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https://doi.org/10.5109/4721

出版情報：九州大学大学院農学研究院紀要. 51 (1), pp.121-124, 2006-02-01. Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

KYUSHU UNIVERSITY

J. Fac. Agr., Kyushu Univ., **51** (1), 121–124 (2006)

Scanning Electron Microscopic Comparison of the Three-Dimensional Collagen Architectures in Rat, Pig and Chicken Adenohypophyses

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(Received October 28, 2005 and accepted November 16, 2005)

The three-dimensional collagen architectures of rat, pig and chicken adenohypophyses were compared using a combination of cell maceration and scanning electron microscopy. The collagen network in the rat adenohypophysis was loose and the compartments of the cell clusters were obscure. The collagen fibrils were more densely worked into the vascular walls than into the walls of the cell clusters. In contrast, the collagen construction in the pig adenohypophysis displayed obvious compartments in the walls of the cell clusters, and the thick bundles of collagen fibrils were usually cylindrical in shape. In the chicken adenohypophysis, distinct cell clusters were separated by membrane-like collagen septa and the walls of these clusters were constructed of a very fine meshwork of collagen fibrils. These results indicate that the collagen architecture in the adenohypophysis differs among animal species, which may be due to differences in their gland sizes, cell distributions and vascular development.

INTRODUCTION

The shape and size of the hypophysis are known to differ among animal species (Hanström, 1966). In general, the adenohypophysis is divided into three subdivisions, namely the pars tuberalis, pars intermedia and pars distalis. The pars tuberalis is a tiny region between the median eminence and the pars distalis in the cranial region of the gland. Some animal species are devoid of a pars intermedia. The pars distalis is the main region for endocrine function.

The adenohypophysis is composed of not only parenchymal cells but also other components, such as blood vessels and extracellular matrix (ECM). Collagen, an ECM component, appears to play important roles in maintaining the morphological structures and strengths of organs and tissues, and may also be involved in the arrangement and distribution of endocrine cells.

We previously investigated the three-dimensional architecture of the collagen network in the goat hypophysis and demonstrated that the architecture differed among the various regions of the adenohypophysis (Nishimura *et al.*, 2004). However, the architecture of the collagen framework in the hypophysis has still not been confirmed in other animal species, despite clear differences in the shape and size of their glands. The present study was performed to compare the collagen network architectures in the pars distalis of the adenohypophysis between rats, pigs and chickens using a combination of cell maceration and scanning electron microscopy.

MATERIALS AND METHODS

Animals

Rats, pigs and chickens were used in the present study. The rats were Wistar strain males at 10 weeks of age purchased from Kyudo Co. Ltd. (Fukuoka, Japan). The pigs were obtained from a local slaughterhouse and their genders and ages were uncertain. The chickens were Red Cornish X New Hampshire crossbred cocks supplied by Yokoo Co. Ltd. (Saga, Japan). The rats were sacrificed by decapitation under ether anesthesia. The heads of pigs were obtained after decapitation in the slaughterhouse. The chickens were exsanguinated after intravascular injection of sodium pentobarbitone. The hypophyses were removed as soon as possible and processed for scanning electron microscopy. This study was carried out according to the guidelines for Animal Experiments in the Faculty of Agriculture and the Graduate Course of Kyushu University, and according to the Law (No. 105) and Notification (No. 6) of the Japanese Government.

Scanning electron microscopy

Cell maceration was employed for observation of the three-dimensional arrangements of the collagen fibers according to the method of Ohtani (1987) with slight modifications. Briefly, the hypophysis was fixed with 3% glutaraldehyde in 0.1 M phosphate buffer (PB; pH 7.4) for 2–4 days at 4 °C. Next, the hypophysis was cut with a razor blade, rinsed with PB and macerated in 8% NaOH solution for 4 days at 25 °C. The NaOH solution was exchanged twice a day. After rinsing with distilled water for 3 days, the tissue was treated with 1% tannic acid for 2 hours followed by 1% osmium tetroxide in PB

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for 2 hours at 4 °C. The tissue was then dehydrated through an ethanol series, placed in 2-methyl-2-propanol and freeze-dried using a TIS-U-DRY FREEZE-DRYER (FTS Systems Inc., Stone Ridge, NY, USA). The dried specimen was mounted on an aluminum holder and coated with Au using an Ion Sputter IB-3 (EIKO Engineering Co. Ltd., Ibaraki, Japan). The specimen was then observed using a SUPERSCAN SS-550 scanning electron microscope (Shimadzu Corporation, Kyoto, Japan) at the Center of Advanced Instrumental Analysis, Kyushu University.

RESULTS

Rat adenohypophysis

The collagen network in the rat adenohypophysis was found to be a relatively loose meshwork (**Fig. 1a**). The compartments of the cell clusters were obscure. On the other hand, the collagen fibrils were more densely distributed in the blood vessel walls than in the walls of the cell clusters (**Fig. 1b**). At higher magnification, several fibrils were observed to be bound into collagen bundles within the framework, although they were not very thick (**Fig. 1c**). However, many fibrils existed separately and were entangled with each other to create a loose connective tissue framework.

Pig adenohypophysis

The collagen construction in the pig adenohypophysis displayed obvious compartments in the walls of the cell clusters (**Fig. 2a**). The inner surfaces of the cell cluster walls were covered by a fine layer of a network of collagen fibrils, while collagen bundles composed the main framework of the walls (**Figs. 2b–c**). The collagen bundles were usually cylindrical in shape, although belt-like bundles were also occasionally observed (**Figs. 2b–c**). The bundles were either straight, winding and

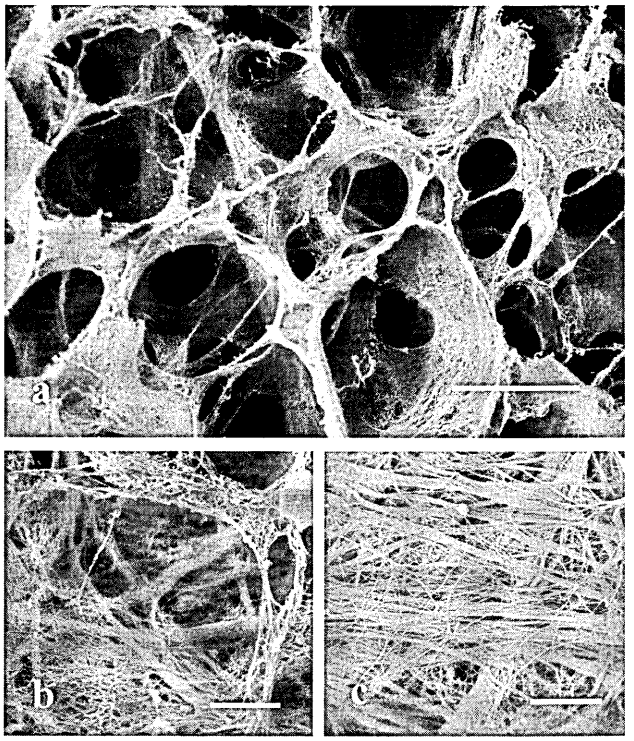

Fig. 2. Scanning electron micrographs of the pars distalis in the pig adenohypophysis. Scale bars: 50 μm (a); 10 μm (b); 10 μm (c).

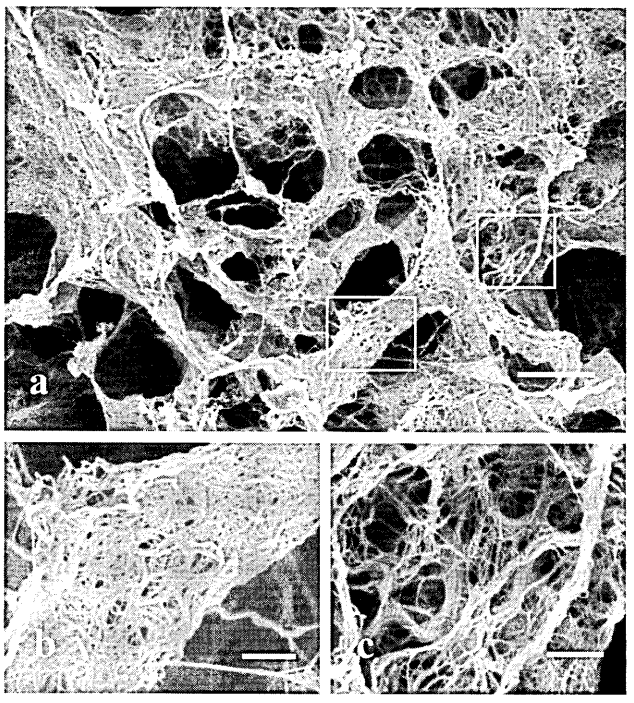

Fig. 1. Scanning electron micrographs of the pars distalis in the rat adenohypophysis. Higher magnifications of the left and right white frames in figure 1a are shown in figures 1b and 1c, respectively. Scale bars: 10 μm (a); 2 μm (b); 2 μm (c).

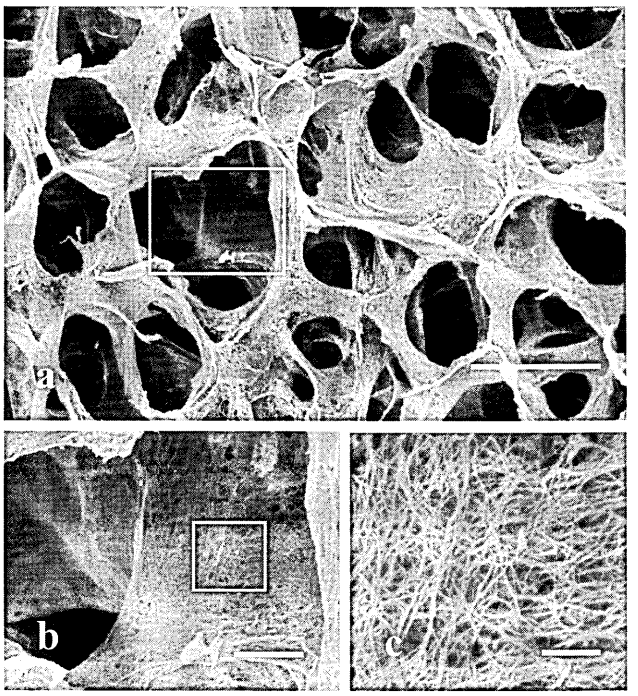

Fig. 3. Scanning electron micrographs of the pars distalis in the chicken adenohypophysis. Higher magnifications of the white frames in figures 3a and 3b are shown in figures 3b and 3c, respectively. Scale bars: 50 μm (a); 10 μm (b); 2 μm (c).

crooked in their course. At higher magnification, the collagen fibrils were revealed to be tangled in a complicated manner, creating a dense collagen framework.

Chicken adenohypophysis

The chicken adenohypophysis was separated into distinct cell clusters by collagen septa at lower magnification (**Fig. 3a**), similar to the case for the pig adenohypophysis. However, the collagen construction was slightly different from that in the pig adenohypophysis. The walls of the cell clusters were composed of a very fine meshwork of collagen fibrils and displayed a membrane-like configuration (**Fig. 3b**). At higher magnification, the collagen bundles were usually not thick, unlike the case in the pig adenohypophysis, and thin bundles and fibrils were intertwined (**Fig. 3c**).

DISCUSSION

The three-dimensional architectures of collagen networks have been studied in many organs of animals. Among these, the architectures in endocrine organs have been studied in the adrenal gland (Kikuta *et al.*, 1991), thyroid gland (Morita *et al.*, 1994) and hypophysis (Nishimura *et al.*, 2004). However, interspecific differences in the architectures have not been elucidated in every organ.

In the goat adenohypophysis, the collagen architecture of the walls of the cell clusters was revealed to be a basket-like configuration woven by flat belt-like collagen bundles of various sizes (Nishimura *et al.*, 2004). In the present study, the three-dimensional architectures of the collagen network in the adenohypophysis displayed different configurations among the rat, pig and chicken adenohypophyses, and also differed from that of the goat adenohypophysis. Specifically, the present results indicate that the collagen network in the cell cluster walls was looser in the rat, firmer in the pig and finer in the chicken. Among these, the architecture in the pig adenohypophysis appears to be the most similar to that in the goat adenohypophysis.

The shape and size of the hypophysis may be important factors for determining the supporting structures in the gland. It is well known that the pars intermedia exists in the rat and pig adenohypophyses, whereas the chicken adenohypophysis is devoid of this region and instead the pars distalis is divided into a cephalic lobe and a caudal lobe. Needless to say, the size of the gland is largest in the pig among these animals. In general, it is supposed that large organs require stronger supporting structures than small organs. Therefore, it is likely that the collagen construction in the pig adenohypophysis was constructed more firmly than that in the rat adenohypophysis.

In general, the pars distalis is supplied by thin-walled sinusoids and composed of thyrotrophs, gonadotrophs, corticotrophs, somatotrophs and mammotrophs (Fawsett and Raviola, 1994). However, the cell distribution patterns in the adenohypophysis differ among animal species. For example, somatotrophs are distributed throughout the pars distalis in the rat adenohypophysis (Takahashi, 1991), while those in the fetal pig adenohypophysis are distributed in all areas except for a large part of the rostral area (Sasaki *et al.*, 1992) and changes in their spatial distribution have been observed in young pigs (Lee *et al.*, 2004). Chicken somatotrophs are restricted in the caudal lobe (Mikami and Takahashi, 1987; Malamed *et al.*, 1997). Therefore, it is possible that the cell composition of the cell clusters and the degree of vascularization may affect the surrounding connective tissue architecture. In the present study, however, we did not observe any striking differences in collagen architecture among the areas of the pars distalis in each animal in relation to the cell type distributions.

Rat adenohypophyseal cells can synthesize types I and III collagens *in vitro* (Kaidzu *et al.*, 2000), although the synthesis and secretion of collagen components by these endocrine cells have not yet been demonstrated *in vivo* (Kaidzu *et al.*, 2000; Nishimura *et al.*, 2004). Together with the relationships to the characteristics of the cell type distributions in the pars distalis, the mechanism for the collagen network construction in the adenohypophysis remains to be elucidated.

In conclusion, the present results indicate that the collagen architecture in the adenohypophysis differs among animal species and may be affected by the shape and size of the gland, the cell composition and the vasculature.

ACKNOWLEDGEMENTS

This research was partially supported by the Japan Society for the Promotion of Science (JSPS), via a Grant-in-Aid for Scientific Research (C), No. 17580257.

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