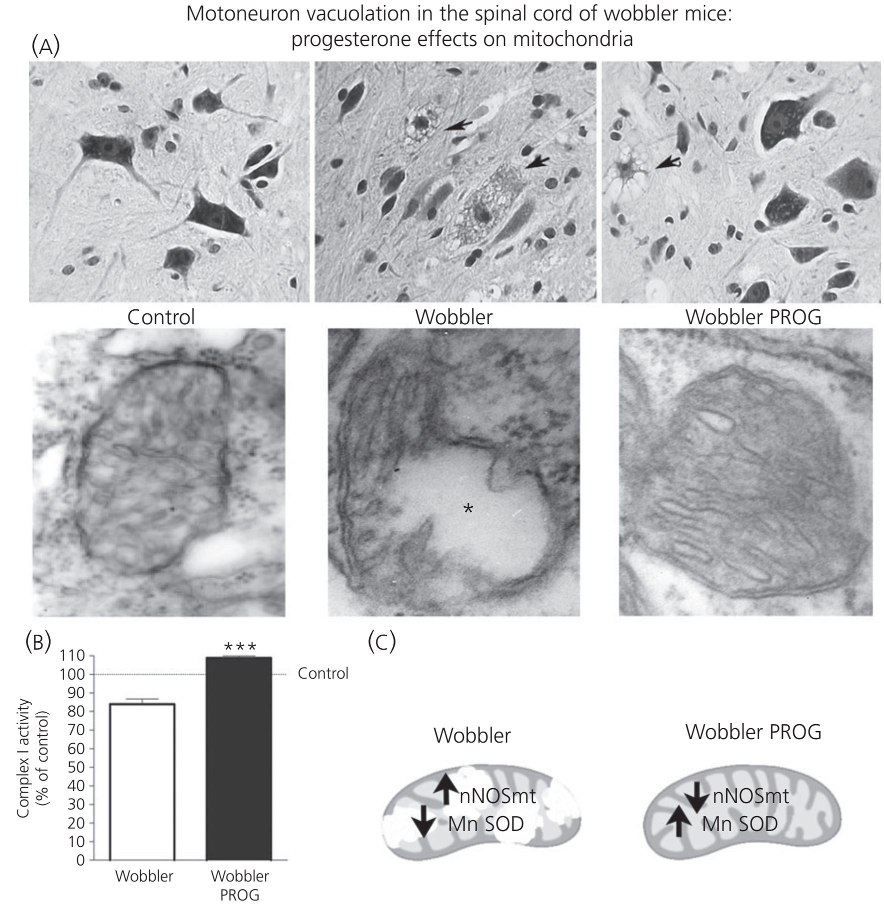

Fig. 2. (A) Digital images of paraffin sections of the ventral horn from the cervical spinal cord stained with cresyl violet. Images show motoneurones with a normal appearance (upper left), two intensely vacuolated cells (arrows) in an untreated Wobbler mouse (middle graph), and four motoneurones with a normal appearance and one vacuolated motoneurone (arrow) in a progesterone-treated Wobbler (upper right) Magnification: × 600. Lower graphs show electron microscopy of mitochondria from a motoneurone of a control mouse (lower left), an untreated Wobbler mouse (middle) and a Wobbler mouse receiving progesterone (PROG) (lower right). Motoneurones from Wobblers show massive vacuolation disrupting the outer, inner mitochondrial membranes and cristae (lower middle graph, asterisk). Motoneurones from PROG-treated Wobblers show some mitochondria with a better conservation of the membrane system, including the cristae. Magnification: × 50 000. (B) Activity of the mitochondrial respiratory enzyme complex I in the cervical region of the spinal cord of Wobbler mice and Wobbler mice receiving progesterone treatment. Progesterone increased complex I activity in cervical spinal cord from Wobblers (***$P < 0.001$ versus Wobbler). Results are expressed as a percentage of complex I activity of control. (C) Content of nNOS and MnSOD in mitochondrial fractions from the cervical cord of Wobbler mice and Wobbler receiving PROG. Wobbler vacuolated mitochondria showed high expression of mitochondrial nNOS (nNOSmt) and low activity and expression of manganese superoxide dismutase (MnSOD). Progesterone treatment in Wobblers significantly modified the high nNOSmt and the low MnSOD contents in mitochondria.

circulation of progesterone may depend on adrenal steroid production (122). Moreover, changes of androgens and oestrogens also occur in ALS and their animal models (123). In some men with ALS, free testosterone levels are in the low to normal range, whereas the finding of a low index-to-ring finger length ratio (2D : 4D ratio) suggests that greater prenatal testosterone exposure may play a role in motor neuronal vulnerability in adulthood (124). By contrast, women ALS patients show higher circulating levels of testosterone than healthy female subjects. In this group of patients, circulating levels of testosterone do not decline with increasing age as it does in controls. The concentration of sex steroids in ALS patients also bears a differential relationship with respiratory status and disease progression. Thus, respiratory symptoms and a decline in forced vital capacity (FVC%) are associated with a shorter survival in ALS patients. ALS patients with higher testosterone levels and a lower progesterone/free testosterone ratio exhibit a greater loss of respiratory function or a more rapid decline of FVC% (122). These results suggest that certain steroids may play a ‘protective’ role, whereas others have a negative influence on parameters vital for the progression and final outcome of the disease. In the transgenic superoxide dismutase-1 and Wobbler mouse models of ALS, it has been reported that ovariectomy leads to a significant acceleration of the disease, whereas oestradiol or progesterone treatment significantly delays disease progression (125).

Given that the biological effects of progesterone are essentially mediated by binding to the classical intracellular PR, Gargiulo-Monachelli *et al.* (126) demonstrated the presence of PR immunoreactivity in the cytoplasm of motor neurones and, more prominently, in axonal processes and large arteries. Indeed, it was reported that PR staining was stronger in nerve roots and large arteries from ALS compared to control spinal cords (126). Immunocytochemistry studies carried out in the rat spinal cord demonstrated that neurones and glial cells localised in the ventral horn are PR positive not only in the cytoplasm, but also in the nucleus (127). Evidence of cytoplasmic PR in the human spinal cord may suggest the intriguing possibility that progesterone could be acting through extranuclear mechanisms of hormone action. The presence of extranuclear PR has also been reported in the pre- and post-synaptic structures in the rat hippocampus that may be linked to the control of neuronal excitability and synaptic plasticity (128). The colocalisation of PR with markers of phosphorylated high molecular weight neurofilaments such as SMI-31 suggests that the PR may have an as yet unknown role in these cells. As noted above, progesterone displays a neuroprotective role in different pathologies of the nervous system (126). In addition, progesterone and DHP reduce axonal supernumerary sprouts and promote nerve repair by influencing the expression of the peripheral myelin proteins P0 and PMP22. The finding that progesterone and DHP are able to interact with the PR further suggests a role for this classical steroid receptor in nerve repair (103). Until the role of PR in ALS is clarified, it should be noted that expression of this receptor in ALS affected spinal cord is not identical to that seen in controls. These findings suggest a role for the PR and progesterone in this disease, probably in relation to regeneration and neuroprotection, as reported for animal models. Consequently, additional studies are needed to clearly establish progesterone neuroprotection in ALS, as may be inferred from previous studies in other neurodegenerative and injury models.

Oestrogen-induced tumourigenesis

Prolactinomas and notch-signalling

Pituitary cell line derived from oestrogen-treated rats: a model for resistant prolactinomas

Prolactinomas are the most frequent tumours in adults, accounting for 60% of all functioning pituitary tumours. They are usually treated with dopaminergic agents and are frequently benign. In addition, 15% may be resistant to classical pharmacological therapy, become invasive and aggressive, and require extirpation. In these cases, the decrease of dopamine D2 receptor (D2R) expression is considered as a hallmark for the loss of dopamine responsiveness, indicating that alternative therapies are needed to treat these tumours.

The oestrogen-treated rat is an interesting and well-studied model of pituitary hyperplasia. Increased pituitary weight, hyperprolactinaemia, lactotrope hyperplasia and reduced dopaminergic action at the pituitary level are physiological consequences in chronically oestrogenised female rats (129,130). The GH3 cell line, one of the best models developed for studying prolactinomas *in vitro*, was generated by treating a rat with high doses of oestrogens, in which a prolactinoma developed (131). By extracting this tumour, the GH3 cell line was generated through cellular culture methods. Interestingly, this cell line lacks D2R, which is the principal feature of clinical dopamine agonist resistant prolactinoma. This feature makes the GH3 cell line a good experimental model for studying prolactinomas and describing the molecular characteristics *in vitro*, or even *in vivo*, in a more physiological context. It is well known that GH3 cells secrete large amounts of prolactin (Prl) and growth hormone (GH), when they are maintained in cell culture or even when used for *in vivo* experiments (131–137).

In humans, Prl-secreting pituitary adenomas arise most commonly from the lateral wings of the anterior pituitary and fill the sella turcica as they progress, leading to compression of the normal anterior and posterior lobes (130,138,139). Tumours range in size from small microadenomas to large invasive tumours with extrasellar extension. Microadenomas, tumours with less than 1 cm in diameter at diagnosis, are observed in high proportions in patients. (140,141)**.**

The physiological symptoms of prolactinomas are galactorrhoea and amenorrhoea in women, and decreased libido or impotence in men (142). Gonadal dysfunction generally associated with amenorrhoea, oligomenorrhoea with anovulation, or infertility is present in approximately 90% of women with prolactinomas (143,144). Gonadal dysfunction in these women is a result of interference with the hypothalamic-pituitary-gonadal axis by the hyperprolactinaemia and, except in patients with large or invasive adenomas, is not a result of destruction of the gonadotrophin secreting cells. On the other hand, in men, the usual manifestations for clinical consultation are those of hypogonadism. The initial symptom is decreased libido, which may be initially regarded by both the patient and physician as a psychological factor; thus, the recognition of prolactinomas in men is frequently delayed and marked hyperprolactinaemia occurs (145–148).

In the last 10 years, a subset population of cells of the pituitary gland called the side population (SP) has been identified. This small subset of SP cells have several characteristics reminiscent of stem/progenitor cells and of early-embryonic pituitary cells (149–151).

Pituitary adenomas were found to contain self-renewing sphere-forming cells, which are considered to be a property of cancer stem cells (CSC). Whole-genome expression profiling performed in SP cells, which contain CSC, compared to the tumour bulk cells from somatotropinomas and nonfunctioning adenomas revealed an up-regulation of several Notch system (receptors and ligands) components in humans (152). The Notch system has a broad expression both in human (153,154) (L. Zubeldia-Brenner, unpublished results) and mice pituitary (149–151), as well as in rat pituitary (L. Zubeldia-Brenner, unpublished results).

The cellular and molecular mechanisms that initiate the formation of prolactinomas are largely unknown. In this regard, the participation of Notch receptors in prolactinoma development has not been studied in detail. The Notch receptors are involved in a wide group of processes during the development of eukaryotic cells, such as proliferation, migration, differentiation and apoptosis (155–158). Thus, the Notch system appears to function as a general developmental tool that is used to direct cell fate and, consequently, to shape a living organism (155,156).

Mammals possess four different Notch receptors, referred to as Notch-1, Notch-2, Notch-3 and Notch-4. The Notch receptor is a single-pass transmembrane receptor protein. It is a hetero-oligomer composed of a large extracellular portion, which associates in a calcium-dependent, noncovalent interaction with a smaller piece of the Notch protein composed of a short extracellular region, a single transmembrane-pass and a small intracellular region. The receptor is normally triggered via direct cell-to-cell contact, in which the transmembrane proteins of the cells in direct contact form the ligands that interact with the extracellular domain of the Notch receptor on an adjacent cell (159). Upon ligand binding, the receptor suffers two proteolytic events as a result of the activation mechanism (160,161). Notch receptors are cleaved near the exterior side of the plasma membrane, and then a posterior cleavage in the transmembrane domain mediated by a gamma-secretase enzyme complex liberates the intracellular domain of the receptor (Notch intracellular domain; NICD) to the cytoplasm. The NICD acts as a transcription factor, translocating to the nucleus after the cleavage to form a multiprotein complex with the ubiquitously expressed CSL (CBF1, Suppressor of Hairless, Lag-1) transcription factor. In the absence of NICD, CSL is complexed with co-repressors. When NICD binds CSL, specific co-activators are recruited, resulting in a transcriptional activation of the target genes (162). The transcriptional targets of the Notch system include differentiation related factors, cell cycle regulators (p21 and cyclin D1) and regulators of apoptosis. Transcription factors of the Hairy/enhancer of split (Hes) and Hes related (HRT/HRP/Hey) families (163) are also part of the principal targets. These proteins belong to the basic helix-loop-helix family of transcription factors and act to bind specific sequences in the promoter region of target genes and repress the transcription via the recruitment of a set of co-repressors.

Notch receptors are involved in several physiological processes. Furthermore, there are several pathologies in which Notch receptors are seriously dysregulated. In cancer and abnormal neoplasic proliferation, Notch receptors are altered, and may be up-regulated or down-regulated depending on the tissue and context involved (164–166). Within the four receptors, Notch-1 and Notch-3 are the most frequently described in tumour development and cancer (153,154,159).

Tumourigenesis and neural development are part of the processes in which Notch-3 has been described. Furthermore, the Notch-3 receptor and its ligands are implicated in cellular differentiation and, in some cases, promote pituitary cell growth and tumour formation in nonfunctioning adenomas (153,154).

Notch-3 is also expressed in the SP cells of the adult anterior pituitary gland. In humans, Notch-3 expression in nonfunctioning pituitary tumours is markedly higher than in normal pituitary tissue (153,154). Strikingly, the Dlk-1 gene deletion, one of the Notch ligands, results in a developmental defect in somatolactotrophs (159).

Notch-1 was also detected in the anterior pituitary of adult mice (151). Recently, L Zubeldia-Brenner, C Cristina, D Becu Villalobos. (unpublished results), detected the additional mammalian Notch receptors (Notch-2, 3 and 4) and other key downstream target genes (Hes-1 and Hes-5, as well as Hey-1 and Hey-2) using a reverse transcriptase-polymerase chain reaction (L Zubeldia-Brenner, C Cristina, D Becu Villalobos, unpublished results).

In recent experiments, xenotropic tumours were generated by injecting GH3 cells into Nude/Nude mice to test the role of Notch signalling in tumour growth and progression. Once the tumours developed, mice were treated with DAPT, a drug that blocks the Notch pathway by inhibiting the gamma-secretase. Tumour volumes were significantly smaller in mice treated with DAPT compared to the controls (L. Zubeldia-Brenner, unpublished data). These results suggest that Notch receptors play a critical role in the hormonal function of these pituitary tumours, as well as in the normal physiological context of the pituitary, enhancing or down-regulating the secretion of Prl and GH.

Summary and conclusions

The present review highlights the important effects of steroid and peptidergic hormones at central and peripheral levels of the neuroendocrine axis and illustrates a wide variety of neural processes in which they participate. The impact of steroids on neuroendocrine processes is very broad and, in this review, we have included their impact on PCO, brain sexual differentiation, ALS and the aetiology of prolactinomas.

Evidence has been presented to show that inappropriate prenatal exposure to environmental oestrogen can alter normal development of the ovary, leading to adult ovarian pathologies. This can be transmitted to the next generation through epigenetic mechanisms that reprogram genes. Moreover, the prenatal hormone milieu also plays an important role in brain sexual differentiation revealing that sexual preferences are also under the control of steroid hormones.

Further examples in which steroid hormones are involved are the protective effect of progesterone in the degenerative spinal cord and ALS, and the role of oestrogen and Notch signalling in prolactin secreting pituitary tumours.

In conclusion, the developmental studies reported in the present study implicate foetal exposure to hormonal and environmental compounds as an important variable that may developmentally reprogram the neural mechanisms regulating adult reproductive function. They reveal important foetal antecedents of adult gonadal dysfunctions that impact fertility and provide new insights into the influence of prenatal hormones on adult sexuality. Moreover, the functional studies of neurodegeneration and tumourigenesis have identified novel cellular and molecular control points regulated by steroid hormones that may constitute future therapeutic targets. The global processes influenced by steroid hormones are tightly regulated and so precise that the presence of hormonal disruptors or imbalances in the neuroendocrine milieu may generate altered phenotypes or predispose individuals to serious pathologies. The insights gained from this research further our understanding of the elaborate and intricate regulation that steroid hormones exert on the central and peripheral nervous systems throughout life.

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