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ORIGINAL PAPER

Histological characterization of gonadotropin-releasing hormone (GnRH) in the hypothalamus of the South American plains vizcacha (*Lagostomus maximus*)

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Abstract In contrast to most mammalian species, females of the South American plains vizcacha, *Lagostomus maximus*, show an extensive suppression of apoptosis-dependent follicular atresia, continuous folliculogenesis, and massive polyovulation. These unusual reproductive features pinpoint to an eventual peculiar modulation of the hypothalamo-hypophyseal-gonadal axis through its main regulator, the gonadotropin-releasing hormone (GnRH). We explored the hypothalamic histological landscape and cellular and subcellular localization of GnRH in adult non-pregnant *L. maximus* females. Comparison to brain atlases from mouse, rat, guinea pig and chinchilla enabled us to histologically define and locate the preoptic area (POA), the ventromedial nucleus, the median eminence (ME), and the arcuate nucleus (Arc) of the hypothalamus in vizcacha's brain. Specific immunolocalization of GnRH was detected in soma of neurons at medial POA (MPA), ventrolateral preoptic nucleus, septohypothalamic nucleus (SHy) and Arc, and in beaded fibers of MPA, SHy, ventromedial hypothalamic nucleus, anterior hypothalamic area and ME. Electron microscopy examination revealed GnRH associated to cytoplasmic vesicles of the ME and POA neurons, organized both in core and non-core vesicles within varicosities, and in neurosecretory vesicles within the myelinated axons of the MPA. Besides the peculiar and unusual features of folliculogenesis and ovulation in the vizcacha, these results show that hypothalamus histology and GnRH immune-detection and localization are comparable to those found in other mammals. This fact leads to the possibility that specific regulatory mechanisms should be in action to maintain continuous folliculogenesis and massive polyovulation.

Keywords Hypothalamus · *Lagostomus maximus* · Plains vizcacha · GnRH · Immunohistochemistry · Electron microscopy

Introduction

Gonadotropin-Releasing Hormone (GnRH)[1] or Luteinizing Hormone-Releasing Hormone (LHRH) is a decapeptide involved in the modulation of the hypothalamo-hypophyseal-gonadal (HHG) axis. According to its amino acid sequence composition, function, localization, and embryonic origin 24 GnRH peptides with similar structures have been identified in the nervous tissue from protochordates to

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[1] 3V: Third ventricle, ac: anterior commissure, AH: anterior hypothalamus, Arc: Arcuate Nucleus of the hypothalamus, ArcM: arcuate hypothalamic nucleus medial, f: formix, E_2: Estradiol, FSH: Follicle-stimulating hormone, GnRH and LHRH: Gonadotropine-Releasing Hormone, HHG: Hypothalamo-hypophyseal-gonadal axis, LH: Luteinizing hormone, : Medial Eminence, MPA: medial preoptic area, Pg: Progesterone, oc: optic chiasm, POA: Preoptic Area of the hypothalamus, RM: recessus mammillaris, SHy: septohypothalamic nucleus, VLPO: ventrolateral preoptic area, VMH: ventromedial hypothalamus, VMN: Ventromedial Nucleus of the hypothalamus, VMPO: ventromedial preoptic area.

vertebrates, in which the NH_2- and COOH-terminal sequences, which are essential for receptor binding and activation, are conserved (Lethimonier et al. 2004; Millar 2005; Tsai 2006; Tsai and Zhang 2008).

GnRH is synthesized by a discrete specialized group of neurons scattered throughout the Preoptic Area (POA) of the basal forebrain, the Ventromedial Nucleus (VMN) of the Hypothalamus, and the Arcuate Nucleus (Arc) in the mammalian postnatal brain (Urbanski et al. 1991, 1992; Silverman and Witkin 1994). The majority of the hypothalamic GnRH secreting neurons project their processes towards the Median Eminence (ME) releasing GnRH into the hypothalamo-hypophyseal portal circulation that transports the hormone to the anterior pituitary gland where it binds to its specific receptor (Krey and Silverman 1978; Silverman et al. 1987; Silverman and Witkin 1994, Witkin et al. 1995; Yin et al. 2009a, b) and stimulates gonadotropins synthesis.

GnRH synthesis and release is under steroid modulation. Progesterone (Pg) and Estradiol (E_2) provide modulation on both pulsatile and basal GnRH secretion (Goodman and Karsch 1980; White et al. 2007; Yin et al. 2009a, b). Androgens also provide negative feedback to GnRH secretion and HHG axis in male rats and monkeys (Kawakami and Winters 1999). In female rhesus monkeys and lambs, an excess of androgens may disrupt communication of negative feedback signals from Pg and stimulate GnRH release (Dumesic et al. 1997; Robinson et al. 1999).

Shortly after birth, GnRH expression increases gradually preceding the increase in GnRH secretion that drives to puberty (Ebling and Cronin 2000). Neurons expressing GnRH are the central regulators of fertility in mammals. Pubertal development and adult reproductive function depend on the activation of the HHG axis. In order to maintain pituitary function, GnRH is released in discrete pulses separated by periods of little to no secretion, from puberty up to menopause, excepting pregnancy (Belchetz et al. 1978). Pulsatile pattern must vary across the reproductive cycle to differentially regulate the releasing of the two gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) responsible for gonadal steroidogenesis and gametogenesis (Marshall and Griffin 1993; Wildt et al. 1981). Low GnRH pulse frequency favors FSH release whereas high pulse frequency stimulates LH (Burger et al. 2008; Ciccone et al. 2010; Wildt et al. 1981).

The South American plains vizcacha (*Lagostomus maximus*) is a caviomorph hystricognatha rodent inhabiting the Southern area of the Neotropical region, especially the Pampean region of Argentina (Jackson et al. 1996). General aspects of its reproductive biology investigated by Barbara Weir (1971a, b) pointed out that *L. maximus* female displays exceptional and unique reproductive characters. These females ovulate up to 800 oocytes per reproductive cycle, representing the highest ovulation rate so far recorded for mammals (Weir 1971a, b). Despite massive ovulation, between 10 and 12 oocytes result successfully fertilized and implanted (5 or 6 in each uterine horn) and only 1 or 2 embryos, those localized nearest the cervix, are gestated to term whereas the remaining anteriorly implanted fetuses are resorbed (Weir 1971b). The massive ovulation in *L. maximus* arises from a strong suppression of apoptosis-dependent follicular atresia that is driven through an over-expression of the anti-apoptotic *BCL2* gene and a basal or absent expression of pro-apoptotic *BAX* gene both in the developing and adult ovary (Jensen et al. 2006; Leopardo et al. 2011). This pattern of gene expression supports a continuous oogenesis and folliculogenesis in the mature ovary that seems to execute constitutive massive germ cell elimination characterizing the mammalian ovary through polyovulation (Jensen et al. 2006, 2008).

Considering the singularity of the reproductive features of female *L. maximus*, specially polyovulation, we explored the histology of the hypothalamic region of *L. maximus* and undertook an extensive analysis on the distribution and localization of GnRH in the main nuclei of the hypothalamus involved in the regulation of the HHG axis.

Materials and methods

Animals

Adult female plains vizcachas (2.5–3.0 kg body weight) were captured from a natural population at the Estación de Cría de Animales Silvestres (ECAS), Ministry of Agriculture, Villa Elisa, Buenos Aires province, Argentina. All experimental protocols concerning animals were reviewed and authorized by the Ethics and Research Committee of Universidad Maimónides, Argentina. Handling and sacrifice of animals were performed in a humane manner and in accordance with all local, state and federal guidelines for the care and utilization of laboratory animals. Husbandry of the animals met the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (Health Research Extension Act of 1985). Appropriate procedures were performed to minimize the number of animals used and suffering. A total of 10 non-pregnant plains vizcachas of similar ages were used in this study. Age was determined through the body size and weight, and dry crystalline lens weight according to Jackson (1986). All animals showed comparable values of serum estradiol (27.2 ± 1.8 pg/ml) and progesterone (1.09 ± 0.21 pg/ml), determined according to Jensen et al. (2008).

Tissue collection

Animals were intraperitoneally anesthetized with a ketamine (Ketamine Clorhidrate, Holliday Scott S.A.): xilacine (Xilacine Clorhidrate, Richmond Laboratories, Veterinary Division) solution (10:1, w/v, 0.3 ml/Kg of body weight), sacrificed by intracardiac injection of Euthanyle (0.25 ml/Kg body weight, Sodic Penthobarbithal, Sodic Diphenilhidanthoine, Brouwer S.A.), and brains immediately removed.

Histological analysis and immunohistochemistry

After removal, five brains were coronally sectioned in blocks of 5–7 mm thick, fixed in cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 72 h, dehydrated through a graded series of ethanol and embedded in paraffin. For each specimen, the brain region containing the hypothalamus was entirely cut to serial coronal sections (5 μm thick) and mounted onto cleaned coated slides. Sections were dewaxed in xylene and rehydrated through a decreasing series of ethanol (100, 95 and 70%). One in ten sections were separated to perform classical Haematoxylin staining to localize hypothalamic nuclei by comparison with mouse (Franklin and Paxinos 2008), rat (Paxinos and Watson 2009), rabbit (Shek et al. 1986), domesticated guinea pig and long-tailed chinchilla (Welker et al. 2010) brain atlases. After localization of the four hypothalamic nuclei: POA, VMN, ME and Arc, adjacent sections were used for immunohistochemical assay. Antigen retrieval was performed by boiling sections in 10 mM sodium citrate buffer (pH 6) for 20 min, followed by 20 min cooling at room temperature. Then, endogenous peroxidase activity was blocked with 2% hydrogen peroxide in phosphate buffer for 30 min. After that, sections were incubated with a blocking solution containing 10% normal horse serum in saline phosphate buffer, pH 7.4, for 1 h. GnRH immunoreactivity was detected by incubating slides overnight at room temperature with a mouse anti-GnRH monoclonal antibody (at 1:200 dilution) that recognizes the N-terminal conservative region of the mature form of the mammalian GnRH subtype of a wide species spectrum (MAB5456 Chemicon—Millipore Corporation, Billerica, MA, USA). Its specificity was corroborated in adjacent sections by omission of the primary antibody or by pre-absorption of the anti-GnRH antibody with LHRH synthetic peptide (10 μg, 1:20 dilution, L7134 Sigma Co, St. Louis, MO, USA) incubated over night in a rotator at room temperature followed by centrifugation for 20 min at 15,000*g*. As it is known the role of GnRH over the function and growth of placenta and on embryo development (Raga et al. 1999; Wolfahrt et al. 1998), plains vizcacha's placenta-to-term sections were employed as positive tissue control. Immunoreactivity was revealed with biotinylated horse anti-mouse IgG followed by incubation with avidin–biotin complex (ABC Vectastain Elite kit, Vector Laboratories, Burlingame, USA). The reaction was visualized with 3,3′ diaminobenzidine and intensification with nickel ammonium sulphate (DAB kit, Vector Laboratories, Burlingame, USA) that yields a black product. Finally, treated sections were dehydrated through a graded series of ethanol (70, 95 and 100%), cleared in xylene and coverslipped.

Electronic microscopy immunohistochemistry

In order to analyze ultracellular GnRH localization, Electronic Microscopy Immunohistochemistry assay was performed according to Goodman et al. (2004). After removal, five brains were coronally sectioned in blocks of 5–7 mm thick, fixed in cold 4% paraformaldehyde, 0.25% glutaraldehyde, in 0.1 M phosphate buffer (pH 7.4, 72 h), and transferred to fresh phosphate buffer. The block of the whole hypothalamus was entirely cut to serial sections (50 μm thick) employing a vibratome (NVSL manual vibroslice, World Precision Instruments Inc., Sarasota, USA). Floating sections were collected in neutral saline buffer (at 0–4°C) and processed for flotation immunohistochemistry to GnRH. Sections were incubated overnight at room temperature with mouse anti-GnRH monoclonal antibody (MAB5456 Chemicon—Millipore Corporation, Billerica, MA, USA, at 1:200 dilution) and visualization was performed with ABC Vectastain Elite and DAB kits (Vector Laboratories, Burlingame, USA) as described above. Following immunohistochemical identification of GnRH by light microscopy, POA and ME regions were dissected out and postfixed in 2% osmium tetroxide containing 1.5% potassium ferricyanide for 2 h, dehydrated in graded alcohols and propylene oxide and embedded in Durcupan (Fluka®). Semithin sections (1 μm thick) were obtained using a Reichert ultramicrotome and examined for GnRH localization in neurons and dendrites. Positive areas were ultrathin sectioned (100 nm), mounted on copper grids, and counterstained with 5% uranyl acetate and 2.5% lead citrate. Specificity was corroborated in adjacent sections by omission of the primary antibody or pre-absorption of the anti-GnRH antibody with 10× LHRH synthetic peptide (L7134 Sigma Co, St. Louis, MO, USA).

Image analysis

Before assays, care was taken on selecting anatomically matching areas among animals for each analyzed hypothalamic nucleus. Histological and immunohistochemical images were analyzed using an optic microscope (Olympus BX40) and captured with an attached digital camera (Olympus Camedia C-5060). Electronic microscopy

sections were examined using a transmission electronic microscope (Zeiss E.M. 10C) and images of GnRH immunoreactivity photographed in a range of 10,000–50,000 magnification. Adobe Photoshop software was used for digital manipulation of brightness and contrast when preparing the shown images.

Results

Histological localization of hypothalamus in the brain of plains vizcacha

Histological examination of coronal brain sections of plains vizcacha compared to brain atlases from mouse, rat, rabbit, guinea pig and long-tailed chinchilla (Franklin and Paxinos 2008; Paxinos and Watson 2009; Shek et al. 1986; Welker et al. 2010) enabled us to identify the localization of the hypothalamic nuclei involved in the regulation of the HHG axis. As shown in Fig. 1, the nuclei of the Preoptic Area (POA) (Fig. 1a), of the Ventromedial Nucleus (VMN) (Fig. 1b), the Median Eminence (ME) (Fig. 1c) and the Arcuate Nucleus (Arc) (Fig. 1d), were identified.

GnRH is distributed throughout the hypothalamus of the plains vizcacha

GnRH was expressed from rostral to caudal coronal regions of plains vizcachas at POA, VMN, ME and Arc (Figs. 2, 3, 4). GnRH immunoreactivity was detected in the cytoplasm and dendrites of neurons scattered throughout the medial preoptic area (MPA) and septohypothalamic nucleus (SHy) (Fig. 2a–c), within beads conformed of circular structures. GnRH immunopositive cells with cytoplasmic immunoreactivity were also detected in the ventrolateral preoptic nucleus (VLPO) (Fig. 2d). Besides, along the VMN and ME, beaded fibers with GnRH immunoreactive varicosities were detected (Figs. 2f, 3a). The ventromedial hypothalamic nucleus (VMH) and the anterior hypothalamic area (AH) of the VMN showed GnRH immunoreactive dendrites, while no immunoreactive soma were found (Fig. 2f). The ME showed GnRH immunoreactive beaded fibers radially orientated with respect to the recessus mammillaris (RM) of the third ventricle (Fig. 3a). Intense GnRH staining was also distributed in the external borders of the ME (Fig. 3a, c), and in terminals of neurosecretory neurons surrounding the primary plexus of the hypothalamic-hypophyseal portal vessels

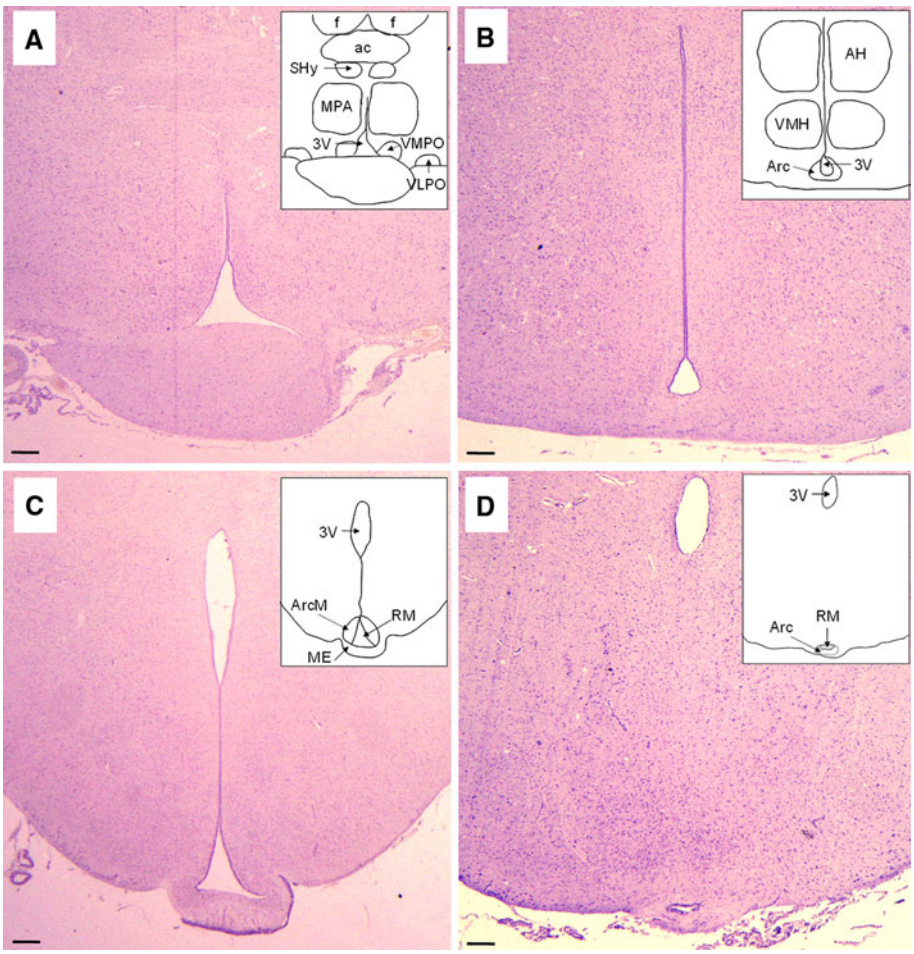

Fig. 1 Histology of the hypothalamus in the brain of plains vizcacha. Representative images of the main hypothalamic nuclei stained with haematoxylin. **a** Preoptic area (POA): medial preoptic area (MPA), septohypothalamic nucleus (SHy), ventromedial preoptic area (VMPO) and ventrolateral preoptic area (VLPO). **b** Ventromedial Nucleus of the hypothalamus (VMN): ventromedial hypothalamus (VMH), anterior hypothalamus (AH) and arcuate nucleus (Arc). **c** Medial hypothalamus: medial eminence (ME) and arcuate hypothalamic nucleus medial (ArcM). **d** Caudal Hypothalamus: Arc. Inserted there are schematic representations of the corresponding hypothalamic nuclei from each region. Third ventricle (3V), anterior commissure (ac), fornix (f), optic chiasm (oc) and recessus mammillaris (RM), were also identified and indicated in the inserted drawings. *Scale bar* 300 μm

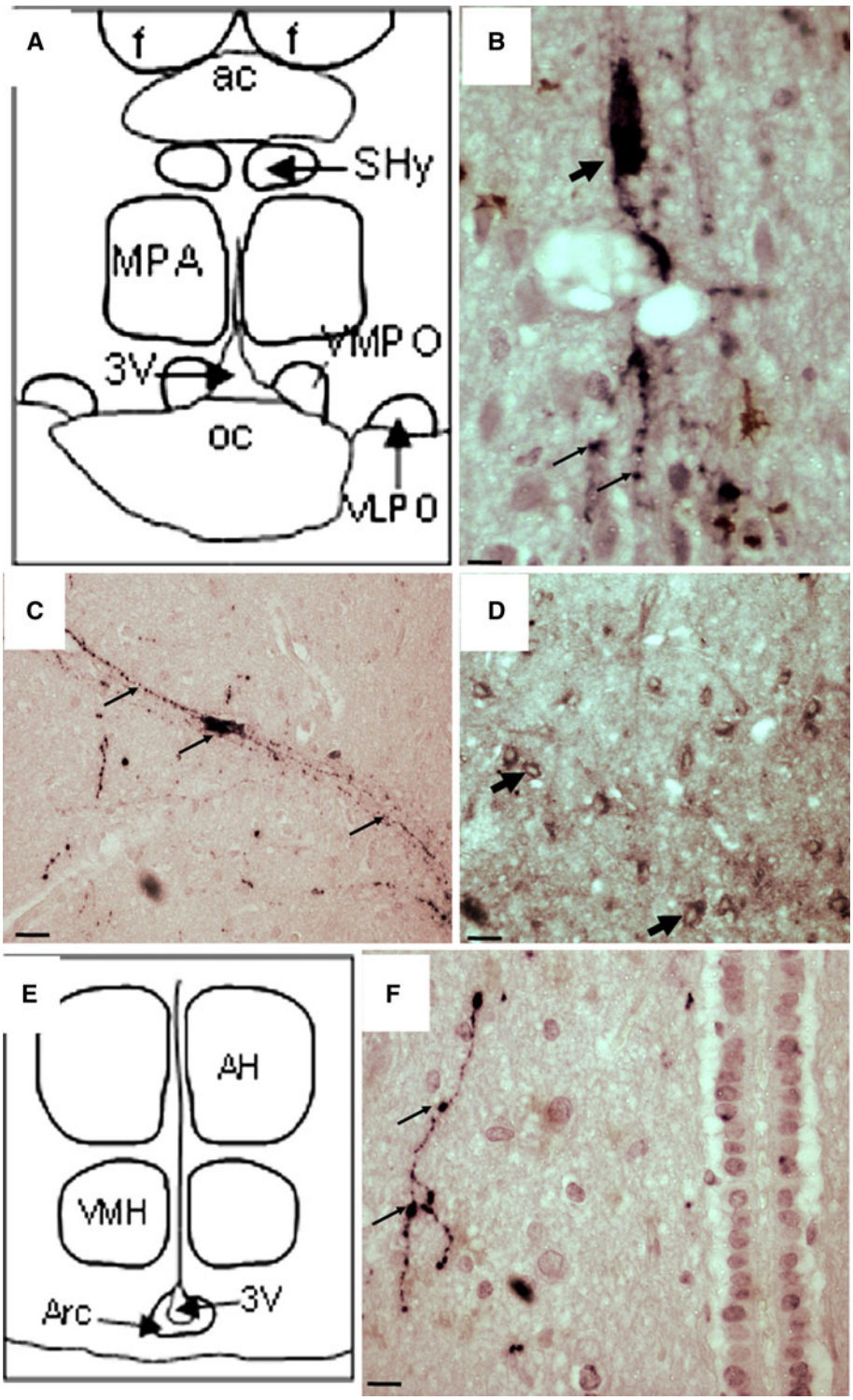

Fig. 2 GnRH localization throughout the rostral hypothalamus of plains vizcacha. **a** Schematic representation of the vizcacha POA of the hypothlamus. **b** GnRH immunoreactivity at soma and dendrites of a neuron in MPA. **c** GnRH immunoreactivity at soma and dendrites of a neuron in SHy. **d** GnRH immunoreactivity at soma of VLPO. **e** Schematic representation of the VMN of the hypothalamus. **f** GnRH immunolocalization in varicosities of dendrites crossing the VMH next to ependymal cells. *Arrows* GnRH immunoreactive varicosities, *arrowheads* GnRH immunoreactive neurons. *Scale bars* **b** 10 μm, **c–d**, **f** 20 μm

(Fig. 3a, d). No GnRH specific labeling was detected after preabsorption of the primary antibody with LHRH synthetic peptide in adjacent ME sections (see arrow in external border of ME and squared vessel in Fig. 3b) or after omission of GnRH primary antibody (not shown). Arc region of the caudal hypothalamus showed GnRH immunoreactive unipolar neurons localized ventrally to the third ventricle (Fig. 4). Cytoplasmic and axonal GnRH staining was observed in this region (Fig. 4c, d respectively). Placenta to term of *L. maximus* was used as positive control tissue

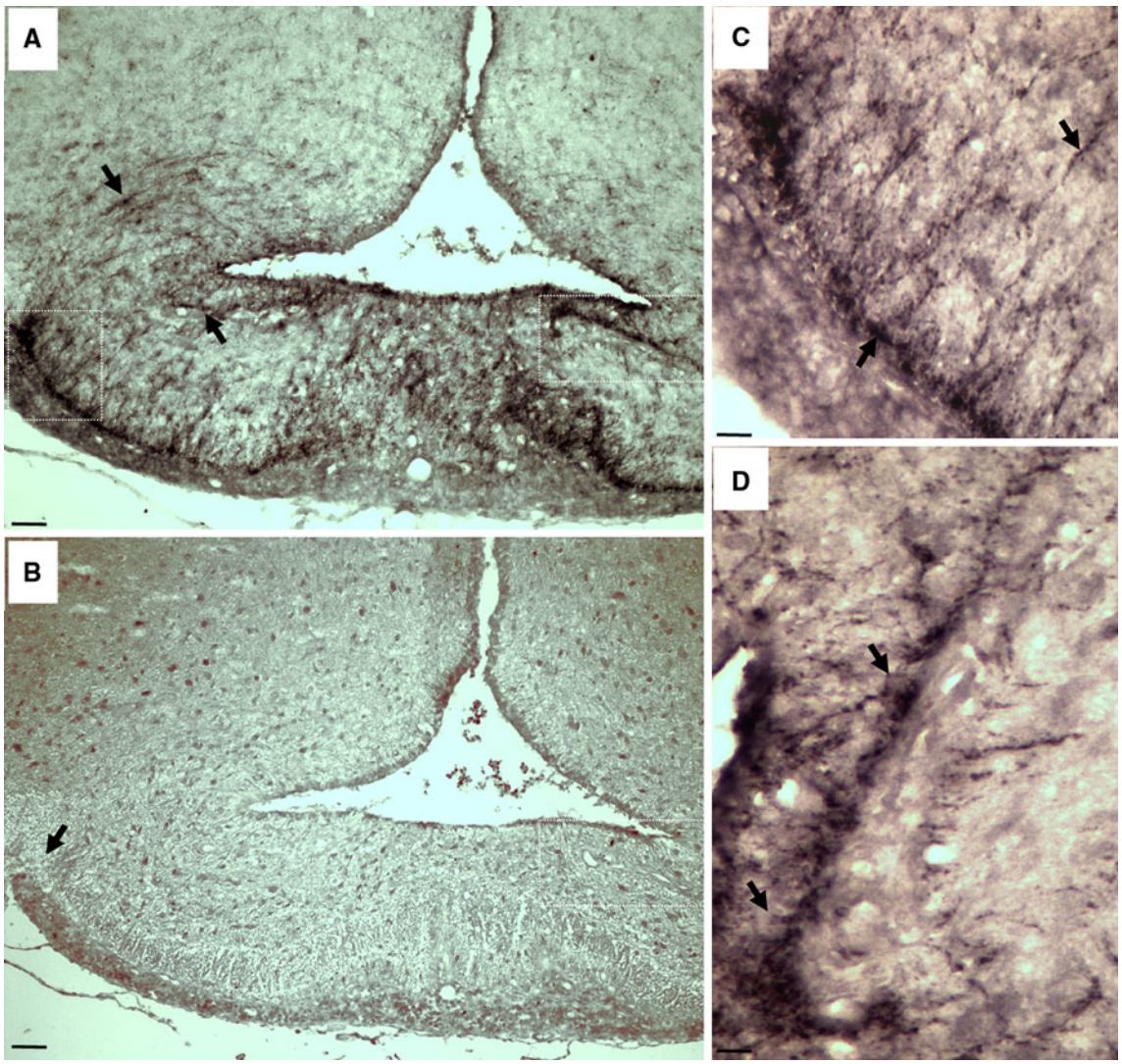

Fig. 3 GnRH localization in the medial hypothalamus of plains vizcacha. **a** GnRH immunoreactivity in varicosities of dendrites at ME (medial eminence). Immunoreactivity is localized in the external borders of ME (*squared region* and **c**), in a radial orientation with respect to the recessus mammillaris (RM) (*arrows* and **c**) and around the primary plexus of the hypothalamic-hypophyseal portal vessels (*squared region* and *arrows* in **d**). **b** Representative image of an adjacent section to **a** with no GnRH specific labeling after preabsorption of the primary antibody with LHRH synthetic peptide. Notice that GnRH immunoreactivity in varicosities is not observed, neither in the external ME zone (*arrow*), nor surrounding the portal vessels (*square*). *Scale bars* **a**–**b** 100 μm, **c** 20 μm, **d** 40 μm

showing specific GnRH immunoreactivity in the maternal-fetal blood exchange area (not shown).

Subcellular localization of GnRH in the hypothalamus of plains vizcacha

High density electron-dense vesicles corresponding to GnRH immunoreactivity were identified throughout the ME and POA of plains vizcachas (Fig. 5). Varicosities containing three types of vesicles: core GnRH immunoreactive (350 nm diameter), non-core GnRH immunoreactive (200 nm diameter) and non-GnRH immunoreactive (250 nm) (Fig. 5a), were observed in the ME. In the POA, clusters of vesicles, 200 nm diameter, in close relationship to axo-dendritic synapses (Fig. 5b), with or without GnRH immunoreactivity were detected (Fig. 5b). In this area, GnRH immunoreactive transfer vesicles near the Golgi apparatus, with a 100 nm diameter, were also evident (Fig. 5c, d). GnRH immunoreactivity over the rough endoplasmic reticulum (Fig. 5a), and over the outer nuclear envelope (Fig. 6b), were also identified. In addition, the ME and the POA showed GnRH immunoreactive neurosecretory vesicles within myelinated axons (Fig. 6c). Sections incubated with the pre-absorbed primary antibody, or after omission of it, did not show GnRH specific labeling neither in the ME, nor in the POA (not shown).

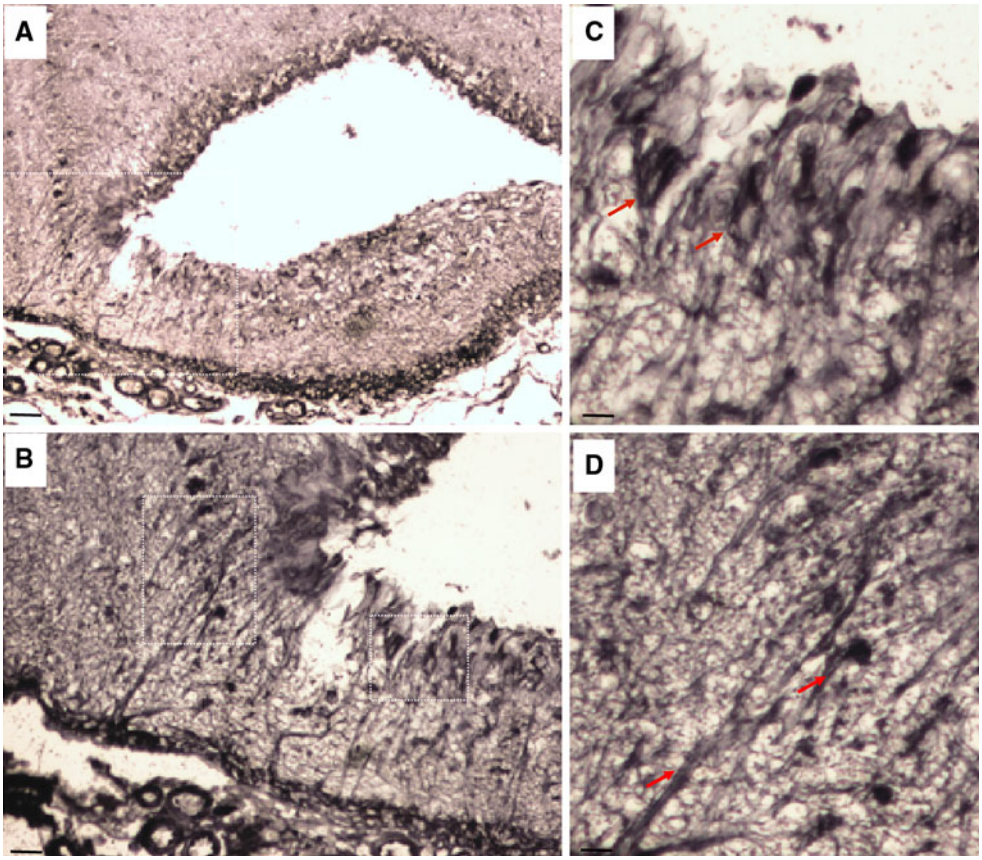

Fig. 4 GnRH localization in the caudal hypothalamus of plains vizcacha. **a** GnRH immunolocalization in the cytoplasm of monopolar neurons of Arc. **b** amplified image of squared region in **a**. **c**, **d** amplified images of left and right squared regions in **b**, showing cytoplasmic (*arrows* in **c**) and axonal (*arrows* in **d**) GnRH immunoreactivity. *Scale bars* **a** 50 μm, **b**–**d** 10 μm

Discussion

The present work is the first reported histological study performed in plains vizcacha's brain. It describes the localization of the hypothalamus with the nuclei involved in the operation of HHG axis, and the expression and distribution of its principal neuropeptide, GnRH.

Three hypothalamic nuclei involved on GnRH synthesis, the POA, the VMN and the Arc, and the ME involved on GnRH secretion, were localized around the third ventricle and below the recessus mammillaris of plains vizcachas's brain. Hypothalamic histological landscape of this animal exhibits no differences with respect to mouse, rat, rabbit, domesticated guinea-pig and long-tailed chinchilla, showing a similar histological pattern and cell distribution around the third ventricle. However, it is worth to note that the forebrain cortex of *L. maximus* shows more pronounced gyrus or folds than the brains of the above mentioned rodents including the hystricognatha long-tailed chinchilla, its closest evolutionary relative (Jackson et al. 1996; Weir 1970).

GnRH localization has been described in the hypothalamus of mouse, rat, guinea pig, lamb and other mammals, by light and electron microscopy (Silverman et al. 1985, 1987, 1990; Shirasawa et al. 2007; Yin et al. 2007). Here we observed GnRH expression at both hypothalamus and placenta-to-term in the vizcacha. Vizcacha showed similar GnRH immunolocalization at cellular and ultracellular levels by light and electron microscopy as previously described in other mammalian species. GnRH distribution was found in dendrites and soma. In dendrites, GnRH expression was restricted to varicosities of the POA, VMN and ME hypothalamic areas, containing GnRH immunoreactive core and non-core vesicles. In soma, GnRH was localized in cytoplasmic vesicles at neurons of MPA, SHy, VLPO and caudal hypothalamic Arc nucleus. In agreement with the previously described package and condensation of GnRH into granules of Golgi apparatus (Naik 1975; King and Anthony 1983; Silverman et al. 1990), we also found GnRH localization in vesicles associated to Golgi apparatus.

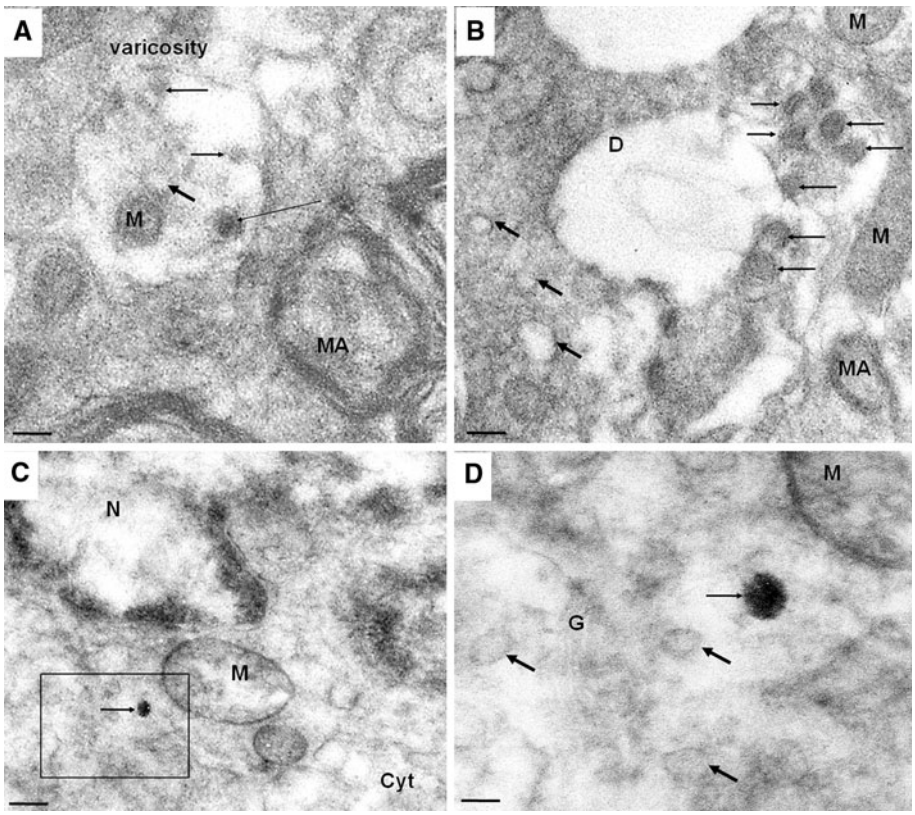

Fig. 5 Subcellular localization of GnRH in vesicles. GnRH immunoreactive vesicles were identified throughout ME and POA of the hypothalamus of plains vizcachas. **a** Representative image of a varicosiy in the ME containing core (*long arrow*) and non core (*short arrows*) GnRH immunoreactive vesicles. Non- GnRH immunoreactive vesicles are also observed into the same varicosity (*thick arrow*). **b** A cluster of GnRH immunoreactive vesicles near an axo-dendritic synapses in the POA (*thin arrows*). Non immunoreactive GnRH vesicles can also be seen in the same picture (*thick arrows*). **c** GnRH immunoreactive transfer vesicle (*thin arrow*) near the Golgi apparatus of a neuron localized in the POA. **d** Magnified image of the inset indicated in **c** showing a GnRH immunoreactive vesicle next to the Golgi apparatus (*thin arrow*) together with non immunoreactive GnRH vesicles (*thick arrows*). *Cyt* cytoplasm, *D* dendrite, *G* Golgi apparatus, *M* mitochondria, *MA* myelinated axon, *N* nucleus. *Scale bars* **a**, **b** 350 nm, **c** 200 nm, **d** 100 nm

GnRH immunoreactive neurons from the POA, or at least a portion of them as suggested by Silverman et al. (1987), are projecting their axons towards the ME, surround the primary plexus of the hypothalamic-hypophyseal portal vessels and release GnRH towards the anterior pituitary gland to modulate ovulation. Distribution of GnRH immunoreactive neurons in the plains vizcacha seems to indicate that this could be the case. However, placement of lesions in the POA of plains vizcacha would shed light on the role of POA on GnRH control of gonadotropin release. Moreover, similar approaches in the Arc or treatments with glutamate monosodium would reveal whether GnRH immunoreactive neurons at the Arc are also projecting towards ME and if they equally contribute to the control of gonadotropin secretion.

Several studies have shown that GnRH can influence the synaptic activity (Dyer and Dyball 1974; Renaud et al. 1975). In line with this, *L. maximus* was found to express GnRH localized near to axo-dendritic synapses of the POA and in neurosecretory vesicles within the axonal fluid of myelinated axons, suggesting that GnRH could be acting as a neurotransmitter besides its central role in the control of ovulation. Detection of GnRH positive neurons in extra-hypothalamic areas in the vizcachas' brain endorses this assumption (data not shown).

The expression of multiple GnRH variants have been reported in a single species. The first identified form of GnRH was isolated from mammalian (mGnRH), porcine and ovine brains (Burgus et al. 1972). Later, two other variants were shown to be expressed in chicken brain (cGnRH or GnRH-I and GnRH-II) together with mGnRH in vertebrates (King and Millar 1982; Miyamoto et al. 1984). A third form was described in guinea pigs (gpGnRH) and also reported in capybara (Jimenez-Liñan et al. 1997; Montaner et al. 2002). In addition, some mammals show a fourth form of GnRH first isolated from salmons (sGnRH) (Sherwood et al. 1986). In the present work, it has been described the hypothalamic localization of GnRH, however, its specific variant is not known. The antibody used in this study identifies the -NH2 group at

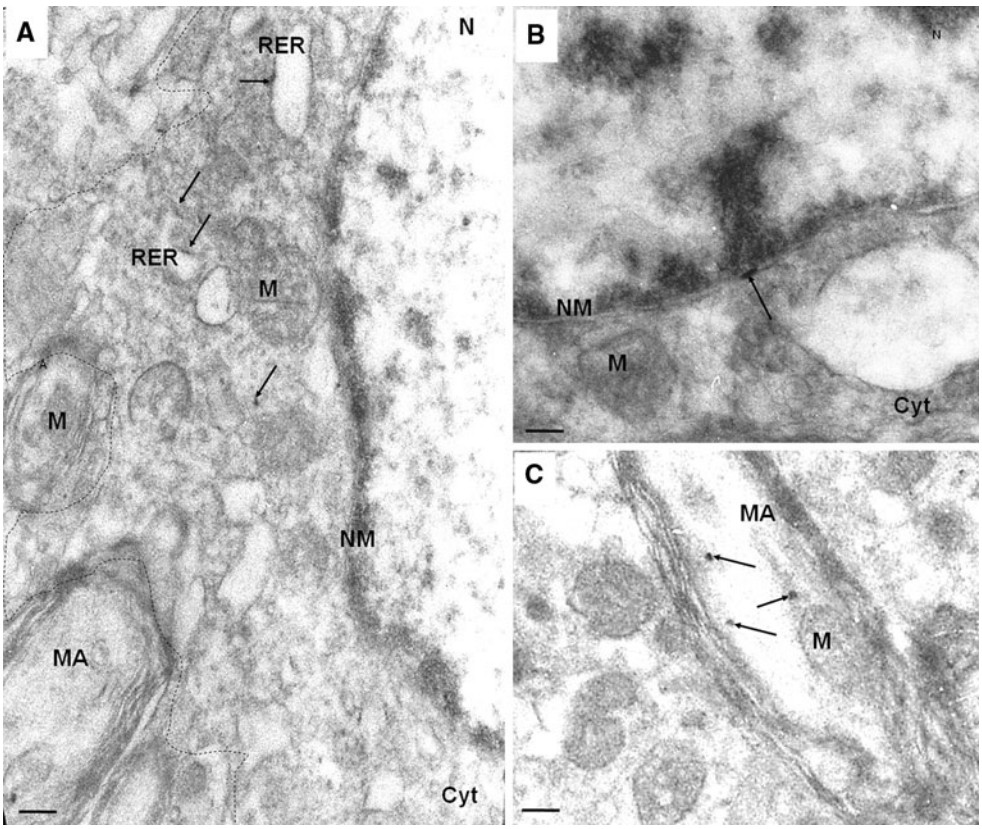

Fig. 6 Subcellular GnRH localization in the hypothalamus of plains vizcacha. GnRH immunoreactivity distributed in neurons of the POA: **a** Representative image of GnRH immunoreactivity over the rough endoplasmic reticulum (*arrows*). *Scratched line* shows the localization of plasmatic membrane. **b** GnRH immunoreactivity over the outer nuclear envelope (*arrow*). **c** GnRH immunoreactivity in neurosecretory vesicles within the fluid of myelinated axons. *Cyt* cytoplasm, *M* mitochondria, *MA* myelinated axon, *N* nucleus, *NM* nuclear membrane, *RER* rough endoplasmic reticulum. *Scale Bars* 200 nm

position 10, a region shared by both mGnRH and gpGnRH. Taking in account that the vizcacha is evolutionary closely related to guinea pig and capibara (Jackson et al. 1996; Weir 1970) it is likely that the immunolocalization of GnRH reported here encompass both variants.

Most mammalian females show a high reduction of germinal mass from birth to puberty that occurs through apoptosis-dependent follicular atresia (Hirshfield 1994). In contrast, the plains vizcacha represents an exception to massive germ cell elimination since it lacks ovarian follicular apoptosis (Jensen et al. 2006, 2008; Leopardo et al. 2011) and shows natural polyovulation reaching up to 800 oocytes per reproductive cycle (Weir 1971a). Those particular features make this animal a valuable experimental model for use in research protocols of fertility and reproduction, giving the opportunity to minimize the number of animals used in each study. On the other hand, the localization of GnRH and its description in the brain of this particular mammal could contribute to a better understanding of HHG axis in ovulation and fertility control. The comparison of plains vizcacha's HHG axis regulation with the HHG axis of other mammals would allow the detection of differential modulation strategies and the finding of possible molecular markers of therapeutic interest.

In conclusion, this research describes cellular and subcellular localization of GnRH in the hypothalamus of plains vizcacha (*Lagostomus maximus*) which has a rather unusual reproductive profile, providing relevant information into the field of comparative biology and an initial step into the understanding of the control of polyovulation in this animal. Future studies to elucidate the modulation of the HHG axis, including transcriptional and translational processing of GnRH, should be developed.

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