Micron 107 (2018) 9–19

Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Effects of nerve stimulation on amphibian oviductal activity

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A R T I C L E I N F O

Keywords:
Amphibian
Oviduct
Innervation
Electric stimulation

A B S T R A C T

The present study describes, for the first time in an anuran amphibian, the nerve stimulation effects on the secretory and motor activity of the oviduct of adult females. The results reveal that in *Rhinella arenarum* oviducts, the epithelial and glandular secretory cells of the mucosa of the pars convoluta respond to nerve stimulation secreting the products synthetized and stored in their cytoplasm. The ultrastructural analysis showed that the cell content released is made up of granular, fibrillar and floccular material, exocytosis being the main secretory mechanism found in epithelial secretory cells, although apocrine and holocrine processes could also be observed. In contrast, in glandular cells only exocytosis processes were found. With respect to the participation of the nervous system in the motility of the duct, observations under our experimental conditions indicated that oviductal nerve stimulation promotes motor activity as manifested by a succession of coordinated contractions and relaxations that generate movements similar to peristaltic waves. These results were observed in oviducts from animals captured during the reproductive and post reproductive periods. However, it is important to note that both the secretory response and duct motility are markedly decreased during the post reproductive period of the species.

1. Introduction

In amphibians, oviducts appear as a pair of elongated ducts located on both sides of the mid line of the body. Structurally, the oviduct wall is constituted in its whole extension by three layers concentrically arranged: the serosa, the muscle layer and the mucosa. The serosa lines the outer surface of the duct and, through the mesothelium, fixes it to the coelomic wall and connects it with the kidney. The muscle layer is characterized by an increasing thickness in the cephalocaudal direction. The innermost layer, which contacts the duct lumen, is the mucosa, which shows structural differences in its organization throughout the organ (Moreno, 1972; Pawar and Pancharatna, 1999; Fernández and Ramos, 2003; Uribe Aranzabal, 2011).

In the oviduct, structurally and functionally, three main zones can be identified: the pars recta (PR), the pars convoluta (PC) and the ovisac or uterus (Fig. 1).

The PR, which is the most cephalic portion of the oviduct, communicates with the coelomic cavity through an opening called ostium. In *Rhinella arenarum*, the mucosa of this segment has a simple organization based only on a single epithelial layer in which ciliated cells (CC) alternate with secretory cells (SC) (Fernández et al., 1989; Winik et al., 1999).

The PR is followed by the PC, which represents the main body of the oviduct and exhibits numerous folds that cause a remarkable increase in its length (Moreno, 1972; Winik et al., 1999; Medina et al., 2007; Uribe Aranzabal, 2011). In this portion of the oviduct, the mucosa is made up of a pseudostratified epithelium arranged in folds oriented toward the lumen of the duct and constituted by CC and by epithelial secretory cells (ESC) that contain granules of different sizes, shapes and electron densities (Winik et al., 1999; Fernández and Ramos, 2003). Beneath the epithelial stratum lies a glandular stratum that exhibits simple tubuloacinar glands made up of glandular secretory cells (GSC) (Crespo et al., 2012). GSC contain granules of medium electron density, many of which exhibit an electron dense core (Winik et al., 1999; Fernández and Ramos, 2003).

The last portion of the oviduct is the ovisac or uterus which connects to the exterior by the cloaca. In this zone the mucosa consists of an epithelial layer with no glands (Moreno, 1972; Uribe Aranzabal, 2011).

In several amphibian species it has been demonstrated that the success of fertilization depends of the product secreted by the SC around the oocytes during their transit trough the oviduct (Hedrick and Nishihara, 1991; Fernández and Ramos, 2003; Crespo et al., 2012).

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https://doi.org/10.1016/j.micron.2018.01.005
Received 6 October 2017; Received in revised form 1 December 2017; Accepted 9 January 2018
Available online 10 January 2018
0968-4328/

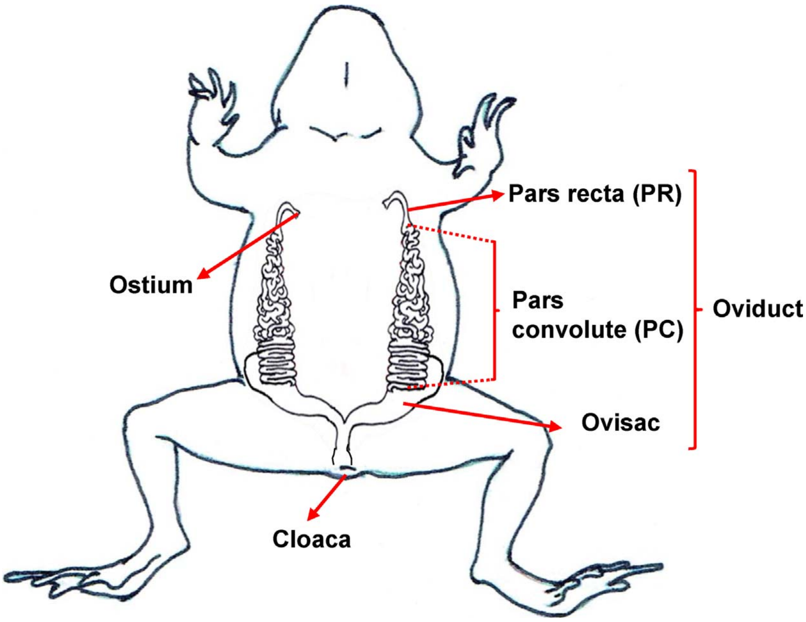

Fig. 1. Diagram of the *Rhinella arenarum* oviduct indicating the three principal zones of the organ.

Two different types of secretion have been identified. The first one, secreted at the PR level, is a low density aqueous product containing a proteolytic enzyme called oviductin (Hardy and Hedrick, 1992). This enzyme, through partial hydrolysis, induces structural and molecular modifications in the oocyte vitelline envelope, making it sensitive to sperm lysins and penetrable by the spermatozoon (Miceli and Fernandez, 1982; Katagiri et al., 1982; Bakos et al., 1990; Llanos et al., 2006).

After passing through the PR, oocytes traverse the PC, zone of the oviduct which synthetizes and secretes the components of the second type of secretion, the jelly, a viscous material arranged in multiple layers that sequentially surround the oocytes during their transit through the duct. The jelly coat is composed of high molecular weight glycoproteins that represent the stable structural component of the jelly to which globular proteins with low molecular weight and mono and divalent cations are attached. It has been demonstrated that the components of the jelly coats are essential for fertilization and especially for the induction of the acrosome reaction of the sperm (Ishihara et al., 1984; Miceli et al., 1987).

Finally, the oocytes surrounded by the components of the jelly enter the ovisac, where they accumulate until the moment of oviposition (Fernández and Ramos, 2003).

Rhinella arenarum females, like most amphibian species, present a reproductive cycle in which two periods can be clearly identified: a reproductive and a post reproductive one. During this cycle the oviduct shows pronounced changes that affect structural and functional aspects (Licht et al., 1983; ndez et al., 1984, 1989; ; Winik et al., 1999).

In the reproductive period the oviductal mucosa reaches a maximum structural and functional development, with a large number of the epithelial and glandular secretory cells fill of secretory products stored in their cytoplasm (Winik et al., 1999; Fernández and Ramos, 2003; Crespo et al., 2012). At the end of this period, the oviduct shows a marked involution due to the release of the secretion products that occurs during the transit of the ovulated oocytes (Winik et al., 1999).

Then the post reproductive period begins, characterized by the recovery of the oviductal mucosa as a result of the gradual increase in the number and volume of the SC. These cells again begin to exhibit an increasing amount of secretory granules (SG) that appear as cytoplasmic granulation (Winik et al., 1999; Crespo et al., 2012).

There are data demonstrating that gonadal steroids, whose secretion profiles show pronounced variations during the reproductive cycle (Iela et al., 1986; Itoh and Ishii, 1990; Medina et al., 2004), are involved in the regulation of the different events that take place at the oviductal level. In fact, estradiol, which reaches its maximum concentrations during the post reproductive period, would be related to the synthesis of specific proteins involved in the fertilization processes (Fernández and Ramos, 2003; Medina et al., 2004). It has been demonstrated that progesterone and dihydrotestosterone, which present highest circulating levels during the preovulatory period, are involved in the control of the secretion of the jelly components by the SC of the PC mucosa (Pierantoni et al., 1984; Medina et al., 2004).

Although hormones are important regulators, neural influence on the oviductal function cannot be ignored, since the organs of the body have both a nervous and an endocrine control. At present, no data exist with respect to the neural control of amphibian oviductal activity. However, studies conducted by our work group allowed us to demonstrate, for the first time in anuran amphibians, that the oviduct of *Rhinella arenarum* receives innervation that reaches the organ through the mesothelium. The nerves are constituted by unmyelinic fibers whose axon terminals are located near the smooth muscle fibers and blood vessels (Cisint et al., 2014).

Taking the above into account, the aim of the present work was to analyze the effects of nerve stimulation on oviductal activity.

2. Materials and methods

2.1. Animals

Sexually mature adult *Rhinella arenarum* females, 100–150 g body weight, were collected in the neighbourhood of San Miguel de Tucumán, Argentina, during the reproductive (n = 6) and post reproductive (n = 6) periods of the sexual cycle. The specimens were used immediately after capture or housed for brief periods (2–3 days) at room temperature and appropriate humidity conditions until use.

Animal maintenance and experimental procedures were carried out in accordance with the Guidelines for the Use of Live Amphibians and Reptiles in Field and Laboratory Research (Herpetological Animal Care and Use Committee of the American Society of Ichthyologists and Herpetologists, 2004).