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Galanin: Presence and distribution in the brain and pituitary of *Rhinella arenarum* (Amphibia: Anura) during development

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Abstract

The immunohistochemical distribution of galanin (Gal) in the brain and pituitary of *Rhinella arenarum* was studied during development. Gal-immunoreactivity was first observed in the brain just after hatching in anterior preoptic area, infundibular area, median eminence and pars distalis of the pituitary as well as in the olfactory epithelium. At the beginning of prometamorphosis new Gal-immunoreactive (ir) cells were observed in the olfactory nerve and bulb. Later in prometamorphosis new Gal-ir cells were observed in the telencephalon, suprachiasmatic nucleus, rostral rhombencephalon and in the pars nervosa of the pituitary. The most numerous accumulations of Gal-ir neurons throughout the larval development were observed in the ventral hyphothalamus where numerous Gal-ir cells of cerebrospinal fluid-contacting type were found. During metamorphic climax and soon after we did not detect Gal-ir neurons in the pallium, medial or pretectal dorsal thalamus.

In the median eminence and pars distalis of the pituitary many Gal-ir fibers were found during development indicating that Gal may play a role in the modulation of hypophyseal secretion. Furthermore, the distribution of Gal-ir elements observed throughout larvae development indicates that galaninergic system maturation continues until sexual maturity.

Keywords: *Bufo arenarum*; Neuropeptide; Development; Nervous system; Pituitary

1. Introduction

Galanin (Gal) is a 29-amino acid peptide, which is present in the central nervous system of vertebrates. The primary structure of this peptide is highly conserved among vertebrates (almost 90%), indicating the importance of the molecule (Wang and Conlon, 1994).

This peptide has multiple biological effects and many studies have demonstrated its involvement in several hypothalamic and hypophyseal functions. Galanin exerts strong neuroendocrine effects by modulating the release of gonadotrophins, prolactin, growth hormone and somatostatin (Vrontakis, 2002). These regulatory actions of Gal are further supported by many reports that show galaninergic innervation of hypophyseal secretory cells in several vertebrate groups including mammals (Moons et al., 1989; Maiter et al., 1990; Olivereau and Olivereau, 1991, 1992; Józsa and Mess, 1993; Jiménez et al., 1994; Liu and Gao, 1998; Liu, 2002). Moreover, the expression of galanin is elevated following estrogen administration, neuronal activation, denervation and/or nerve injury, as well as during development (Vrontakis, 2002).

The use of immunohistochemical methods has revealed a wide distribution of galanin in the brain of several vertebrate groups (mammals: Pérez et al., 2001; Jacobowitz et al., 2004; birds: Józsa and Mess, 1993; Azumaya and Tsutsui, 1996; reptiles: Jiménez et al., 1994; amphibians: Lázár et al., 1991; Olivereau and Olivereau, 1992; González-Nicolini et al., 1995; fish: Batten et al., 1990; Olivereau and

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Olivereau, 1991; Jadhao and Meyer, 2000; Rodriguez et al., 2003; Adrio et al., 2005). These investigations have revealed a conserved pattern of distribution of galaninergic structures in preoptic–hypothalamic regions.

Furthermore, the developmental origin of Gal-immunoreactive (Gal-ir) neurons from the olfactory epithelium has been described in mice, where the Gal-ir neurons were demonstrated to migrate to the CNS along the olfactory/vomeronasal nerve (Key and Wray, 2000).

To our knowledge nothing is known about the development of the galaninergic system during metamorphic process in amphibians. Therefore here, we study in detail the development of the galanin system throughout the entire brain and the pituitary of the larva of the anuran *Rhinella arenarum* by means of immunohistochemistry.

2. Materials and methods

2.1. Animals

Rhinella arenarum tadpoles were obtained by *in vitro* fertilization according to Paz et al. (1995). Amphibian systematics is in a state of flux due to a series of large scale taxonomic changes recently proposed by several papers. Because of this reason many species formerly included in the genus *Bufo* have been recently accommodated in other genera. The new taxonomy is still changing as new data are gathered, and so many taxonomic changes are still expected in the near future (Frost, 2007). Be aware that the species that now is called *Rhinella arenarum* has recently been called *Chaunus arenarum*, and previously *Bufo arenarum*, the name that has been used in nearly most publications.

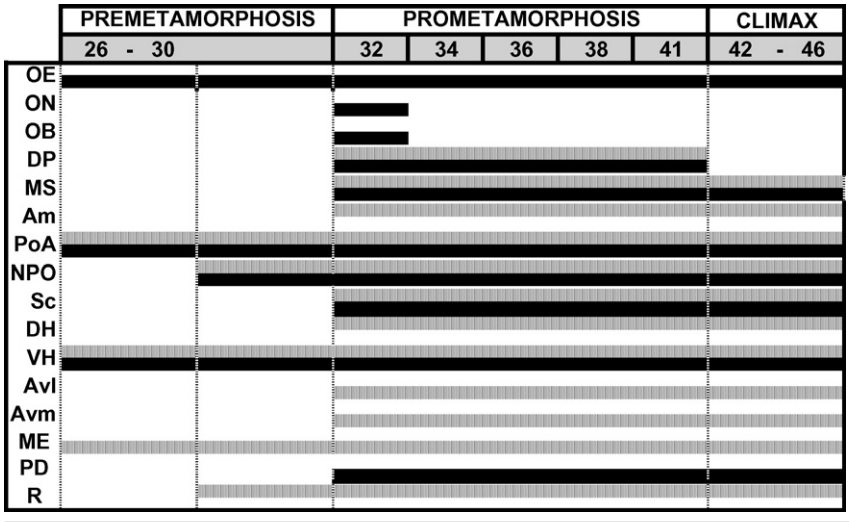
The tadpoles were held in tanks containing dechlorinated tap water, exposed to 12–12 h light–darkness cycles and constant temperature (22 ± 1 °C). At least three individuals, in each stage of development after hatching and metamorphs were used. The developmental stages were classified according to Gosner (1960).

All the procedures were in accordance with the principles of laboratory animal care of the Institutional Care and Use Committee of the Facultad de Ciencias Exactas y Naturales, UBA Res CD: 140/00, and the principles of the NIH (publication 8523, revised 1985). The tadpoles were anesthetized by immersion in 0.1% MS222 (tricaine methanesulfonate, Sigma St. Louis, MI) and fixed in Bouin's solution for 24 h at 4 °C. Then, they were dehydrated and embedded in Histoplast (Biopack, Buenos Aires, Argentina). Serial transversal and sagittal sections were cut at 7 μm and mounted on gelatin-coated glass slides.

2.1.1. Immunohistochemical procedures

Tissue sections were deparaffined, rehydrated and washed in phosphate-buffered saline. Sections were treated with 5% hydrogen peroxide (H_2O_2) solution to quench endogenous peroxidase activity. Non-specific binding sites were blocked by treating tissues with TNB blocking reagent (Cat. FP1020, NEN Life Science Products, Boston, MA) and subsequently incubated 24 h at 4 °C with the primary antiserum. The antiserum used was rabbit anti-Galanin (human), (dilution 1:1000) (Peninsula, Belmont, CA). Then, sections were treated with the biotinylated Anti-Rabbit antibody (dilution 1:1000) (Vector Laboratories, Burlingame, CA) followed by avidin–horseradish peroxidase–biotin complex (Vectastain ABC kit, Vector Laboratories). The color reaction was visualized by exposure to 3,3′-diaminobenzidine

Table 1
Time table of appearance of Galanin immunoreactive cells groups (black lines) and fibers (gray lines) in the central nervous system of *Rhinella arenarum*



OE: Olfactory epithelium; ON: olfactory nerve; OB:olfactory bulb; DP: dorsal pallium; MS: medial septum; Am: medial amygdala; PoA: anterior preoptic area; NPO: preoptic nucleus; Sc: suprachiasmatic nucleus; DH: dorsal hypothalamic nucleus; VH: ventral hypothalamus; Avl: ventrolateral thalamic area; Avm: ventromedial thalamic area; ME: median eminence; PD: pars distalis; R, rostral rhombencephalon.