Original Article

Immunochemical detection of growth hormone and prolactin cells in the pituitary gland of the mountain frog (*Rana blythii*)

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Abstract

Immunocytochemistry and immunogold techniques were employed to classify and localize the cell producing growth hormone (GH) and prolactin (PRL) in the pars distalis of the mountain frog (*Rana blythii*) pituitary. The pituitary glands obtained from mature frogs during the breeding season (November-February) were fixed in Bouin's fluids for an immunocytochemical study, and in 0.2% glutaraldehyde, 2% paraformaldehyde, and 0.5% picric acid in 0.05M Millonig's phosphate buffer pH 7.4 at 4°C for an immunogold study. By immunocytochemical study, GH immunoreactive cells appeared as round or oval shaped, concentrated mainly in the dorso-posterior region of pars distalis. PRL immunoreactive cells were elongated in shape and scattered throughout the pars distalis. By immunogold technique, GH cells were identified by the presence of lipid droplets and contained three types of granules; round-shaped (356.2±4.1 nm in diameter), rod-shaped (196.1±4.1 nm in width and 345.6±5.3 nm in length), and irregular shaped (139.5-310.8 nm in width and 223.3-459.7 nm in length). PRL cells exhibited round granules of moderate electron density (355.2±5.3 nm in diameter).

Key words: pars distalis, *Rana blythii*, GH cell, PRL cell, immunocytochemistry

1. Introduction

Pars distalis of pituitary gland of anurans composed of a variety of hormone-producing cells, which are difficult to identify by the routine staining technique. There were extensive studies on the classification of corresponding cell types using various special staining methods. Among various stains, it appears that Mallory's trichrome is probably the most ideal one for studying the histology of pars distalis in frogs. This dye stains both acidophils and basophils (Barrington and Jorgensen, 1968). Despite its usefulness, Mallory's trichrome staining could not distinguish both subtypes of acidophils and basophils. Immunocytochemical and immunogold techniques have been applied to identify with more specificity each cell type in pars distalis of frogs. Previous immunocytochemical studies revealed that growth hormone (GH) cells were located in the dorsal and antero-ventral zone in pars distalis of *Rana ridibunda*, which were round or oval in shape (Yon *et al.*, 1991). In *R. catesbeiana* (Sretarugsa *et al.*, 1996), they were angular in shape and scattered in the postero-dorsal region. In *R. tigerina*, GH cells were concentrated in the postero-dorsal and some in the centro-ventral zone. These type of cells were elongated in shape (Pakdeeronachit *et al.*, 2001). Prolactin (PRL) cells were found throughout pars distalis in *R. catesbeiana* (Sretarugsa *et al.*, 1996; Yamamoto *et al.*, 2004), and *R. tigerina* (Pakdeeronachit *et al.*, 2001). They were elongated or irregular in shape in *R. catesbeiana*, whereas round or oval in shape in *R. tigerina*. In contrast, PRL cells of *R. ridibunda* (Akgün-Dar *et al.*, 2004) were mainly localized in the anterior part, and they were spherical, triangular or polygonal in shape. By immunogold technique, GH cells in

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R. catesbeiana (Sretarugsa *et al.*, 1996) contained spherical granules with a diameter of 370-420 nm. In *R. ridibunda* (Yon *et al.*, 1991), these cells contained large polymorphic granules (200-700 nm). In *R. tigerina*, GH cells were identified by the presence of lipid droplets. They contained round, densely packed high electron-dense granules (345±3.5 nm) (Pakdeeronachit *et al.*, 2001). The granules in PRL cells of *R. ridibunda* (Akgün-Dar *et al.*, 2004) and *R. tigerina* (Pakdeeronachit *et al.*, 2001) were round shaped with a diameter of 105-281 nm and 500±5.9 nm, respectively. In contrast, these cells of *R. catesbeiana* contained ovoid granules (315-430 nm) (Sretarugsa *et al.*, 1996).

However, data in *R. blythii* have not been identified. Thus, the purpose of this study is to classify and localize the cells producing GH and PRL in pars distalis of *R. blythii* by means of immunocytochemistry and immunogold technique.

2. Materials and Methods

2.1 Experimental animals

Mature mountain frogs (*Rana blythii*) (n = 5) during breeding season (November-February) were deeply anesthesized and the pituitary glands were removed immediately after decapitation.

2.2 Immunocytochemistry

Pituitary glands were fixed in Bouin's solution for 6 h and processed by conventional light microscopic method. Five-micron thick sections were collected on slides, deparaffinized, rehydrated and treated with 3% H_2O_2. Nonspecific binding of antibody was blocked in serum. Then, sections were incubated in rabbit anti-sera against bullfrog GH (dilution 1:5,000) or PRL (dilution 1:5,000) for 30 min and washed in 0.05M phosphate buffer saline (PBS). They were incubated in biotinylated secondary antibody for 10 min, rinsed in PBS, incubated in streptavidin-peroxidase, and visualized by colored reaction of diaminobenzidine tetrahydrochloride (DAB). Finally, the sections were rinsed in distilled water, dehydrated, mounted, and examined under light microscope. Control sections were incubated with PBS instead of primary antibodies.

2.3 Immunogold technique

Samples were fixed in 0.2% glutaraldehyde, 2% paraformaldehyde, and 0.5% picric acid in 0.05M Millonig's phosphate buffer pH 7.4 at 4°C for 2 h and postfixed in 0.5% OsO_4. Then, they were dehydrated and embedded in Spurr's resin. Ultrathin sections were cut, picked up on nickel grids, immersed in 3% H_2O_2 and in saturated sodium metaperiodate solution. The grids were treated in 4% BSA-PBS and incubated overnight at 4°C in rabbit anti-sera against bullfrog GH (dilution 1:500) or PRL (dilution 1:500). After they were washed, the grids were incubated in gold conjugated (20 nm)

goat anti-rabbit IgG at dilution 1:100 for 2 h at 4°C. Then, they were washed again, stained with uranyl acetate and lead citrate, and observed under a transmission electron microscope.

3. Results

3.1 Immunocytochemistry

Based on immunocytochemical staining, GH immunoreactive cells were concentrated mainly in the dorsoposterior region of pars distalis, and some of them appeared throughout the central zone except in the anterior region of the gland (Figure 1A). The cells were oval or round shaped (Figure 1B). PRL immunoreactive cells were distributed throughout the gland. They appeared in a high density at the postero-dorsal and antero-ventral zones of pars distalis (Figure 1C). Most of them were elongated in shape (Figure 1D). Both GH and PRL cells did not stain in the pars intermedia and pars nervosa. GH and PRL immunoreactivity did not occur in the control sections incubated with PBS instead of the primary antibodies.

3.2 Immunoelectron microscopy

At the ultrastructural level, GH and PRL cells were identified by immunogold technique, as GH cells of *R. blythii* have a distinctive feature. Their cytoplasm contained lipid droplets, which were dispersed among the secretory granules (Figure 2A,B). They contained irregularly shaped nuclei located at the base of the cells. GH immunoreactive
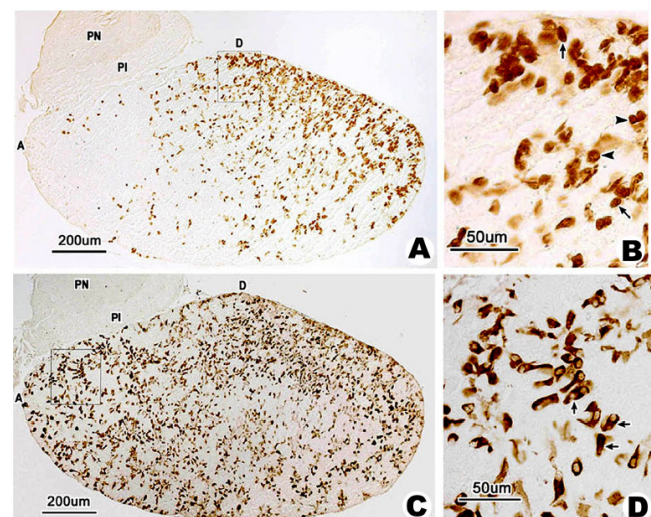

Figure 1. Immunocytochemistry of saggital section of the pars distalis of *R. blythii*, illustrating the distribution of GH cells (A) and PRL cells (C). High magnification of GH cells demonstrating the oval (arrows) or round shaped (arrowheads) (B), and PRL cells showing the elongated in shape (arrows) (D). A = anterior, D = dorsal, PI = pars intermedia, PN = pars nervosa
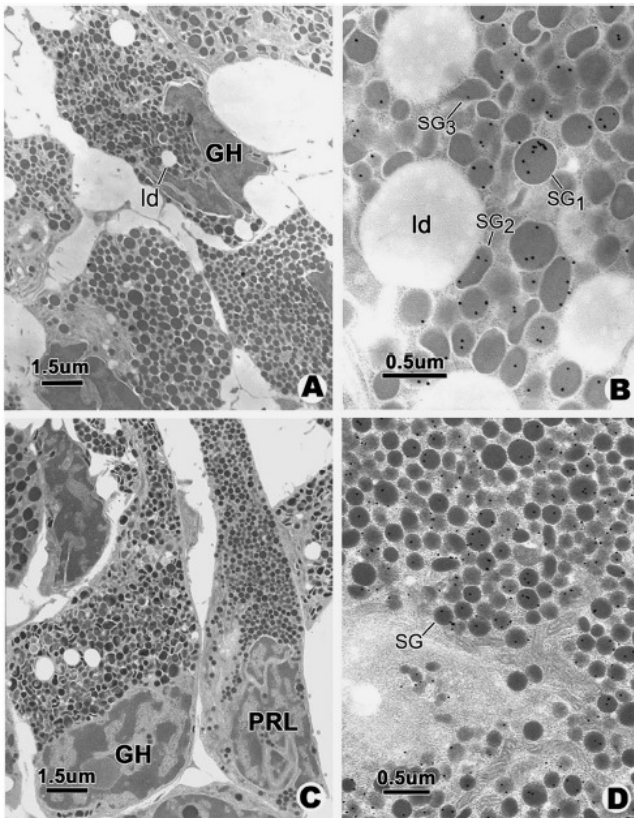
Figure 2. Electron micrographs of immunogold technique, demonstrating the characteristic of GH cells (A,B) and PRL cells (C,D). ld = lipid droplet, SG1 = secretory granule type I (round), SG2 = secretory granule type II (rod), SG3 = secretory granule type III (irregular), GH = growth hormone cell, PRL = prolactin cell

cells contained numerous electron-dense granules. These granules could be divided into three types based on their shapes. Type I granules were round (356.2±4.1 nm in size) (Figure 2B). They were the most numerous ones and distributed throughout the cytoplasm. Type II granules were rod shaped (345.6±5.3 nm in length and 196.1±4.1 nm in width) and loosely distributed (Figure 2B). Type III granules were irregular in shape (139.5-310.8 nm in width and 223.3-459.7 nm in length) (Figure 2B). They were the fewest in number among all types of GH granules. PRL cells contained numerous secretory granules. These granules were round shaped with moderate electron density and densely packed together. The size of granules were about 355.2±5.3 nm in diameter. PRL cells consisted of lobulated nuclei (Figure 2C,D).

4. Discussion

The present study is the first to demonstrate the growth hormone (GH) and prolactin (PRL) immunoreactive cells in *R. blythii* pituitary. It shows that GH cells were concentrated in the postero-dorsal area of pars distalis, and some

of them appeared throughout the central zone. The distribution pattern of GH immunostaining is in line with earlier studies in other anuran species, such as in *R. ridibunda* (Yon *et al.*, 1991), *R. catesbeiana* (Sretarugsa *et al.*, 1996), and *R. tigerina* (Pakdeeronachit *et al.*, 2001). At the ultrastructural level, GH cells of *R. blythii* contained lipid droplets, which was not reported in any anurans except in *R. tigerina* (Pakdeeronachit *et al.*, 2001). These cells contained three types of granules (round, rod, and irregular shaped). Most granules in GH cells were round shaped (356.2±4.1 nm). This is in contrast to the characteristic of GH cells in other species, which contain only one type of granule. The size of GH granules in *R. blythii* were rather similar to that reported in *R. tigerina*. In other species they were either smaller or larger (Sretarugsa *et al.*, 1996; Yon *et al.*, 1991). The granules of GH cells in *R. blythii* were densely packed similar to those of *R. tigerina*.

The growth hormone functions as a growth promotion (Okada and Kopchick, 2001). In amphibian, it plays important role in the regulation of growth of adult organs, such as hind limbs (Kikuyama *et al.*, 1993). This hormone is associated with the stimulation of body growth and metabolism (Harvey *et al.*, 1995), increasing the body weight and length (Yu *et al.*, 2006). During the reproductive period, GH regulates the vitellogenesis and ovarian growth of female frog in *R. esculenta* (Carnevali *et al.*, 2000).

PRL cells in *R. blythii* were dispersed throughout the pars distalis. This finding corresponds with the distribution of PRL cells in *R. catesbeiana* (Sretarugsa *et al.*, 1996; Yamamoto *et al.*, 2004), and *R. tigerina* (Pakdeeronachit *et al.*, 2001). In contrast, a different pattern of distribution was observed in *R. ridibunda* (Akgün-Dar *et al.*, 2004), where the PRL cells were mainly localized in the anterior region of the gland. By immunogold technique, it was found that the PRL cells were characterized round shaped secretory granules with moderate electron density (355.2±5.3 nm). The shape of granules in *R. blythii* were similar to that reported in *R. ridibunda* (Akgün-Dar *et al.*, 2004) and *R. tigerina* (Pakdeeronachit *et al.*, 2001), while ovoid shaped granules were exhibited in *R. catesbeiana* (Sretarugsa *et al.*, 1996). The size of granules was in line with previously reported data from *R. catesbeiana*, while PRL granules were smaller in *R. ridibunda* (105-281 nm) and larger in *R. tigerina* (500±5.9 nm). In *R. blythii*, the PRL cells contained densely packed granules similar to the PRL cells of *R. tigerina*.

Prolactin exhibits many roles in amphibians. It helps in the promotion of growth in larval stage, as it increases the body weight and tail length of larvae (Hasunuma *et al.*, 2004). This hormone antagonizes the action of thyroid hormone to suppress metamorphic changes (Tata, 2006) by inhibiting the tail resorption (Huang and Brown, 2000). PRL stimulates one of the adult-typed features of amphibian skin, the Na^+ transport (Takada, 2005). It participates in the reproduction such as courtship behavior (Toyoda *et al.*, 2005). This hormone enhances the synthesis of the female attract-

ing pheromone (sodefrin) and the responsiveness of the olfactory epithelium to sodefrin (Kikuyama *et al.*, 2005). PRL results in the accumulation of oocyte sensitivity to the action of the physiological ovulation inducers (Ramos *et al.*, 2005).

These results provide the basic information about the localization and the features of GH and PRL producing cells in pars distalis of the mountain frog, *R. blythii*. This is very beneficial for the future study of the frog's pituitary gland.

Acknowledgement

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