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# Morphological evidence of the relevance of aromatase P450 in autoparacrine regulation and pathology of the hypophysis

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## SUMMARY

Aromatase P450, the enzyme responsible for the catabolism of aromatizable androgens to estrogens is involved in sexual dimorphism and several pathologies induced by estrogenic effects. The present work reports evidence of pituitary aromatase synthesis, its regulation by gonadal steroids, and the physiological roles of aromatase on pituitary endocrine cells. The involvement of aromatase, through the auto-paracrine production of estradiol, in the pathogenesis of pituitary tumors -mainly prolactinomas- is reviewed.

**Key words:** Pituitary gland – Aromatase – Prolactin – Paracrine regulation

## INTRODUCTION

Aromatase P450 is an enzyme that catabolizes the metabolism of some sexual steroids, in particular aromatizable androgens such as testosterone. Testosterone acts by binding to a specific protein receptor, called the androgen receptor, triggering alterations in gene expression and in cellular functions (Carson-Jurica et al., 1990; Sar et al., 1990).

Testosterone is metabolized intracellularly to develop its biological actions through two main pathways (Martini, 1982): on one hand, it is transformed into 5α-dihydrotestosterone through the action of 5 α-reductase; the second pathway transforms testosterone into 17-β-estradiol through the action of aromatase P450, an enzyme that forms part of the cytochrome P450 macrofamily (CYP19). A similar process occurs in the metabolism of androstendione to estrone or of 16α-hydroxyl androgens to estriol. The estrogens thus formed inside the cell interact with estrogen receptors, a protein receptor system completely different from the androgen receptor (McCarthy, 1994).

Aromatase P450 has been defined as an enzyme complex formed by two components; cytochrome P450 and NADPH cytochrome P450 reductase, located in the membranes of the endoplasmic reticulum. This oxidase catalyzes a reaction that first removes the methyl group of carbon 19 after the hydroxylation of testosterone to form formic acid and then reorganizes the A ring towards an aromatic structure. The reaction requires NADPH and consumes three moles of oxygen

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(Gore-Langton and Dorrington, 1981).

Hickey et al. (1990) described the amino acid sequence of aromatase P450. This sequence results from the transcription and later translation of the aromatase CYP 19 gene. The aromatase P450 sequence has been conserved phylogenetically, mainly between birds and mammals (Simpson et al., 1987; Hall et al., 1987; McPhaul et al., 1988; Harada, 1988; Harada et al., 1990; Toda et al., 1990; Matsumine et al., 1991; Polymeropoulos et al., 1991; Harada, 1992; Miyashita et al., 2000; Böhne et al., 2013; Chen et al., 2014). These (birds and mammals) partially differ in the carboxy-terminal amino acid sequence, which could account for some of the differences observed by different authors in the location of the enzyme. Physiologically, aromatase has been implicated as being directly responsible for sexual dimorphism in the nervous system.

Aromatase P450 represents a key checkpoint in the metabolism of steroid hormone metabolism since it is the limiting enzymatic step in the rate of estrogen synthesis. It is composed of two proteins: a specific microsomal hemo-glycoprotein (cytochrome P450 aromatase) and a microsomal flavoprotein (NADPH-cytochrome P450 reductase). The first one is a monooxigenase, a member of the cytochrome P450 superfamily that catalyzes the insertion of oxygen atoms into organic molecules to give rise to the formation of hydroxyl groups. The second one is a ubiquitous and non-specific microsomal flavoprotein that transfers electrons from NADPH to any cytochrome P450 (Simpson et al., 1994).

Aromatase P450 is the product of the expression of the CYP19 gene. This gene is present as a single copy in the haploid genome of most mammals and belongs to the P450 gene superfamily, in turn formed by more than 300 members –shared among 36 gene families (Nelson et al., 2013). The different aromatase transcripts derive from the expression of the 9 exons of the gene, beginning as exon II.

The promoters are untranslated regions of exon I and vary from one type of tissue to another (Toda and Shizuta, 1993). In the placenta, aromatase P450 synthesis is driven by promoter I.1 (Jenkins et al., 1993); in the ovary by promoter II, in adipose tissue by promoter 1.4 (Harada et al.., 1993), and in the brain by promoter 1f. (Honda et al., 1994). The activity of promoter PII is regulated by cyclic AMP and requires the participation of CREB, CREM and the steroidogenic response factor (SF-1) (Simpson 2000).

In mammals, the tissue-specific expression of CYP19 is regulated using different regions of the promoter and alternative splicing (Simpson et al., 1994). This leads to the generation of transcription variants of CYP19 with different untranslated 5' regions (YTRs), but with identical coding sequences.

In humans, nine untranslated exons have been identified, coding for 5 UTRs and their corresponding promoter regions. The different promoter regions and exons span more than ninety kb pairs, while the coding exons are restricted to 30 kb of the genomic sequence (Sebastian and Bulun, 2001; Kamat et al., 2002).

In the mouse, the Cyp19 locus spans at least 60 kb and is located on chromosome 9 (Youngblood et al., 1989). The tissue-specific expression of aromatase P450 is also achieved by alternative splicing and/or the use of different promoters (Golovine et al., 2003). The transcripts derived from two different regions of the promoter have been identified. The conserved proximal promoter is responsible for driving the ovary transcripts, while the promoter located distally is mainly active in the brain (Honda et al., 1996; Yamada-Mouri et al., 1996). All the CYP19 transcripts of somatic cells and testicular germinal cells derive from the proximal promoter II (Lanzino et al., 2001).

In the mouse, the regulatory regions represented by the untranslated exons and their adjacent promoters occupy more of the locus than all the nine coding exons together. This reveals the complex mechanism of the transcriptional regulation of this gene. The distal-most promoter (Pbr) is found at least at 31 kb distant from the first coding exon. This distance correspond ds to that estimated for the human homologue, the 1.f-specific promoter of the brain (Sebastian and Bulun, 2001), suggesting the unvarying nature of this genomic. In the human gene, the promoter region located most distally, which is responsible for the strong placenta-specific expression (PI.1), is present at more than 90 kb before the translation start site.

Some cognitive aspects, such as sexual behavior, depend on the conversion of testosterone into estrogens (Sachs and Meisel, 1989), as long as the action of 5$\alpha$-reductase and the production of 5 $\alpha$-dihydrotestosterone are maintained (De Bold and Clemens, 1978; Powers et al., 1985; Steel and Hutchison, 1988; Roselli, 1991). This is important when considering that only aromatized androgens stimulate sexual behavior during copulation (McDonald et al., 1970; Feder, 1971; Whalen and Luttge, 1971), such stimulation being suppressed when the cerebral synthesis of estrogens is inhibited or anti-estrogens are used (Luttge, 1975; Beyer et al., 1976; Morali et al., 1977; Bonsall et al., 1992).

## AROMATASE AND THE HYPOTHALAMUS

The formation of estrogens from androgens is crucially involved in the sexual differentiation of neuronal structures and of reproductive behavior (McEwen et al., 1977; Parsons et al., 1984). It is also known that the in situ aromatization of androgens is an important determinant of the occupation

of estrogen receptors in the brain of adult male rats (Lieberbug and McEwen, 1977; Lieberbug et al., 1979; Krey et al., 1982). Additionally, it has been demonstrated that estrogens generated by aromatase activity act on estrogen receptors (McCarthy, 1994).

In the male rat, the action of aromatase during pre- and early post-natal development on the medial preoptic area leads to a sexual differentiation of the hypothalamus; pre- and post-natal treatment with aromatase inhibitors elicits important alterations in the sexual behavior of males and seems to induce a trend towards homosexuality (Houtsmuller et al., 1994). The critical period of action has been established at before the 12th day post-partum (González and Leret, 1994).

At hypothalamic level, testosterone stimulates the activity of aromatase through its action on androgen receptors (Steimer and Hutchison, 1981; Roselli et al., 1984, 1987; Hutchison et al., 1991a, b) and induces an increase in the number of hypothalamic neurons immunoreactive to the enzyme (Dessi-Fulgheri and Lupo, 1982; Hutchison et al., 1995). Nevertheless, aromatase activity may not be completely correlated with the circulating levels of testosterone, such that other factors could be related to the activity of the enzyme (Compaan et al., 1994). In species other than the rat, estradiol stimulates cerebral aromatase (Schumacher and Balthazar, 1986; Hutchison and Steimer, 1986; Pasmanik et al., 1988; Negri-Cesi et al., 1989). Estradiol, acting on estrogen receptor $\alpha$, could regulate aromatase expression and activity via the promoter I.f in the mouse hypothalamus (Yilmaz et al., 2009).

There is considerable coincidence between the hypothalamic areas showing aromatase activity and the presence of androgen and estrogen receptors, such as in the case of the anterior preoptic area (Sar and Stumpf, 1975; Stumpf et al., 1975; Selmanoff et al., 1977; Roselli et al., 1985). This topographic coincidence has also been demonstrated with immunocytochemical techniques (Beyer et al., 1994b). The anatomical differences in the expression and clearance of aromatase could be differentially sensitive to androgens and estrogens and could depend on the presence of receptors for sexual steroids (Balthazart et al., 1992).

It seems clear that an exogenous regulation is exerted (mainly by sexual steroids) on the aromatase activity in the diencephalic neurons of rodents. The activity and mRNA levels of the enzyme increase in the brains of these animals as prenatal development progresses, but little is known about the factors involved in regulating this increase (Lephart et al., 1992b; Harada and Yamada, 1992; George and Ojeda, 1982; Michnovicz et al., 1987; Lephart et al., 1992a, b).

There is some evidence obtained from in vitro studies that suggests the possibility that the increase in aromatase mRNA levels observed in diencephalic neurons during development could be an endogenous characteristic and that it is probably determined genetically as from the 12th day of development (Abe-Dohmae et al., 1996). Without ruling out this possibility, the existence of a synergy between androgens and estrogens in the pretranslational induction of aromatase mRNA in the brain has been described. Along general lines, this would affect the whole of the brain, the medial preoptic area being the zone showing the strongest response (Harada, 1993).

Other findings suggest that the differentiation of sexually dimorphic structures does not only take place in the perinatal period but that it also occurs, above all in the anterolateral periventricular nucleus of the rat, during puberty (Davis et al., 1996). This confirms previous observations in other animal species in which cerebral aromatase activity was detected in sexually active phases that depend on gonadal function (Hutchison et al., 1992).

Since the formation of neurosteroids in cells of the glial lineage was described, some authors have analyzed the cell types that display immunoreaction for aromatase. According to the studies of Beyer et al. (1994a), the immunoreactive cells are exclusively of the neuronal type and not glial, both in the brain cortex and in the hypothalamus, in agreement with the findings obtained in vitro upon analyzing the enzymatic activity of aromatase (Negri-Cesi et al., 1992).

## HYPOPHYSEAL AROMATASE

Since the hypophysis is a sexually dimorphic endocrine gland whose secretion is affected by the action of gonadal steroids, the possibility that the gland might produce aromatase and that this might exert its action at local level may be of particular relevance.

At our lab, working with human beings and rodents, we have described the immunohistochemical expression of aromatase in the hypophysis (Carretero et al., 1999a; 2002; 2003a). Later, these findings were confirmed by other authors working with different animal species (Menuet et al., 2003; Goto-Kazeto et al., 2004; Galmiche et al., 2006a,b; Nocillado et al., 2007; Kadioglu et al., 2008; Trubiroha et al., 2012). The possible role played by the transformation of testosterone into estradiol with respect to the secretion of the gland remains to be fully elucidated. It could be debated whether the events reported in different studies might occur in the hypothalamus, in the hypophysis or in both at the same time, such as the fact that the administration of aromatase inhibitors increases circulating LH levels (Boyar et al., 1973; Marynick et al., 1979; Plant, 1986), and there is evidence to suggest a potential physiological or physiopathological importance for hypophyseal aromatase.

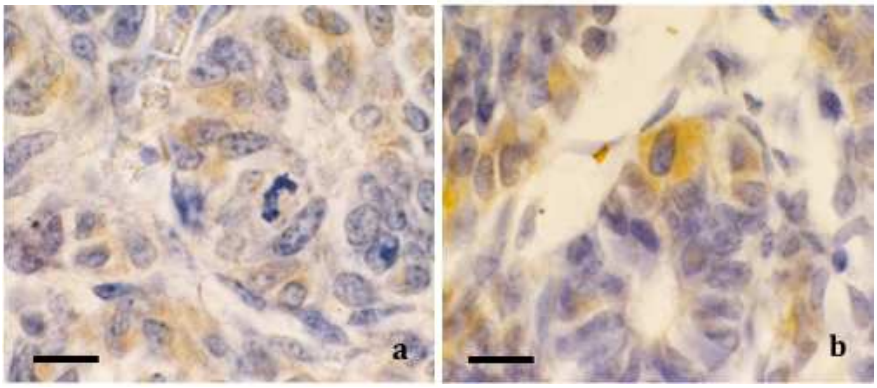



**Fig. 1.** Micrographs of pituitary of rats during embryonic development at E17 **(a)** and E19 **(b)**, in which aromatase positivity (brown) is seen after the immunocytochemical technique. Scale bars: 10 µm. Images reprinted from Carretero et al. (1999b).

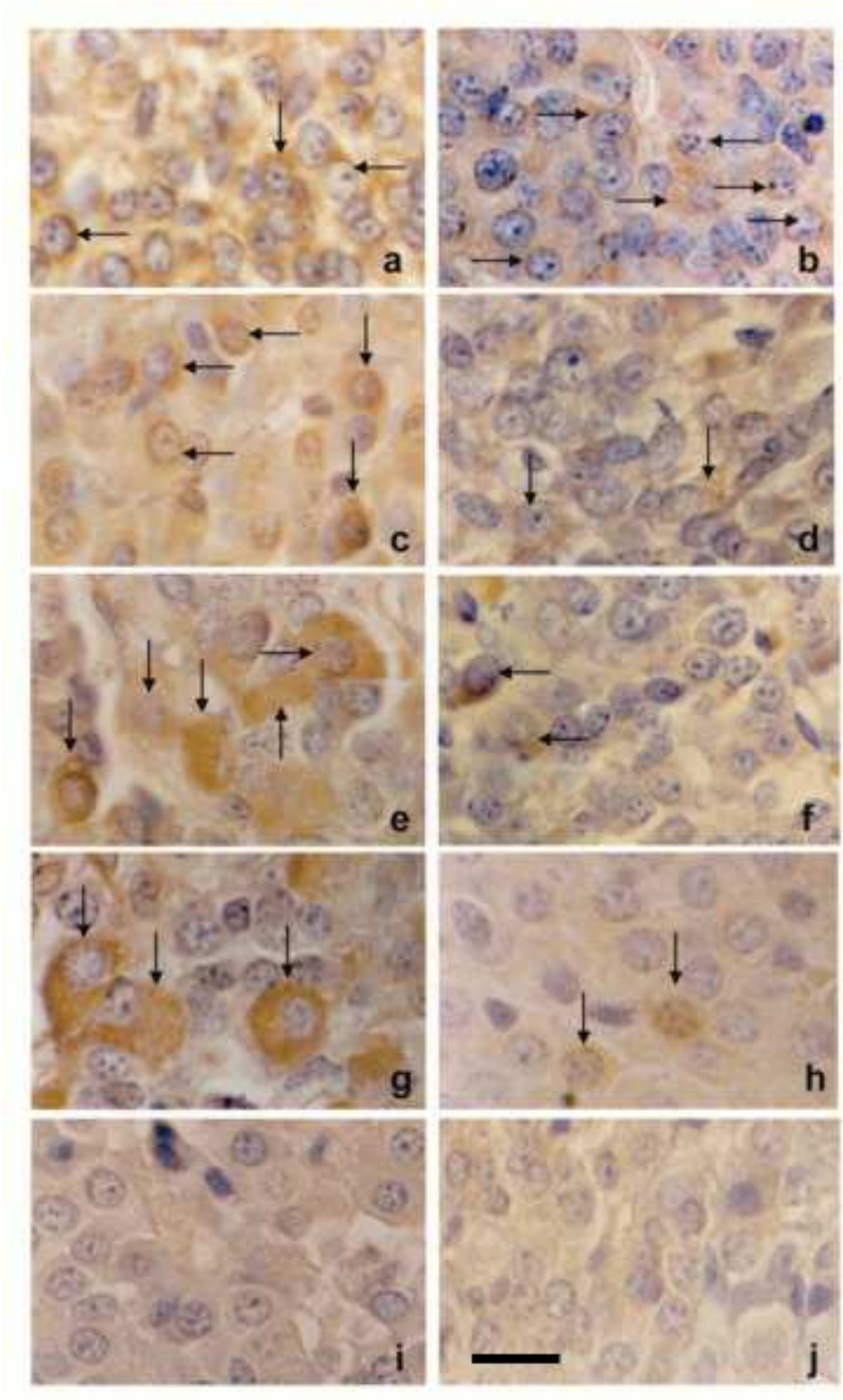



**Fig. 2.** Aromatase immunoreactivity (arrows) in the cells of the pituitary gland of female and male rats at different ages. (a) Male, 7 days; **(b)** Female, 7 days; **(c)** Male, 14 days; **(d)** Female, 14 days; **(e)** Male, 21 days; **(f)** Female, 21 days; **(g)** Male, 2 months; **(h)** Female, 2 months; **(i)** Male, 24 months; **(j)** Female, 24 months. Scale bar a-j (in j): 16 µm. Images reprinted from Carretero et al. (2003a).

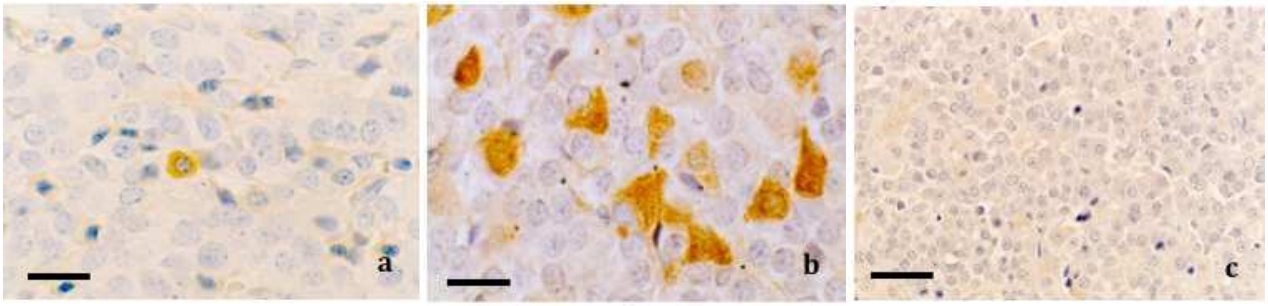



**Fig. 3.** Hypophysis of an adult female **(a)**, adult male **(b)** and aged male **(c)** showing aromatase positivity (brown) after the immunocytochemical technique. Scale bars: a, b: 15 µm; c: 25 µm. Image **(b)** reprinted from Carretero et al. (1999a) and **(c)** from Carretero et al. (2002), with permission from Springer Science+Business Media.

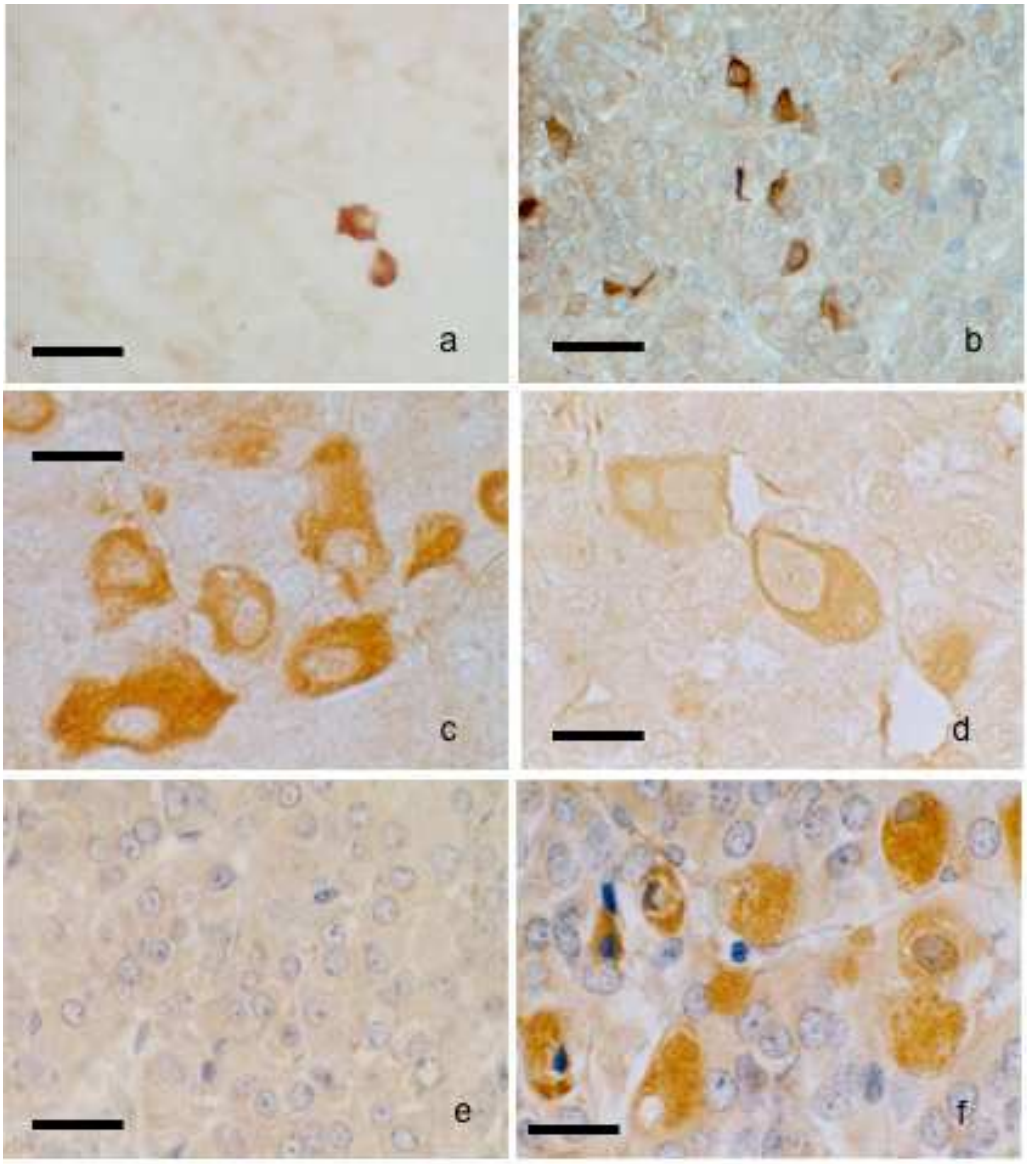



**Fig. 4.** Pituitaries from adult untreated **(a)**, castrated **(c)** and castrated and treated with estradiol **(e)** female rats; and untreated **(b)**, castrated **(d)** and castrated and treated with testosterone **(f)** male rats, showing the effects of peripheral gonadal steroids on pituitary aromatase-positive cells (brown) identified by immunohistochemistry. Scale bars: a, b: 40 µm; c, d, f: 16 µm; e: 20 µm. Images reprinted from Carretero et al. (2011).

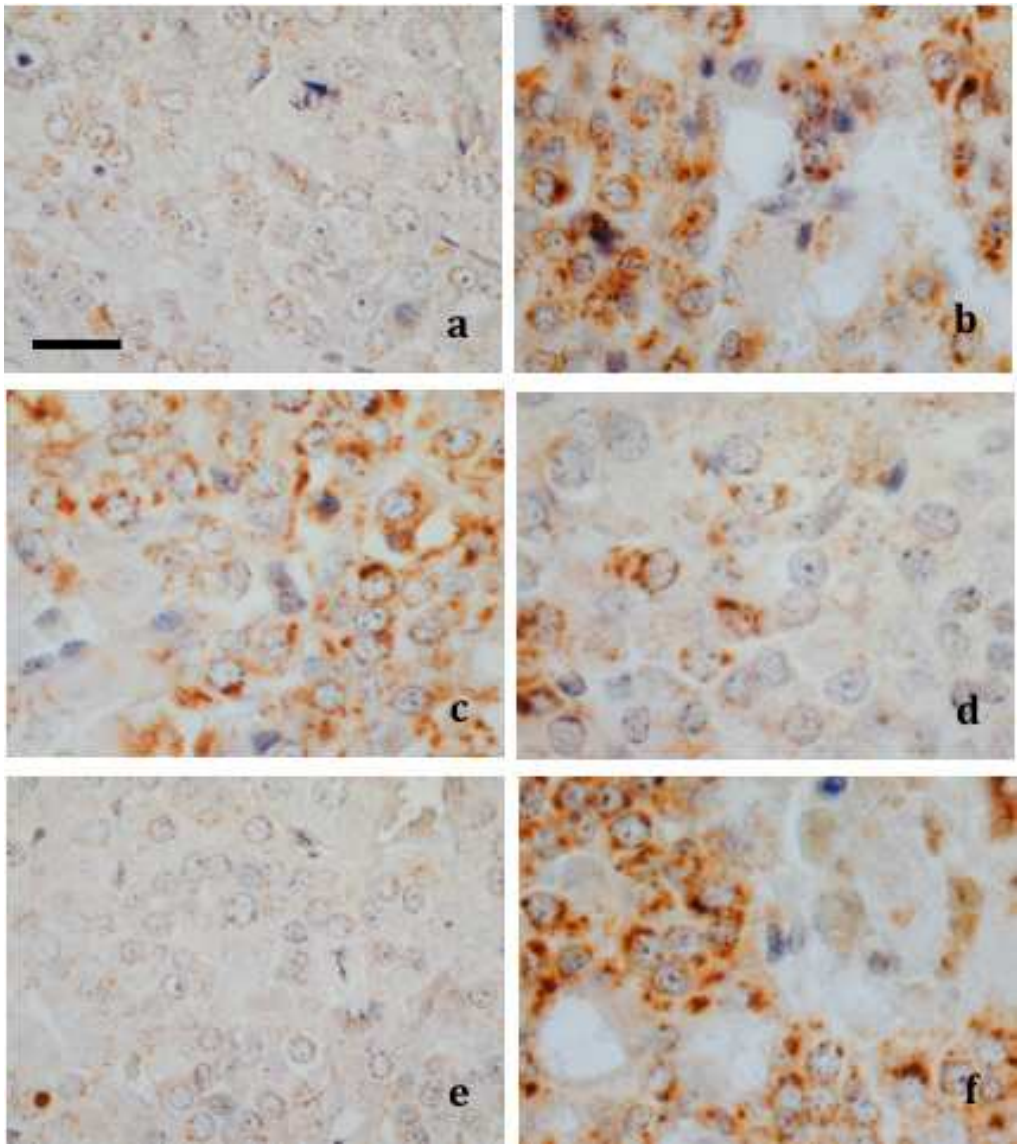



**Fig. 5.** Pituitaries from adult untreated **(a)**, castrated **(c)** and castrated and treated with estradiol **(e)** female rats; and ) untreated **(b)**, castrated **(d)** and castrated and treated with testosterone **(f)** male rats, showing the effects of peripheral gonadal steroids on aromatase mRNA identified by in situ hybridization (brown). Scale bar a-f (in a): 18 µm. Images reprinted from Carretero et al. (2011).

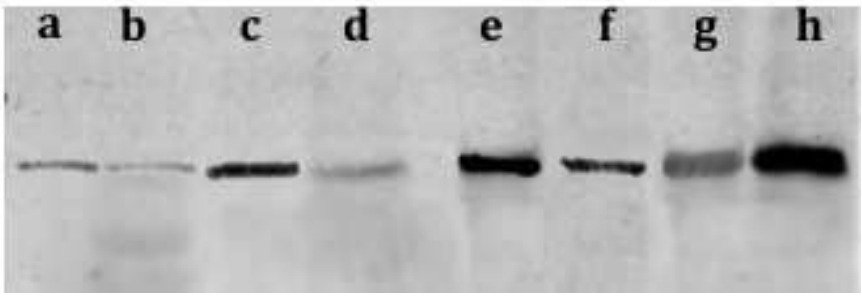



**Fig. 6.** Western blot bands for aromatase P450 obtained from hypophyseal lysates. The bands are representative of the effects induced by the presence or absence of gonadal steroids (a: untreated females, b: sham-operated females, c: castrated females, d: castrated estradiol-treated females, e: untreated males, f: sham-operated males, g: castrated males, h: castrated and testosterone-treated males). Image reprinted from Carretero et al. (2011).

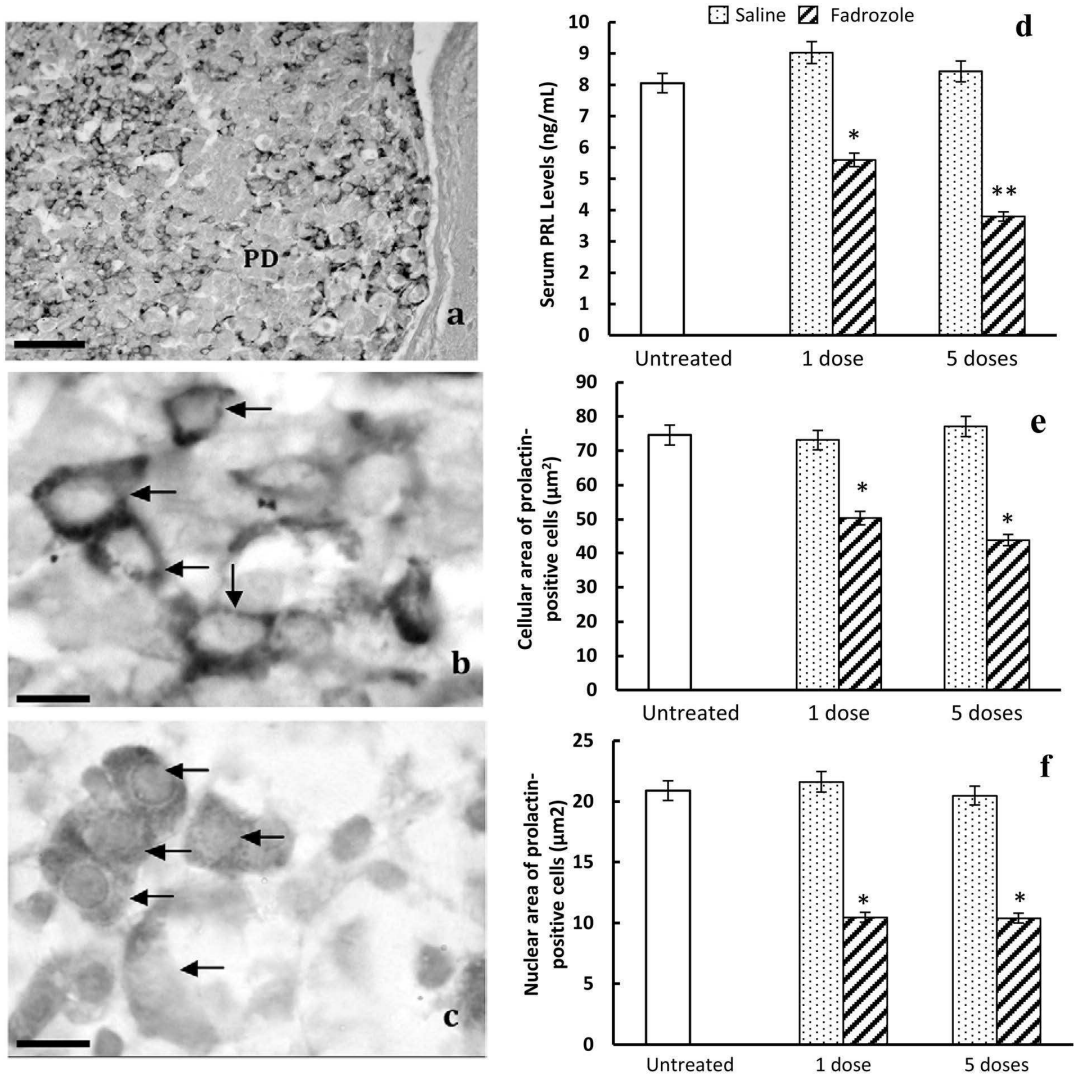



**Fig. 7.** Effects of fadrozole on prolactin-positive cells in the pituitary of male rats. **(a)** General disposition of prolactin-positive cells in the gland. **(b)** Irregular and strongly stained prolactin-positive cells (arrows) in untreated male rats. **(c)** Polygonal and weakly stained prolactin-positive cells (arrows) after 5 doses of fadrozole. **(d)** Plot showing the *in vivo* effects of the 1 or 5 doses of fadrozole on the serum levels of prolactin (*p<0.05, **p<0.01, with respect to untreated animals). Morphometric effect of treatment with fadrozole on cellular **(e)** or nuclear **(f)** areas; in both cases fadrozole induces decreases in the sizes (*p<0.01). Scale bar: a: 100 µm; b, c: 12 µm. PD: Pars distalis of pituitary gland. Images reprinted from García-Barrado et al. (2014).

By means of immunohistochemistry, we have previously demonstrated (Carretero et al., 1999a) that aromatase is expressed in the rat hypophysis as from day 17 of prenatal development, the cells positive for the enzyme being more prominent and present in greater numbers as from day 19 up to birth (Fig. 1). Postnatally, towards puberty (around 21 days), differences between males and females begin to appear (Carretero et al., 2003a). Thus, the immunohistochemical expression of aromatase P450 in the adult rat hypophysis is sexually dimorphic (Carretero et al., 1999b). Finally, non-tumoral hypophyses from aged rats hardly express the enzyme (Figs. 2 and 3).

Gonadal steroids play an important regulatory role in the expression of aromatase in the hypophysis of adult rats (Figs. 4, 5, 6). The mRNA signal is more abundant in males than in females; moreover, male pituitaries show more immunohistochemical-positive cells than females, and by western blotting the enzyme has been seen to be more abundant in males than in females. The expression of this enzyme is clearly sex-dependent and it can also be modified by castration and gonadal steroid administration (Carretero et al., 2011).

Treatment with aromatase inhibitors induces morphometric alterations and changes in the cellular proliferation of some hypophyseal lineages, such as lactotroph cells (Figs. 7,8) or LH-producing gonadotroph cells in mice knocked-out for aromatase (Fig. 9) (see García-Barrado et al., 2014).

Although the above findings are suggestive of a defined role for hypophyseal aromatase, the immunohistochemical expression of the enzyme does not necessarily imply that it exerts a major

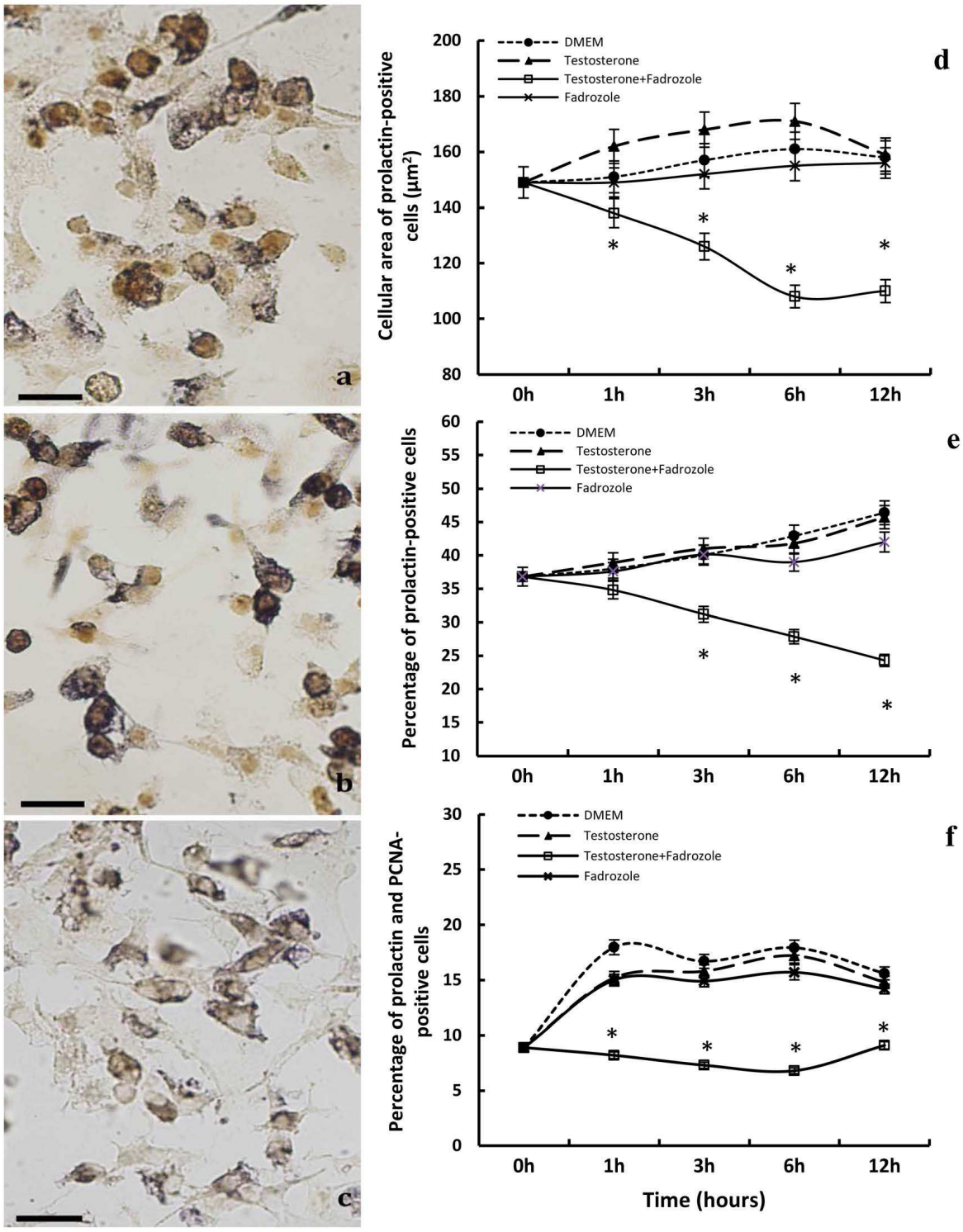



**Fig. 8.** Effects of *in vitro* treatment with fadrozole on prolactin-positive cells. Double immunocytochemical staining for PCNA (brown) and prolactin (dark blue-grey) in control dishes **(a)**, testosterone-treated dishes **(b)**, and testosterone- and fadrozole-treated dishes **(c)**. **(d)** Plot showing the decrease in cellular area at the different time-points assayed; from 1 to 12 hours of treatment a significant decrease (*p<0.05) was observed. **(e)** The percentage of prolactin-positive cells decreases as from 3 to 12 hours of treatment (*p<0.01). **(f)** The percentage of proliferating prolactin-positive cells decreases as from 1 to 12 hours of treatment (*p<0.01). Scale bar: a, b, c: 50 µm. Images reprinted from García-Barrado et al. (2014).

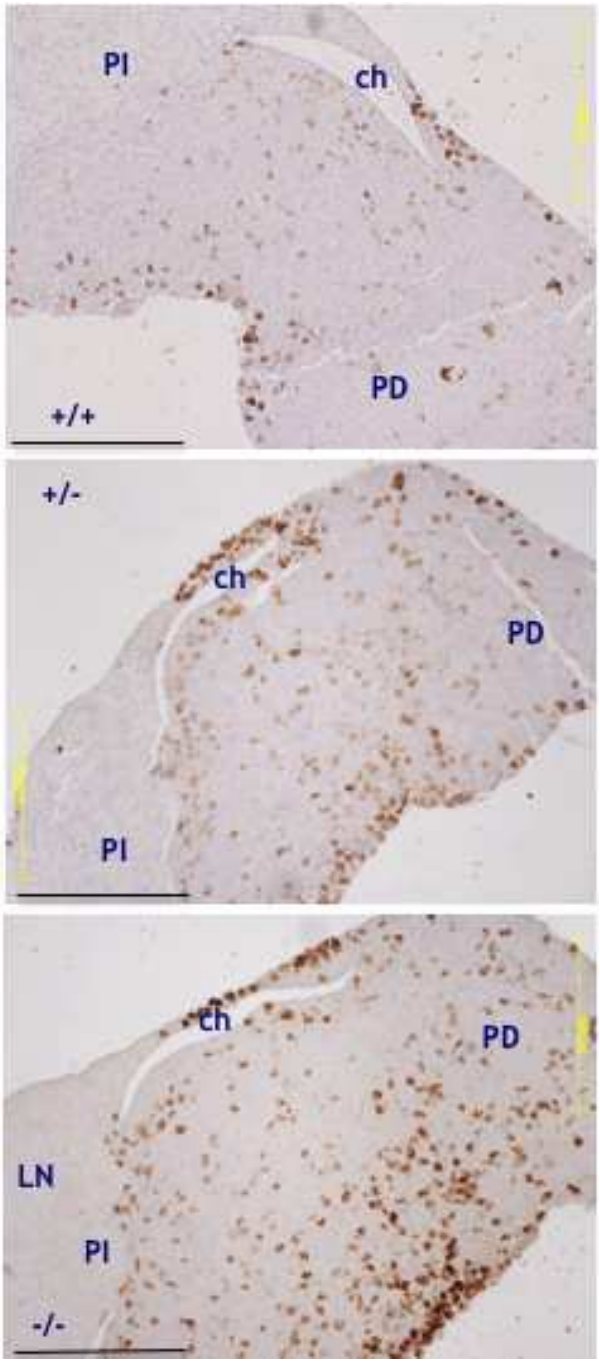



**Fig. 9.** Frontal sections of the hypophysis of female rats of 22 months of age, in which it is possible to note the increase in LH-positive cells in the aromatase knock-out mice with respect to the wild-type and the heterozygotes. **+/+**: wild type. **+/-**: heterozygote. **-/-**: knock out. PD: Pars distalis, PI: Pars intermedia, LN: Neural lobe, ch: pituitary cleft. Scale bars: 500 μm.

biological action at hypophyseal level. Nevertheless, the observation of the strong correlation of the expression in the same hypophyseal cell (Fig. 10) of aromatase and of the estrogen receptor α (Carretero et al., 2009) suggests that the enzyme would exert a hypophyseal auto-paracrine effect.

## AROMATASE P450, ESTROGENS AND PROLACTIN

For some time it has been clear that estrogens, and in particular estradiol, are important regulators of prolactin synthesis (Baker et al., 1963; MacLeod et al., 1969; Neil et al., 1971; Maurer and Gorski, 1977). This is done through a biphasic transformation (Shull and Gorski, 1984, 1985, 1986; Carrillo et al., 1987) that culminates in an increase in prolactin mRNA levels (Shupnik et al., 1979; Vician et al., 1979; Maurer, 1982), estradiol being an agent with a direct action on the gene able to alter transcription. The lactotroph cells that produce the hormone have estrogenic receptors (Friend et al., 1994).

It is unanimously accepted that estrogens cause increases in prolactin release (Carr et al., 1977; Giguère et al., 1982; Chang et al., 1982; Deyo and Miller, 1982; Barraclough et al., 1986; Pérez et al., 1986; Kubota et al., 1989; Veldhuis et al., 1989) and that chronic treatment with estradiol elicits, in an initial phase, clear signs of hyperactivity and hyperplasia in lactotroph cells (Goluboff and Ezrin, 1969; Poole et al., 1980; Scheithauer et al., 1990). It also causes hyperprolactinaemia, with a clear decrease in hormone accumulation within hypophyseal cells and the disappearance of secretory granules as seen under the electron microscope (Fig. 11).

Lactotroph cells show differential signs, depending on sex (Carretero et al., 1989). After the administration of estradiol to adult male rats, lactotroph cells acquire secretory properties and morphological characteristics similar to those found in females (Carretero et al., 1991), and estrogens are able to transform GH cells into prolactin cells or into mamo-somatotroph cells able to produce both hormones (Kineman et al., 1992).

Estrogens act through the coupling of the estrogen to estrogen-receptor complex to the response

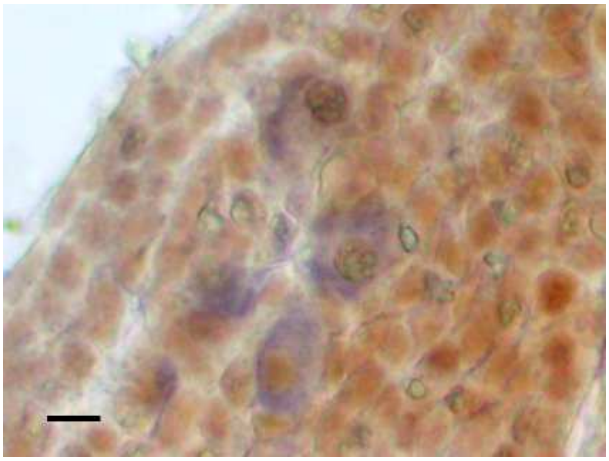


**Fig. 10.** Coexistence of aromatase P450 and estrogen receptor α in cells of the pituitary gland of a male rat. Scale bar: 10 μm. Image reprinted from Carretero et al. (2009).

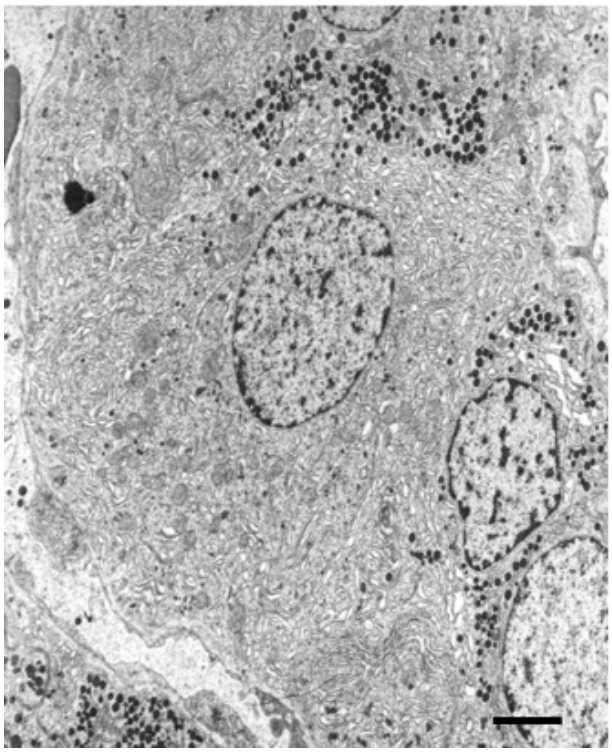


**Fig. 11.** Degranulated appearance, with a strong development of the rough endoplasmic reticulum and the Golgi apparatus, typical of prolactin cells after long-term treatment with estradiol. Scale bar: 1.5 µm.

elements specific to estrogens in the promoter of the gene encoding prolactin synthesis (Somasekhar and Gorski, 1988; Waterman et al., 1988; Crenshaw et al., 1990; Lannigan and Notides, 1990; Schaufele, 1999). Additionally, they play an important mitogenic role, stimulating the proliferation of lactotroph cells (Lloyd et al., 1975; Yamamoto et al., 1986; Pérez et al., 1986; Amara et al., 1987; Lloyd et al., 1988). They act directly on these cells, although these effects could be partially mediated by a similar effect on VIP-producing hypophyseal cells (Prysor-Jones et al., 1988; Lam et al., 1990; Kasper et al., 1992; Carrillo and Phelps, 1992; Carretero et al., 1992, 1995, 1998). VIP is a peptide that, in an auto-paracrine way, stimulates prolactin and lactotroph cells (Carretero et al., 2006). The estrogenic effects on prolactin cells could also be mediated by galanine (Kaplan et al., 1988; Hsu et al., 1990; Vrontakis et al., 1989), which regulates the basal or stimulated activity of lactotroph cells (Wynick et al., 1993).

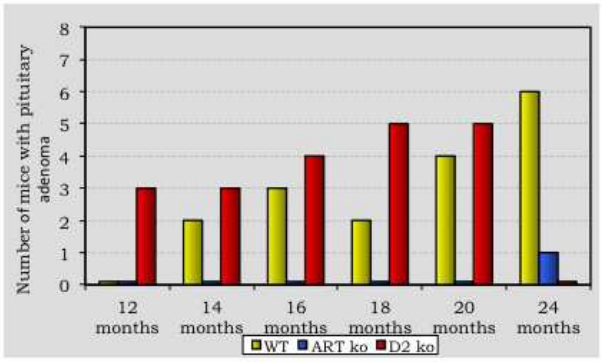
Although other factors may be involved in these processes, such as interleukins (Carretero et al., 2013), dopamine (Gibbs and Neill, 1978; Kelly et al., 1997; Saiardi et al., 1997), or TRH (Tashjian et al., 1971; Bowers et al., 1971; Jacobs et al., 1971; Carretero et al., 2003b), the direct action of estrogens on lactotroph cells is well documented.

Hypophyseal aromatase is produced in different cell types (Galmiche et al., 2006a), among them normal and tumoral lactotroph cells (Carretero et al., 2002). This is of particular relevance since the transformation of aromatizable androgens into estradiol means that in males the androgenic inhibitory effect on prolactin is transformed locally and intracellularly into a stimulatory effect, allowing the population of lactotroph cells to be high in this sex. The treatment of male rats with aromatase inhibitors elicits a decrease in the activity and proliferation of lactotroph cells (Fig. 8).

### *Possible involvement of aromatase P450 in the pathogenesis of prolactinomas*

Hypophyseal tumors that develop from adenohypophyseal cells are one of the commonest types in intracranial pathology, representing around 10% of all brain tumors. They have often been detected in autopsy series from undiagnosed patients (see the comparative study of Molitch, 2002). Most are formed from neoplastic clones rather than as hyperplasias in response to alterations in endocrine regulation (Herman et al., 1990; Jacoby et al., 1990), mainly hypothalamic, although currently the mechanisms involved in the genesis of hypophyseal tumors are not fully understood.

Prolactinomas are the most frequent tumors among the functioning tumors of the hypophysis (Horvath and Kovacs, 1991), together with adenomas of gonadotroph cells (Molitch, 2002), and some lines of evidence suggest that their appearance would be due to a heterogeneous disorder with a complex pathogenesis and a multifactorial etiology.



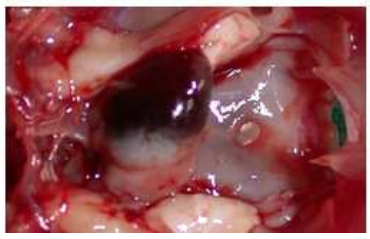



**Fig. 12.** Macroscopic image of a pituitary adenoma observed in a mouse knocked-out for the dopaminergic D2 receptor. As shown in the image, spontaneous pituitary adenomas are relatively frequent in wild-type mice from 14 months of age, their increasing prevalence significantly at 20 and 24 months (yellow bars). These adenomas appear infrequently in mice knocked-out for aromatase (blue bars) and are more prevalent in mice knocked-out for the D2 receptor (red bars).

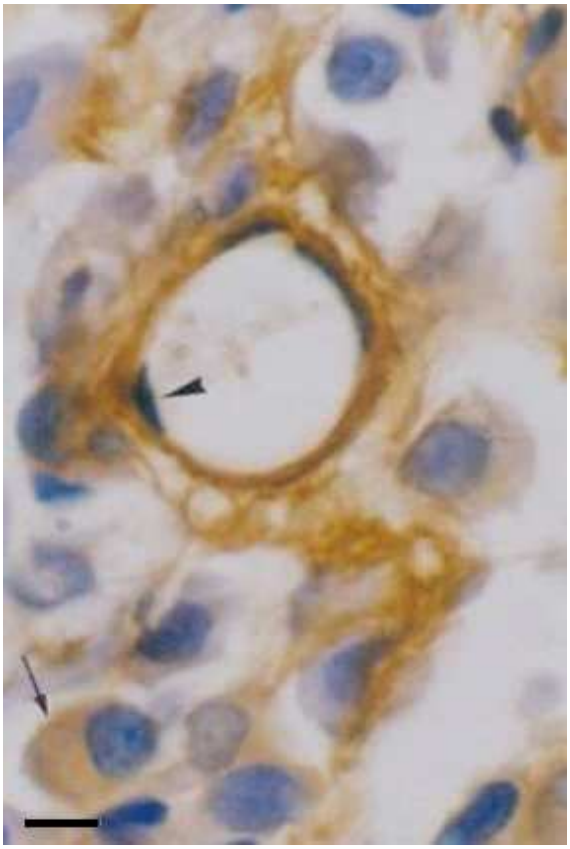


**Fig. 13 (above).** Aromatase positivity (brown) according to the immunocytochemistry in the endothelial cells (arrowhead) and glandular cells (arrow) of a rat prolactinoma. Scale bar: 5 µm. Image reprinted from Carretero et al. (2002), with permission from Springer Science+Business Media.

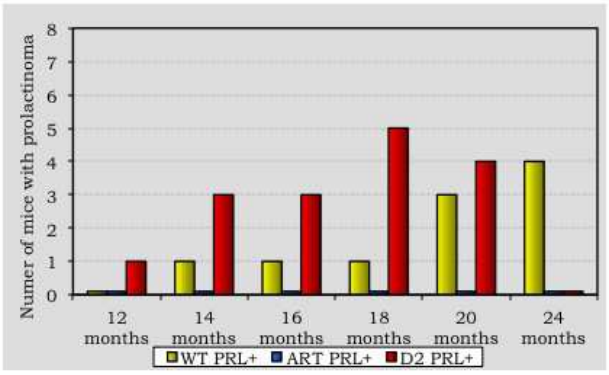



**Fig. 15 (above).** Graph showing the absence of spontaneous prolactinomas in mice knocked-out for aromatase (blue bars) as compared with the occurrence of these tumors with the passage of time in wild-type mice (yellow bars) and mice knocked out for the D2 (red bars) receptor.

**Fig. 16 (right). (a)** Western blot of aromatase in spontaneous human prolactinomas revealing the overexpression of aromatase in the bands. **(b)** Section of a human prolactinoma showing aromatase positivity (brown) after *in situ* hybridization for human aromatase mRNA. Scale bar: 10 µm. Image reprinted from Carretero et al. (2013), with permission from Elsevier.

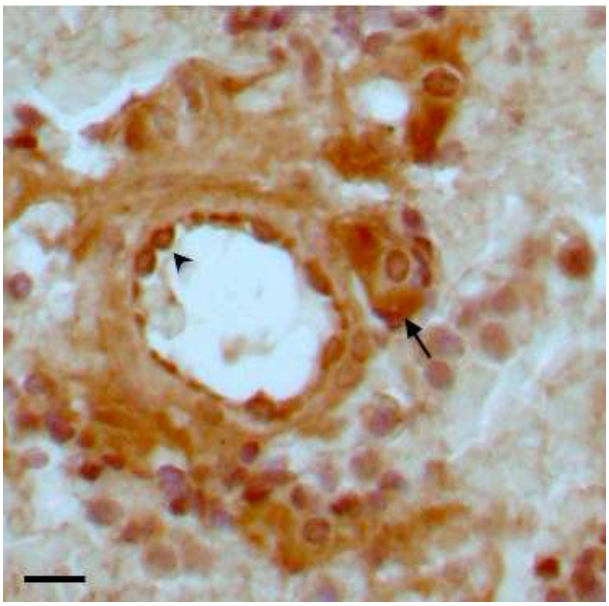
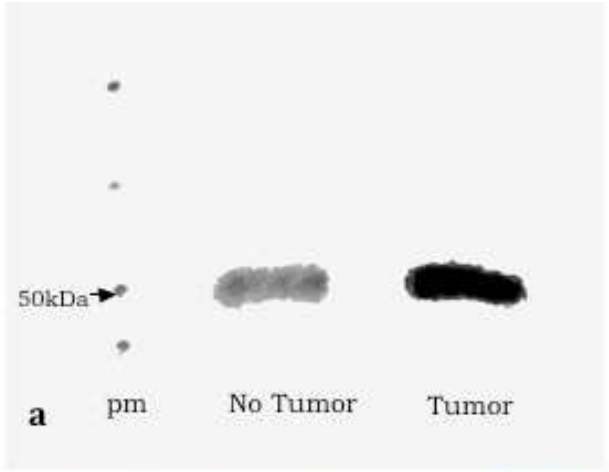
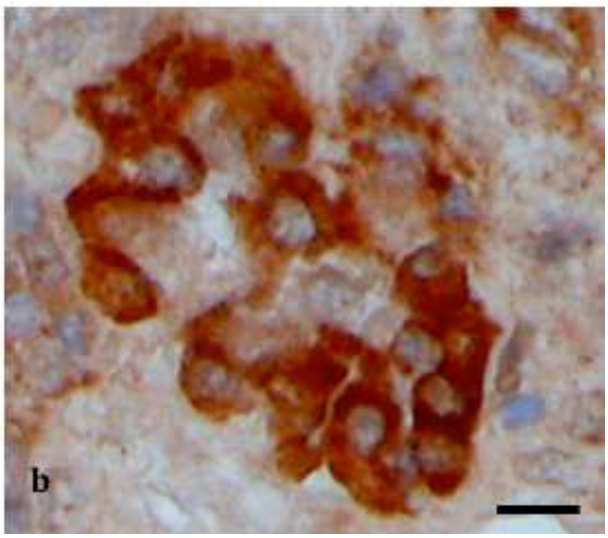


**Fig. 14 (above).** Spontaneous prolactinoma in a woman, similar to the situation in rodents. A positive immunocytochemical reaction to aromatase can be seen in endothelial (arrowhead) and glandular (arrow) cells. Scale bar: 16 µm.



It should be also considered that deficits in the dopaminergic inhibition of prolactin secretion cause hypertrophy of lactotroph cells (Olivereau et al., 1988; Schechter et al., 1988; Rossi et al., 1992) and, probably, the development of these tumors. In fact, mice knocked out for the D2 receptor develop these tumors, although they require gonadal integrity for them to occur (Hentges and Low, 2002). At our laboratory, we have observed an increase in the frequency and an advance in the age of appearance of hypophyseal adenomas in mice KO for the D2 receptor (Fig. 12).

One of the factors that have been implicated in the genesis of prolactinomas is estradiol (Wingrave et al., 1980; Phelps and Hymer, 1983; Gooren et al., 1988; Molitch, 2001; Heaney et al., 2002), because the tumor develops following chronic treatment with the steroid. Since the pituitary produces estradiol through the action of aromatase, it is not unreasonable to speculate that the development of pituitary tumors, and in particular the development of prolactinomas, could be related to the local production of estradiol from testosterone through the action of aromatase.

We also have collected evidence suggesting an important relationship between the pathogenesis of prolactinomas and the overexpression of aromatase in the hypophysis of rodents and humans. For this, we analyzed two series, one of 105 adenomas obtained from female Sprague-Dawley rats of 24 months of age and the other of 87 spontaneous adenomas obtained during surgical treatment of women aged between 23 and 67 years old. In both cases, immunoreactivity to the enzyme appeared in endothelial cells (arrowhead in Figs. 13 and 14) and glandular cells (arrow in Figs. 13 and 14).

The importance of the involvement of aromatase in the development of prolactinomas is seen upon observing that mice KO for aromatase do not develop prolactinomas (Figs. 15 and 16).

The overexpression of aromatase in human prolactinomas is associated with the presence of the estrogen receptor α and overexpression of the mitogen coactivator AIB1 (Fig. 17). Of special relevance is the intracellular distribution of the coactivator in prolactinomas since, when it is expressed intranuclearly, cell proliferation is increased and apoptosis is inhibited, quite the opposite occurring

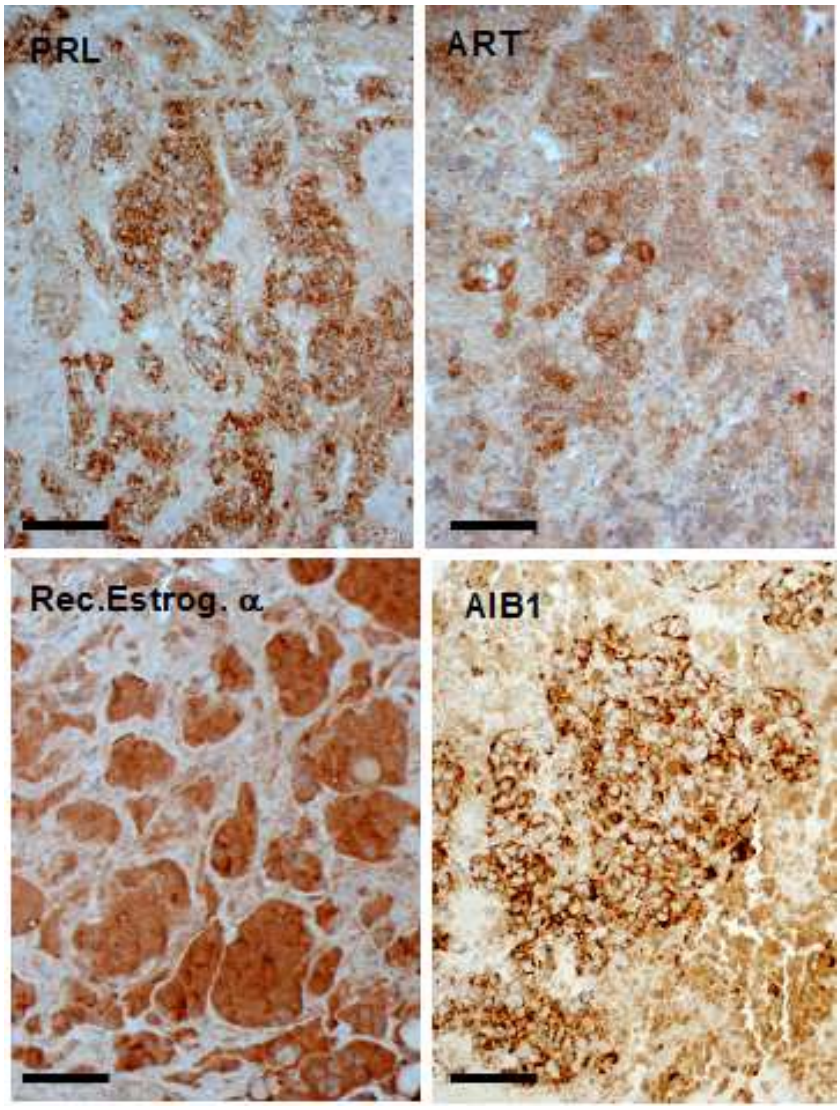



**Fig. 17.** Sections from the same human prolactinoma, where it is possible to observe the immunoreactivity to prolactin (PRL), aromatase (ART), estrogen receptor α and the mitogen coactivator AIB1. Scale bars: 50 µm. Images reprinted from Carretero et al. (2013), with permission from Elsevier.

when the coactivator is arrested in the cell cytoplasm (Carretero et al., 2013). Other authors have corroborated the expression of aromatase in prolactinomas and its relationship with the behavior of the tumor (Akinci et al., 2013). It has been demonstrated clinically that although dopaminergic agonists are effective at braking the growth of prolactinomas this is accompanied by a hypergonadism that is only reversed after testosterone has been added to the treatment; however, this treatment induces an exacerbation of the prolactinoma. This clinical observation is not seen if the treatment with testosterone is associated with the use of aromatase antagonists (Heidari et al., 2010).

In sum, the available experimental evidence attributes to the pituitary gland (thanks to the involvement of aromatase) the capacity to produce estradiol locally in several types of endocrine cells. This would make aromatase an auto-paracrine regulatory factor for the maintenance of the population of lactotroph cells or modulation of the action of central or peripheral regulators. Its overexpression seems to be involved in the pathogenesis of prolactinomas.

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