Acta Histochemica xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Acta Histochemica

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

Immunomorphometric variations of sustentacular cells of the male viscacha adrenal medulla during the annual reproductive cycle. Effects of androgens and melatonin

Luis Ezequiel Gallol[a,b], Fabian Heber Mohamed[a,*]

[a] Cátedra de Histología, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Av. Ejército de los Andes 950-2° Piso- CP (5700), San Luis, Argentina

[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), San Luis, Argentina

ARTICLE INFO

Keywords:
Viscacha
Adrenal medulla
Sustentacular cells
Reproductive conditions
Androgen
Melatonin

ABSTRACT

The adrenal medulla is crucial for the survival of species facing significant environmental changes. The parenchyma is composed mainly of chromaffin cells, ganglion cells and sustentacular cells (SC). The male viscacha exhibits seasonal variations of gonadal activity and other metabolic functions. The aim of this work was to investigate the influence of the reproductive conditions on the morphology of SC of this rodent. In addition, the effects of testosterone and melatonin on these cells were studied. Immunoexpression of S100 protein, GFAP and vimentin were analyzed. Furthermore, the distribution of adrenergic and noradrenergic chromaffin cells subpopulations was studied for the first time in this species. SC present long cytoplasmic processes in contact with chromaffin cells, probably generating an intraglandular communication network. Significant differences ($p < 0.05$) in the %IA (percentage of immunopositive area) for the S100 protein were observed according to winter (4.21 ± 0.34) and summer (3.51 ± 0.15) values. In castrated animals, the %IA (6.05 ± 0.35) was significantly higher in relation to intact animals (3.95 ± 0.40). In melatonin-treated animals the %IA (3.62 ± 0.23) was significantly higher compared to control animals (2.65 ± 0.26). GFAP immunoexpression was negative and no noradrenergic chromaffin cells were detected suggesting an adrenergic phenotype predominance. Vimentin was observed in SC, endothelial cells and connective tissue. Results indicate that SC exhibit variations along the annual reproductive cycle, along with castration and the melatonin administration. Our results suggest that in this rodent SC are not only support elements, but also participate in the modulation of the activity of the adrenal medulla; probably through paracrine effects.

1. Introduction

The adrenal medulla is a major component of the sympathetic nervous system, constituting what is known as the sympathoadrenal system. Unlike the adrenal cortex, the medulla originates from the migratory cells of the embryonic neural crest (Lumb and Schwarz, 2015). The parenchyma of the adrenal medulla is composed mainly of chromaffin cells, sympathetic ganglion cells and sustentacular cells. The chromaffin cells are neuroendocrine cells that release catecholamines. These hormones exert numerous functions in the organism, like adipose tissue lipolysis and hepatic glicogenolysis, with the consequent energetic reserves mobilization (Barth et al., 2007). Due to the capability of catecholamines to regulate the organism energy metabolism, the activity of the adrenal medulla is crucial for the survival of species facing significant environmental changes. Furthermore, the role of catecholamines is equally important in the regulation of hydro-electrolyte balance (Schrier et al., 1976; Correia, 2015). The function of chromaffin cells has been widely studied and understood. However, the role of sustentacular cells, in close relation with chromaffin cells, is not completely known and were initially considered as passive supporting cells (Iwagana and Fujita, 1984).

The sustentacular cells have been characterized by the expression of glial cell markers like S100 protein, vimentin and glial fibrillary acidic protein (GFAP). While vimentin and GFAP are structural proteins that constitute the intermediate filaments of the cytoskeleton, the S100 protein belongs to a family of calcium-binding proteins of the EF-hand type. Numerous functions have been attributed to S100 protein family, especially as regulators of intracellular calcium levels and signal transducers of calcium-dependant intracellular signaling pathways involved in proliferation, differentiation, apoptosis, energy metabolism

* Corresponding author.
E-mail address: fhmo@unsl.edu.ar (F.H. Mohamed).

https://doi.org/10.1016/j.acthis.2018.03.009
Received 15 December 2017; Received in revised form 12 March 2018; Accepted 29 March 2018

and migration. They can even act as extracellular signaling proteins which are recognized by cell surface receptors (Donato et al., 2013). GFAP has been reported to be key in the maintenance of the cytoskeleton mechanical strength of astrocytes and in the response to injury in the central nervous system (Yang and Wang, 2015). In the peripheral nervous system, the expression of GFAP has been observed in non-myelinating Schwann cells and in sustentacular cells of rat adrenal medulla (Suzuki and Kachi, 1995). Vimentin, initially described as a characteristic component of mesenchymal cells, has been observed in different cell types in a range of maturation states, particularly in immature glial cells where this protein constitutes a major component of the cytoskeleton (Bignami et al., 1982; Schnitzer et al., 1981).

Our experimental model, the viscacha (*Lagostomus maximus maximus*), is the largest member of the Chinchillidae family. This rodent inhabiting semiarid zones from Paraguay to central Argentina. The male viscacha exhibits seasonal variations of gonadal activity throughout its annual reproductive cycle and in many other metabolic functions. These variations are key to ensure survival and are synchronized with environmental factors, such as photoperiod length, temperature, food availability, rainfall patterns and social interactions. These nocturnally active rodents emerge from their burrows during night-time to fulfill their food needs on the vegetation (Weir, 1971). The reproductive cycle of the male viscacha is characterized by a period of maximum activity during summer and early autumn. In winter, these animals undergo a significant testicular regression with the subsequent loss of the reproductive activity. Testicular recovery occurs during spring before the start of a new cycle (Fuentes et al., 1991; Muñoz et al., 1997; Aguilera-Merlo et al., 2005; Filippa et al., 2005; Godoy-Pieri et al., 2014). In the viscacha, the natural photoperiod constitutes the main environmental factor that regulates reproduction through the release of melatonin from the pineal gland (Fuentes et al., 2004). Adult male viscachas show an increased activity of the pineal hydroxyindole-O-methyl transferase with the consequent increment in the production and secretion of melatonin during winter a period that, as mentioned before, is characterized by a gonadal regression status (Piezzi et al., 1984).

The aim of the present study was to investigate the influence of the reproductive conditions on the morphology of sustentacular cells of the adrenal medulla of adult male viscachas captured in their natural habitat during the annual reproductive cycle. Since all of the existing studies on sustentacular cells have been carried out in laboratory animals kept under artificially controlled conditions, our study was performed on animals subjected to environmental conditions in their habitat and will provide new information on these cells. In addition, the effects of testosterone and melatonin on these cells were studied through castration and melatonin administration experiments. Furthermore, the relation between sustentacular cells and chrommafin cells adrenergic and noradrenergic subpopulations was studied for the first time in this species.

2. Materials and methods

2.1. Animals

A total of thirty-two adult male viscachas were captured in their habitat near San Luis, Argentina (33°20′ S, altitude 760 m), using a system of rectangular traps conveniently placed near the entrance of their burrows. Solar irradiation values, expressed as heliophany, and seasonal mean values of precipitations and temperature were provided by the Red de Estaciones Meteorológicas de San Luis and the Servicio Meterológico Nacional (Table 1). All captured animals were immediately transferred to the animal facility where they were sacrificed or subjected to experimental conditions. In every case, the animals were intramuscularly anesthetized with a combination of ketamine (ketamine 50; Vetanarcol; König S.A., Barcelona, Spain) and xylazine (xylazine 20, PharmaVet, LaboratoriosPharmaVet S.A. Rosario, SantaFe, Argentina) at a dose of 12 and 0.4 mg/kg bodyweight, respectively. The adrenal glands were quickly excised and dropped into Bouin's solution, or glutaraldehyde, as soon as possible in order to prevent postmortem destructive alterations in the tissue. Whenever necessary blood samples were obtained by cardiac puncture. Animals were sacrificed by intracardiac injection of Euthanyl (0.25 ml kg^{-1} body weight, sodium pento-barbitone, sodium diphenylhydantoin, Brouwer S.A., Buenos Aires, Argentine).

Table 1
Seasonal environmental conditions.

	SUMMER	AUTUMN	WINTER	SPRING
PRECIPITATION (mm)	272	44	33	189
AVERAGE TEMPERATURE (°C)	25	14	8	20
HELIOPHANY (h)	9.38	7.09	6.82	9.09

Average values of precipitations, temperatures and heliophany in San Luis, Argentina (2016).

The experimental design used in this work was approved by the local Ethics Committee and was in accordance with the guidelines of the National Institute of Health (NIH, USA) guidelines for the use of experimental animals. In addition, the Biodiversity Control Area of the San Luis Environment Ministry (Argentina) approved a study protocol for conducting scientific research within the territory of this province (Resolution No. 47 PBD, 2015).

2.2. Annual reproductive cycle

In order to study the influence of the annual reproductive cycle on the sustentacular cells, the expression and distribution of the S100 protein, GFAP and vimentin were studied. The animals were captured in their natural habitat during the following seasons: February to March (summer, maximum reproductive activity; n = 4), April to May (autumn; n = 4); July to August (winter, gonadal regression; n = 4) and September to October (spring, gonadal recovery period; n = 4). The animals were immediately transferred to the animal facility and the adrenal glands were obtained as described in previous sections. Gonadal activity in all groups was assessed through careful histological examination of testes according to the observations of Fuentes et al. (1991) and Muñoz et al. (1997).

2.3. Castration

Eight adult male viscachas captured in autumn were used. Animals in the experimental group (n = 4) were subjected to surgical castration, while the animals in the control group (n = 4) underwent sham-castration. All animals were kept in isolated boxes with ad libitum access to food and water and were maintained under a 14L:10D photoperiod at 20 ± 2 °C. After 6 weeks, all animals were sacrificed and the adrenal glands were excised. This castration time length has been used in previous experiments for this species (Filippa et al., 2014), and it is similar to the castration time length in which notorious changes were observed in the histoarchitecture of the adrenal gland of other rodents (Hall and Korenvchesky, 1983; Benmouloud et al., 2014). Gonadal activity in all groups was assessed through careful histological examination of testes.

2.4. Melatonin administration

For this experiment, eight adult male viscachas captured during autumn were used. The rodents were kept in isolated boxes with ad libitum access to food and water at 20 ± 2 °C. They were maintained under a 14L:10D photoperiod. The experimental group (n = 4) received two daily subcutaneous injections of melatonin (Sigma, 100 µg kg^{-1} body weight in peanut oil solution) at 9:00 A.M and 5:00 P.M. The control group (n = 4) received only the diluent. After 9 weeks of