Anat. Histol. Embryol. **36,** 182–185 (2007)
ISSN 0340–2096
doi: 10.1111/j.1439-0264.2006.00732.x

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Interaction between Chromaffin and Sustentacular Cells in Adrenal Medulla of Viscacha (*Lagostomus maximus maximus*)

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With 5 figures

Received November 2005; accepted for publication June 2006

Summary

New evidence provides valuable information about the participation of sustentacular cells in chromaffin cell catecholamine secretion. In this process, calcium ions play an important role. It has been shown that there is an intense ionic traffic between both types of cells. Moreover, sustentacular cells take an active part in calcium metabolism, regulating levels of the ion and indirectly, the synthesis and release of catecholamines. This background information encouraged us to study the sustentacular population of *Lagostomus* adrenal medulla and its morphologic relationship with the chromaffin population. The animals were captured, transported to the animal facilities, anaesthetized and killed. The adrenal gland was processed by immunohistochemistry using antiserum against S-100 (subunit α and β), a specific marker. Through the morphological and immunohistochemical study, it was found that there are sustentacular cells in deferent regions of adrenal medulla, mainly in the basal zone of chromaffin cells, which constitute the glomerular structure around blood capillaries. Cytoplasmic extentions of sustentacular cells penetrate into chromaffin cells and make contact with the basal membrane of the capillary endothelium. The relationship among chromaffin cells, capillaries and sustentacular cells suggests that they may intervene actively in the adrenal medulla metabolism.

Introduction

The adrenal medulla is an endocrine organ of neural origin located in the central region of the adrenal gland, and surrounded by the cortex. The predominant cellular population consists of modified neurones called chromaffin cells, specialized in catecholamine secretion, besides neurones and sustentacular or glial cells (Parker et al., 1990). The presence of these cell types confirms the nervous nature of the adrenal medulla (Parker et al., 1993; Dagerlind et al., 1995).

The glial cells are considered to be supporting cells (Susuki and Kachi, 1996). However, there is evidence of their participation in the modulation of the neuronal activity in the central nervous system, and of chromaffin cells in adrenal medulla (Cocchia and Michetti, 1981; Kameda, 1996; Laming et al., 2000). It has also been determined that the sustentacular cells of the adrenal medulla exhibit characteristics similar to supporting cell types located in other endocrine organs, such as the pituitary pars distalis and the pineal gland (Susuki and Kachi, 1995; Heizmann et al., 2002). The presence of a S-100 subfamily belonging to the EF hand-binding protein family, related to calcium metabolism, has been demonstrated in these cell types (López-Muñoz et al., 1992; González-Martínez et al., 2003). It has been postulated that the sustentacular cells might regulate the calcium levels in the extracellular compartment (Chao et al., 1994; Newman and Zahs, 1998; Mäler et al., 2002). The calcium ion constitutes one the most important second messengers involved in the exocytosis of hormonal products. There is evidence of its participation in the mechanism of liberation of catecholamines, mainly noradrenaline (Burgoyne et al., 1993; Villalobos et al., 2002; Camacho et al., 2003; Crivellato et al., 2004). It has been demonstrated that the sustentacular cells are mainly located in the neighbourhood of the noradrenergic cells (Susuki and Kachi, 1994).

Adrenaline synthesis from noradrenaline is stimulated by the glucocorticoids produced in the fasciculata zone of the adrenal cortex (Schober et al., 2000; Carrasco-Serrano and Criado, 2004). These hormones reach the adrenal medulla through an extended capillary network that relates to both zones anatomically. The glucocorticoids cause a stimulatory effect on the activity of the phenylthanolamine *N*-metyltransferase (PNMT), an enzyme that catalyses the transformation of noradrenaline into adrenaline (Yonekubo et al., 2003). The attraction of glucocorticoids by adrenergic cells is also likely to be modulated by calcium levels.

The anatomic and histoarchitectural study of the adrenal gland of *Lagostomus* shows that this organ exhibits similarities and differences with the description made for other rodents. The anatomic position, blood irrigation and the lateral dimorphism that both glands exhibit are typical in most of the mammals. However, there are two particular parameters that correspond to the adrenal gland of our experimental model. The first parameter refers to the association of the right adrenal with a medium-calibre vein. The cortex is interrupted in the contact surface, and the adrenal medulla enters into close contact with the vein wall. The close relation between the medullar parenchyma and the circulation permits to postulate that the right adrenal medulla is likely to acquire fundamental importance for the secretion of adrenaline caused by the stress stimuli as the hormone seems to reach the systemic circulation immediately, thus triggering a series of physiological events that lead to an integral behaviour change causing an effective and quick response. The left adrenal gland probably follows the response intensity, potentiating and extending in time the physiological processes characteristic of the stress. The second differentiating parameter refers to the histoarchitectural

Grant Sponsor: Project 22/Q303–UNSL.

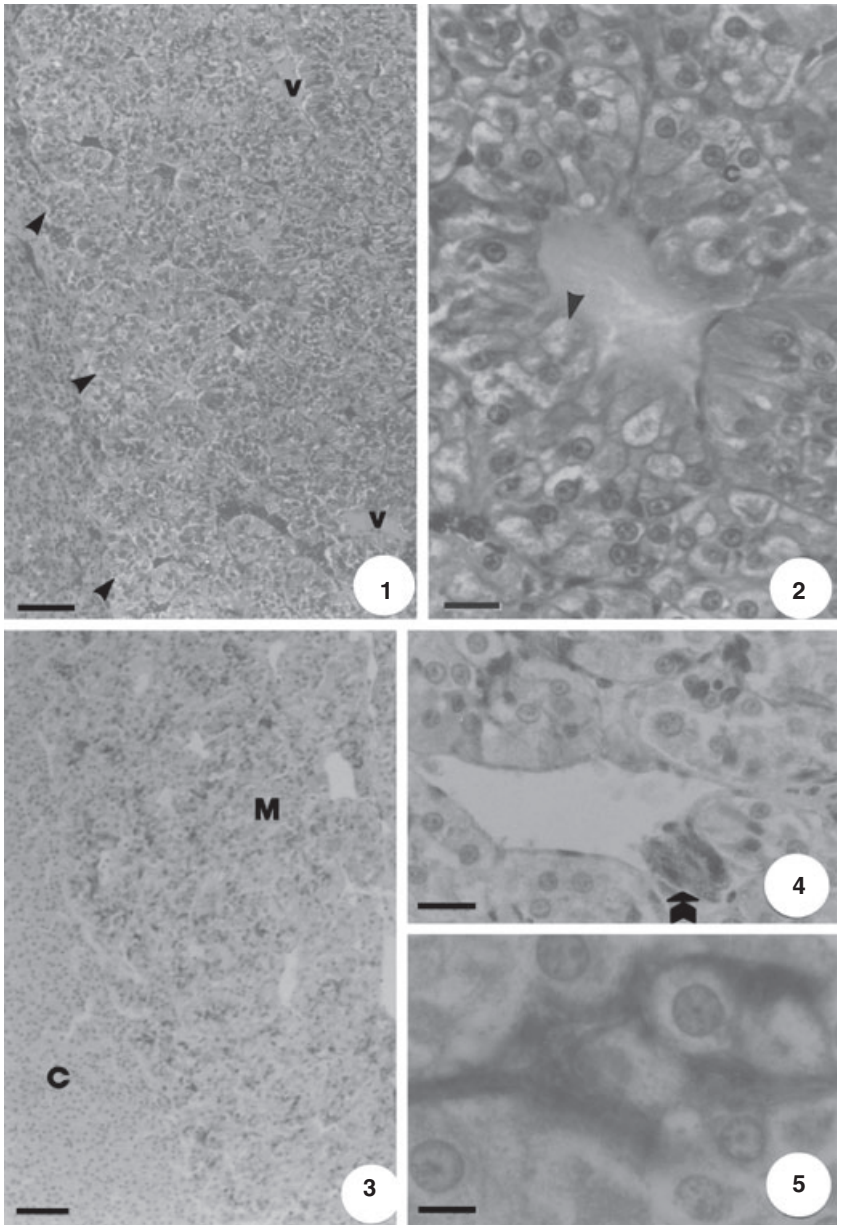

Fig. 1. Adrenal medulla of viscacha. The chromaffin cells are forming glomerular structures surrounding blood vessels (V). The arrowheads indicate the limit between adrenal medulla and cortex. Masson's trichrome. Scale bar: 100 μm.
Fig. 2. The micrograph shows chromaffin cells (c) surrounding a blood vessel of thin walls. The apical cytoplasm (arrowhead) is empty suggesting a recent hormonal secretion. Masson's trichrome. Scale bar: 25 μm.
Fig. 3. Adrenal medulla immunolabeled with anti S-100 protein. The immunolabelling is observed only adrenal medulla (M). There is no positive immunolabelling in the adrenal cortex (C). Scale bar: 100 μm.
Fig. 4. Image showing glial cells immunolabeled with anti-S-100. These cells have cytoplasmic processes extending towards blood vessels (arrow). Scale bar: 25 μm.
Fig. 5. Micrograph showing cytoplasmatic processes of the glial cells immunolabelled with anti-S-100 surrounding chromaffin cells. Scale bar: 10 μm.

organization of the glomerulose zone because of the presence of tubulous structures in the cellular organization (Ribes, 2000).

Our experimental model is the viscacha (*Lagostomus maximus maximus*), a photoperiodic rodent having nocturnal habit. Under natural conditions, the adult male exhibits an annual reproductive cycle (Piezzi et al., 1984). In winter, males of this species show minimum pituitary (Mohamed et al., 2000) and gonadal activity (Muñoz et al., 1997), while the pineal gland shows maximum activity (Dominguez et al., 1987) and this is reversed in summer. The objective of the present investigation was to study the morphological distribution of the sustentacular cells in the parenchyma of the adrenal medulla of viscacha, their relationship with chromaffin cells and the blood capillaries. S-100 protein as a specific marker of sustentacular cells was used for this study.

Materials and Methods

Eight male adult viscachas were captured in their natural habitat near San Luis, Argentina (33°20′S, 760 m altitude) in 2004 using traps placed in their burrows. After capture, animals were immediately taken to the laboratory, anaesthetized with Nembutal (pentobarbital) and killed by decapitation. The adrenal gland was rapidly exposed and excised. They were fixed in Bouin's fluid and embedded in paraffin. Serial sections were cut at 5 μm, mounted on glass slides and stained with haematoxylin–eosin and Masson's trichrome.

Immunohistochemical technique

Serial sagittal sections (5 μm thick) were cut and first deparaffinized with xylene and hydrated through decreasing concentrations of ethanol. Slides were incubated for 20 min in a solution of 3% H_2O_2 in water to inhibit endogenous peroxidase activity. They were then rinsed with distilled water and phosphate-buffered saline (PBS, 0.01 M, pH 7.4). Non-specific binding sites for immunoglobulins were blocked by incubation for 15 min with 0.25% casein in PBS and rinsed with distilled water and PBS. Sections were then incubated overnight in a moist chamber at 4°C with the following primary antisera-developed polyclonal antibody to S-100 protein (subunit α and β), diluted 1:200 (BioGenex, San Ramón, CA, USA). After they were rinsed with PBS for 10 min, the immunohistochemical visualization was carried out using the Super Sensitive Ready-to-Use Immunostaining Kit (BioGenex) at 20°C. The biotin–streptavidin amplified (BSA) system was used as follows: sections were incubated for 30 min with diluted biotinylated anti-mouse IgG, and after being washed in PBS, they were incubated for 30 min with horseradish peroxidase-conjugated streptavidin, and finally washed in PBS. The reaction site was revealed by 100 μl 3,3′-diaminobenzidine-tetrahydrochoride (DAB) chromogen solution in 2.5 ml PBS 50 μl H_2O_2 substrate solution. The slides were counterstained with haematoxylin for 1 min, dehydrated and mounted.

In all cases, two controls for specificity of the primary antibodies were made: (1) omission of primary antibody, and (2) pre-absorption of primary antibody with viscacha brain homogenates.

Results

The adrenal medulla of the *L. maximus maximus* is profusely irrigated by numerous blood vessels of big size and thin walls. The boundaries between this region and the reticularis zone are not precise because of the developed parenchyma of the medulla of this rodent that pervades the cortex (Fig. 1).

The cell types present are chromaffin cells, sympathetic neurones and supporting cells of glial nature. The chromaffin cells constitute glomerular and cordonal structures separated by subtle connective fibres and fibroblasts. These cells are markedly polarized. The nuclei are located in the basal region, and the apical cytoplasm is in close contact with the blood vessels (Fig. 2).

The immunolabelling with anti-S-100 protein are observed only in the adrenal medulla. On the contrary, there is no immunolabelling in the adrenal cortex (Fig. 3).

The immunohistochemical reaction for S-100 protein reveals that the sustentacular cells are generally located in the basal region of the glomerular structures constituted by the chromaffin cells placed around the blood capillaries (Fig. 4). Immunolabelling is also observed between the lateral membranes of the chromaffin cells (Fig. 5), which would indicate that the cytoplasmic processes of the sustentacular cells through the interstitial spaces reach the basal membrane of the capillary endothelium. Moreover, sustentacular cells are observed in all the medullar parenchyma, distributed among the chromaffin cells that do not constitute glomerules but cellular clusters or cordonal structures.

Discussion

The disposition of the S-100 immunoreactive cells in the basal region of the glomerular structures constituted by chromaffin cells confirms the basic supporting function of these cells, as described for other mammals (Magro and Grasso, 1997). Besides, there is an abundant population of sustentacular cells in the cordonal structures, which reaffirms their supporting function (Edenfeld et al., 2005). The adhesion factor among the membranes of both cellular types produced by the sustentacular cells permits an increase in the intercellular cohesion (Grant et al., 1992; Lee et al., 2000; Oguievetskaia et al., 2005). Moreover, it has to be emphasized that their processes adopt interstitial positions, penetrating among the lateral membranes of the chromaffin cells, thus performing the sustentacular function almost completely. However, the close relation between the processes and the endothelial basal membrane suggests other functions such as the stimulation in the attraction of the plasmatic glucocorticoids caused by the adrenergic cells, as described by Wong et al. (1998) and Peters et al. (2005). The production of an endothelial growth-stimulating factor in the sustentacular cell should not be discarded, as postulated in the folliculo-stellate cells of the pars distalis of the rat pituitary (Maretová and Maretta, 2004). This cellular type is also of glial origin (Nakajima et al., 1980). The S-100 protein is related to the production of this factor (Kobayashi and Coupland, 1993). In addition, the S-100 protein is likely to join the cytoplasmic calcium, modulating the levels of the ion that might act on the noradrenergic cells facilitating the secretion of noradrenaline. The previous description permits to conclude that the sustentacular cells may fulfill an important function in the support and regulation of the chromaffin cells, modulating in a way the production and secretion of catecholamines. This modulation might be carried out through a differential mechanism upon the noradrenergic and adrenergic cells.

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