DOI : 10.5958/2277-8934.2015.00041.7 *Vol 22 No 2, p 251-256*

# IMMUNOHISTOCHEMICAL STUDIES ON THE ENDOCRINE CELLS IN THE THYMUS OF THE ONE-HUMPED-CAMEL (*Camelus dromedarius*)

**H.I. Ismail[1] and A.M. Ali[2]**

[1]Department of Anatomy, College of Veterinary Medicine, University of Bahri, Khartoum, Sudan

[2]Department of Anatomy, Faculty of Veterinary Medicine, University of Khartoum, Sudan

## ABSTRACT

Endocrine cells in the thymus of the one-humped camel (*Camelus dromedarius*) were studied using immunohistochemical methods. Serotonin, insulin, gastrin, glucagon, calcitonin, substance P, Pancreatic polypeptide (PPP)-, CCK, vasoactive intestinal peptide, galanin and somatostatin-cells were detected in the camel thymus by Avidin-Biotin-Complex technique. These immunoreactive cells, except for calcitonin cells, restrictively observed in the medulla. These endocrine cells were shown to be oval, elongated or triangle in shape. Calcitonin-immunoreactive cells were observed both in the cortex and medulla. Hassall's corpuscles were immunoreactive for all neuropeptides except for gastrin, calcitonin, CCK and VIP. Thymocytes were reactive only for gastrin. The obtained data indicate the presence of different neuropeptides in the camel thymus and suggest a role for neuroendocrine hormone-mediated mechanisms in the regulation of the thymichomopoesis in the camel.

**Key words:** Camel, endocrine cells, immunohistochemistry, thymus

The thymus is a primary lymphatic organ containing both epithelial cells and lymphoblasts derived from the bone marrow. The thymus is also known as an endocrine organ responsible for thymopoesis. Increasing evidence suggests the existence of cross talk between the endocrine and the immune system with shared ligands and receptors used as common mechanism of communication between the two systems (Screpanti *et al,* 1993).

Interactions between endocrine cells and epithelial cells, mediated by different hormones, peptides and neuropeptides have been proposed in chicken thymus (Atoji *et al,* 1997; Kawai, 1993).

Although there were few studies dealing with the morphology of the thymus in the camel (Aly e*t al,* 1988; Moustafa *et al,* 1969), comprehensive data on the endocrine cells, peptides or neuropeptides are virtually lacking.

Visualising the morphological basis for endocrine cells and neuropeptides in the thymus of the one-humped camel (*Camelus dromedarius*) is considered as a prerequisite to understand the endocrine-immune system interaction. The current investigation was undertaken using a group of polyclonal antibodies against some hormones and neuropeptides.

## Materials and Methods

A total of 10 camel calves of both sexes and at different ages (6 months-2 years) were used in this study. The thymus glands were collected from slaughterhouse immediately after the animals were slaughtered. All glands were free from pathological changes or lesions when examined prior to tissue sampling.

Collected fresh thymus gland were further cut into small pieces and fixed in neutral buffered formalin, paraformaldehyde solution or Bouin's fluid for routine histological and immunohistochemical purposes.

Three to five micrometre thick paraffin sections, prepared by routine procedures, were stained with either haematoxylin-eosin for general histological observation or with immunohistochemistry for endocrine cells and neuropeptides.

A panel of polyclonal antibodies against different hormones or neuropeptides were used and their characteristics are shown in Table 1.

Immunohistochemistry: Avidin-biotin-peroxidasecomplex (ABC) method was applied to demonstration of endocrine cells and neuropeptides. Briefly, the tissue sections were treated with 0.08 hydrogen peroxide and then with absolute

SEND REPRINT REQUEST TO ISMAIL HAIDER IBRAHIM email: haideribrahim1@hotmail.com

**Table 1.** Showing the primary and secondary antibodies, their sources and dilutions.

| Primary antibody | Source | Working dilution | Secondary antibody | Working dilution |
|---|---|---|---|---|
| 1- Serotonin | Serotec-UK | 1:20 | Biotinylated rabbit IgG | 1:100 |
| 2- Somatostatin | Serotec-UK | 1:20 | Biotinylated rabbit IgG | 1:100 |
| 3-Calcitonin | Serotec-UK | 1:500 | Biotinylated rabbit IgG | 1:100 |
| 4- CCK-8 | Serotec-UK | 1:250 | Biotinylated rabbit IgG | 1:100 |
| 5- Substance P | Serotec-UK | 1:500 | Biotinylated rabbit IgG | 1:100 |
| 6- VIP | Serotec-UK | 1:200 | Biotinylated rabbit IgG | 1:100 |
| 7- Insulin | Serotec-UK | 1:50 | Biotinylated rabbit IgG | 1:100 |
| 8- Glucagon | Serotec-UK | 1:5 | Biotinylated rabbit IgG | 1:100 |
| 9- Gastrin | Dako | 1:5 | Biotinylated rabbit IgG | 1:100 |
| 10- Galanin | Serotec-UK | 1:5 | Biotinylated rabbit IgG | 1:100 |
| 11- PPP | Dako | 1:30 | Biotinylated rabbit IgG | 1:100 |

methanol for 30 min in room temperature in each step to remove endogenous peroxidase activities. Subsequently, non-specific reactions were blocked by incubating in 5% goat normal serum in 1% bovine serum albumin (BSA) for 30 min at room temperature, then incubated in the primary antibody for 24 hr at 4°C (Table 1). Following rinsing in phosphate buffer saline (PBS), sections were then incubated with biotinylated secondary antibody at 1: 100 dilution for 1 hr at room temperature. Thereafter, the sections were incubated with avidin-biotin-peroxidase complex (Dako) at 1: 100 dilution for 1 hr at room temperature. The antigen-antibody reaction was visualised with 3,3′-diaminobenzidine-HCL (DAB) solution (using tabs in tris buffer) and slightly counterstained with Mayer's haematoxylin.

Appropriate negative controls, including omitting the primary antibody (using PBS instead) or using an irrelevant antibody were prepared. Positive controls were also used.

## Results

Similar to other animals the thymus gland was enclosed by a connective tissue capsule. The gland was further divided into lobules by connective tissue septa extending from the capsule. The lobules had an outer cortex and an inner lightly stained medulla. Numerous granulated cells of different shapes and sizes were observed in the interlobular septa and occasionally adjacent to the cortex (Fig 1 a and b).

Immunohistochemical analysis of the thymus gland for various endocrine cells and neuropeptides revealed a distinctive distribution at different animals ages and sexes. Immunoreactivity for the various components of the thymus is shown in Table 2.

**Serotonin:** Few serotonin reactive cells were found in the centre of the medulla. These cells were usually large of different shapes showed the reactivity in the peripheral region (Fig 2, a). Nevertheless small reactive cells were also present. Some epithelial cells around the thymic corpuscles, and the thymic corpuscles were also intensively reactive (Fig 2, a). Thymocytes showed weak reactivity. No reactive cells were noticed in the cortex or in the septa between the lobules. Granulated cells shown by haematoxlin and eosin staining were not reactive to serotonin.

**Table 2.** Showing the reactivity of hormones on some components of the thymus.

| Primary antibody | ER | TC | GC | Thymocytes | Region |
|---|---|---|---|---|---|
| Serotonin | +++ | + | - | - | medulla |
| Insulin | +++ | + | + | - | medulla |
| Gastrin | +++ | - | - | + | medulla |
| Glucagon | +++ | + | - | - | medulla |
| Calcitonin | ++++ | - | - | - | medulla/ cortex |
| Substance P | + | + | + | - | medulla |
| PPP | + | + | - | - | medulla |
| CCK | ++ | - | - | - | medulla |
| VIP | +++ | - | - | - | medulla |
| Galanin | ++ | + | - | - | medulla |
| Somatostatin | + | + | - | + | medulla |

**ER** epithelial reticular cells, **TC** thymic corpuscles, **GC** granular cells. For **ER** intense immunoreactivity is designated as ++++, less intense as +++, moderate as ++, sparse as +, while - signifies lack of immunoreactivity. The presence of immunoreactivity within thymic corpuscles is marked by +.

**Insulin:** A few cells of different shapes and sizes showed reactivity to insulin antiserum. They were usually present in the centre of the medulla. Intense reaction was notified in the thymic corpuscles (Fig 2, b). Those cells located in the cortex were not reactive.

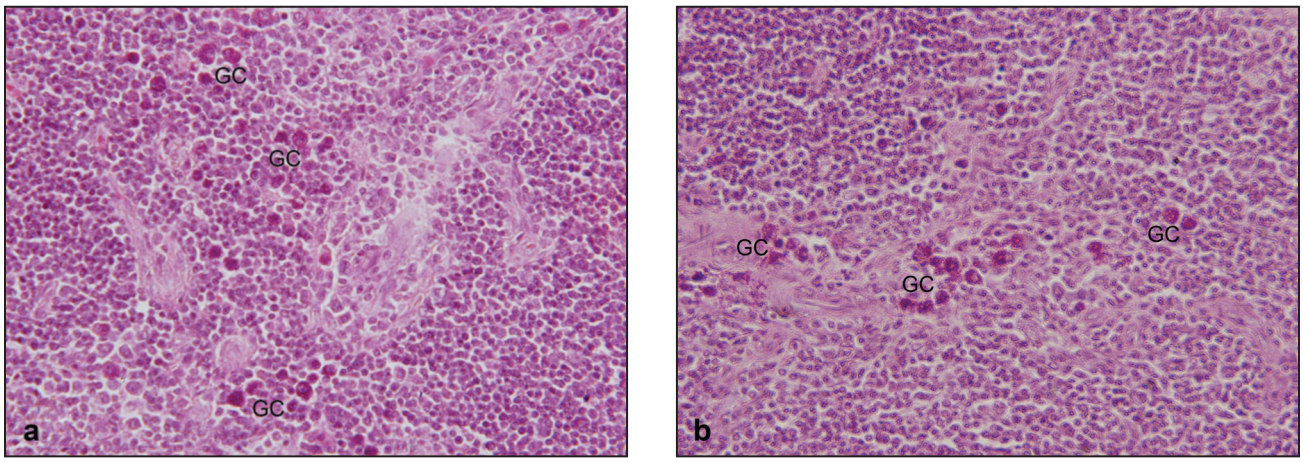


**Fig 1.** (a and b): Thymic medulla and paracortical areas showing many granulated cells (GC). HEX400.

Numerous granulated cells in the septa between the lobules showed intense reactivity (Fig 2, b).

**Gastrin:** Positive cells to gastrin were found in the medulla and were usually of different shapes and sizes (Fig 2, c). Some reactivity could be seen in the thymocytes. Thymic corpuscles were not reactive. No reaction was seen in the cells of the cortex or in the granulated cells of the septa between the lobules.

**Glucagon:** Large reactive cells of various shapes were present in the medulla, while the cortex and septa between the lobules contained non-reactive cells (Fig 2, d). A positive reaction was also demonstrated in the thymic corpuscles, in their center and at their peripheries. Granulated cells were not reactive. A weak reaction was observed in the connective tissue between the lobules (Fig 2, d).

**Calcitonin:** Abundant cells showed reactivity to calcitonin were located both in the cortex and medulla. These cells were usually large and of different shapes (Fig 2, e). Thymic corpuscles and granulated cells between the lobules were not reactive. Strong reaction was also noticed in the connective tissue between the lobules.

**Substance P:** Intensive reactivity was observed in few large rounded cells in the medulla and in the connective tissue adjacent to the cortex (Fig 2, f). Strong reactivity was noticeable in the granular cells (Fig 3, a). Thymic corpuscles were also reactive, while no reactivity was shown in the cortex.

**PPP:** While only a few reactive cells of different shapes were noticed in the medulla, thymic corpuscles were intensively reactive (Fig 3, b).The cells of the cortex and the granulated cells in the septa between the lobules were non-reactive.

**CCK:** Many reactive cells of different shapes and sizes were encountered in the medulla and septa between the lobules (Fig 3, c). The cells of the cortex did not show reactivity. No granulated cells in the septa between the lobules or thymic corpuscles showed reactivity.

**VIP:** A few number of reactive cells were found in the medulla and near the thymic corpuscles (Fig 3, d). However, the thymic corpuscles, granulated cells and the cells of the cortex were not reactive.

**Galanin:** Intense reactive cells of different shapes and sizes were noticed in the medulla (Fig 3, e). Thymic corpuscles and the connective tissue were also positive to galanin. No positive reaction was observed in the cortex or in the granulated cells in the septa between the lobules.

**Somatostatin:** Only few reactive cells could be detected in the medulla (Fig 3, f), whereas thymic corpuscles were markedly reactive, granulated cells in the septa between the lobules or near the cortex were not reactive.

## Discussion

No single paper is available demonstrating the endocrine cells or neuropeptides in the thymus of the camel. The present investigation is the first description of these cells in the thymus of the one-humped camel (*Camelus dromedarius*).

In the current study we have shown the presence of eleven endocrine cells in the thymus of the camel. These hormones and neuropeptides were serotonin, insulin, gastrin, glucagon, calcitonin, substance P, CCK, vasoactive intestinal peptide (VIP), galanin and somatostatin (SOM). These immunoreactive cells were restrictively observed in the centre of the medulla and subcortical area. The existence of this vast number of various endocrine cells in the thymus gland, provides further evidence that immunoendocrine interactions are likely to occur in the camel thymus.

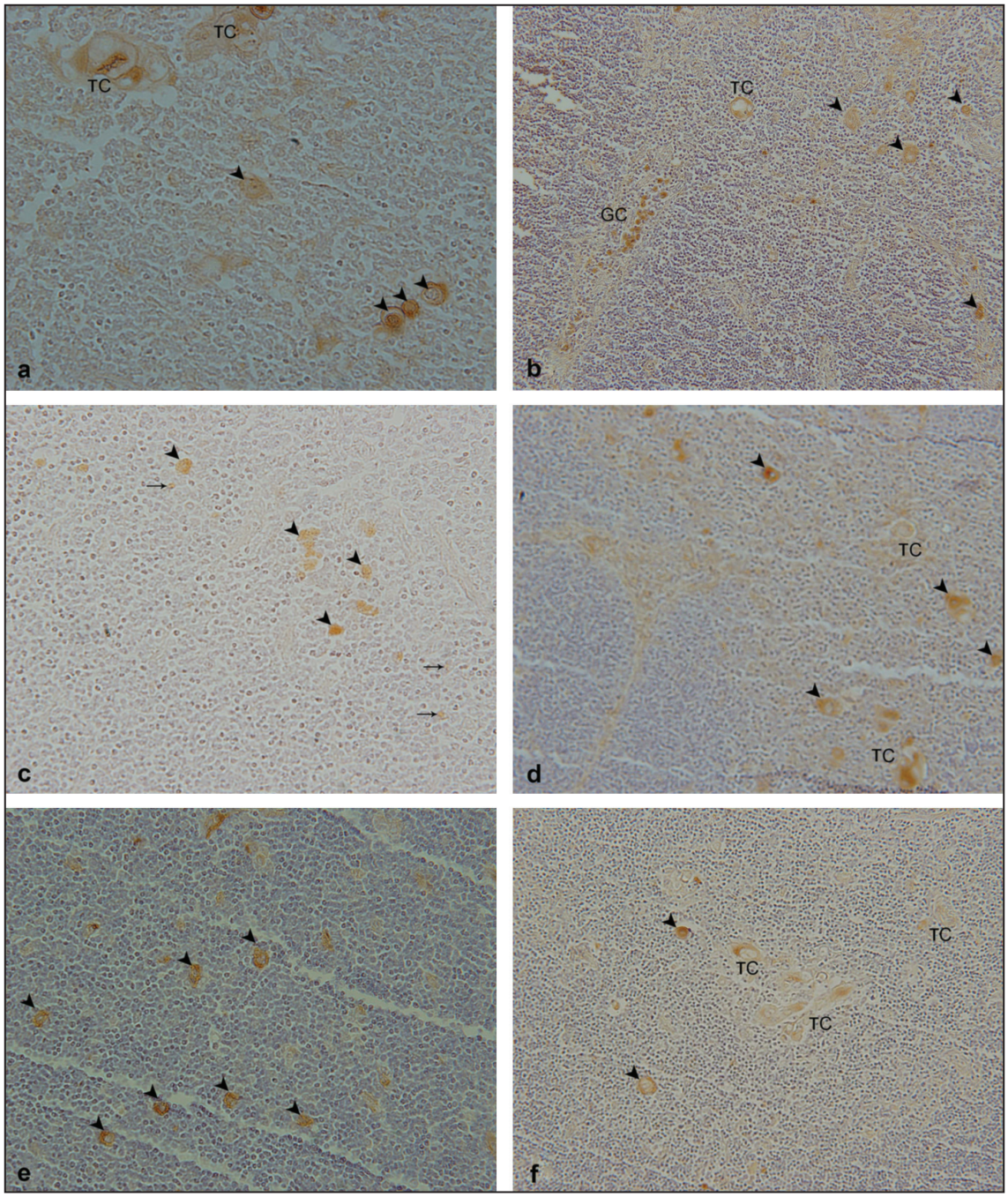


**Fig 2.** Immunoreactive staining for endocrine cells in the camel thymus. Serotonin-, insulin-, gastrin-, glucagon-, calcitonin- and substance P-immunoreactive cells are shown in the medulla (a, b, c, d, e and f, respectively). Thymocytes and endocrine cells exhibiting immunoreactivity are marked by arrows and arrowheads, respectively. Immunoreactive thymic corpuscles (TC) and granulated cells (GC) are also shown. ABC method, X400.

The presence of serotonin-, dopamine-, gastrin/CCK, CCk8, neurotensin, peptide YY, glucagon, somatostatin, insulin, calcitonin, growth hormone, and SP-1/chromogranin-immunoreactive cells was detected in the thymus of the chick (Kawai, 1993). Similar findings were obtained in the camel thymus. However, in the camel thymus calcitonin was present in both the medulla and cortex whereas in the chicken thymus it was demonstrated only in the cortex. Neuropeptide Y, substance P, encephalin and VIP were also demonstrated in the chicken thymus (Atoji e*t al,* 1997) while (Zhang e*t al,* 2009) studied carboxypeptidase E and chromgranin A.

The expression of hormones and neuropeptides in the other species thymuses has been thoroughly investigated. Immunoreactivity for VIP, CGRP and

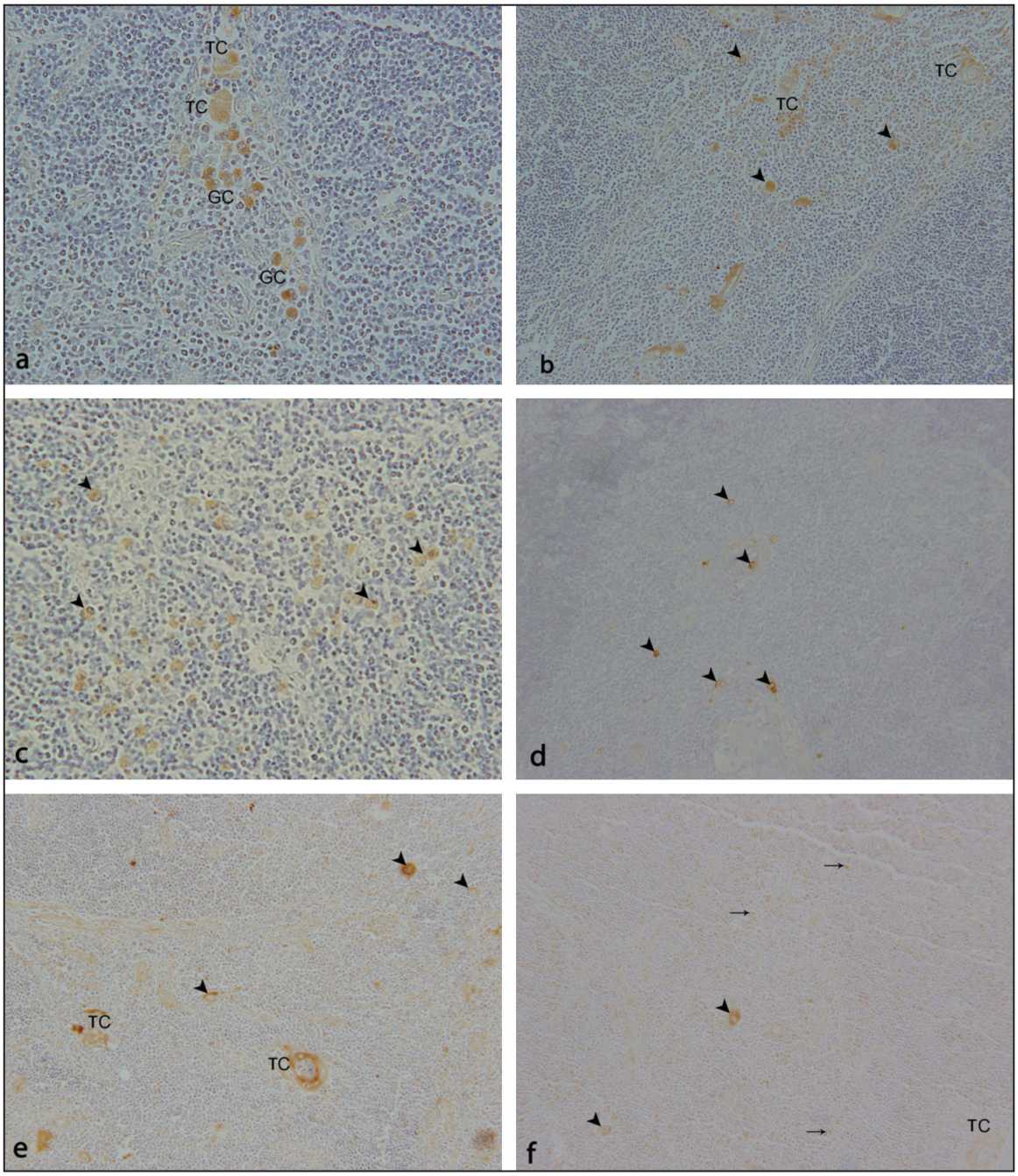


**Fig 3.** Immunoreactive staining for endocrine cells in the camel thymus. Substance P-, PPP-, CCK-, VIP-, galanin- and somatostatin-immunoreactive cells are shown in the medulla (a, b, c, d, e and f respectively). Thymocytes and endocrine cells exhibiting immunoreactivity are marked by arrows and arrowheads, respectively. Immunoreactive thymic corpuscles (TC) and granulated cells (GC) are also shown. ABC method, X400.

Substance P was found before in chicken and rodent thymic cells (Ericsson e*t al,* 1990; Gomoriz e*t al,* 1990; Gomoriz e*t al,* 1993; Soder and Hellstrom, 1989; Weihe e*t al,* 1989). In the human thymus, Leposavic *et al* (2011) demonstrated the immunoreactivity for VIP, CGRP, SP, bompesin and motilin. Neuropeptide expression for CGRP, NPY, SOM, SP and VIP was also shown in the thymus of zebrafish, xenopus, avian, rodent, porcine and equine (Screpanti *et al,* 1993).

These endocrine cells encountered in the thymus of the camel appeared to be thymicreticulo-epithelial cells. The heterogeneous origin of the thymic epithelial cells may explain the presence of this vast number of hormone and neuropeptide cells in the thymus. It was stated that the epithelial reticular cells of the

camel could be divided into three types (Aly *et al,* 1988). Heterogeneity of the thymic stromal cells was claimed by various authors (Batanero *et al,* 1992; Bodey *et al,* 2000; Dardenne *et al,* 1991; Ferone e*t al,* 1999; Silva *et al,* 2006; Zhang *et al,* 2009). Oxytocin and vasopressin immunoreactive cells were detected in the stromal cells in the subcapsular cortex and in the medulla of the human thymus. It was demonstrated that these cells were subset of epithelial cells (Robert *et al,* 1991). Four functional subtypes of medullary reticuloepithelial cells were identified (Bodey, 2007).

In conclusion, the present study confirms the expression of vast number of hormones and neuropeptides by the thymus of the camel. These hormones may act in a paracrine or autocrine fashion in order to regulate the thymus homeostasis in the camel. The obtained data support the concept that the thymus in addition to being a central lymphoid organ is also an endocrine organ which produces various hormones and neuropeptides that may influence the function of this gland. Furthermore, it is suggested that these hormones and neuropeptides produced by the thymus of the camel may play an important role in T-cell development and provide an evidence of cross-talk between the immune and endocrine systems.

## References


Aly AE, Abdo MS, Algaily S and Prentis P (1988). Electron microscopic studies on the thymus of the Arabian camel (*Camelus dromedarius*). Anatomischer Anzeiger 167:119-127.

Atoji Y, Yamamoto Y, Komatsu T and Suzuki Y (1997). Localisation of neuropeptides in endocrine cells of the chicken thymus. Journal of Veterinary Medical Science 59:601-603.

Batanero E, de Leeuw FE, Jansen GH, van Wichen DF, Huber J and Schuurman HJ (1992). The neural and neuro-endocrine component of the human thymus. II. Hormone immunoreactivity. Brain, Behaviour, and Immunity 6:249-264.

Bodey B (2007). Thymicreticulo-epithelial cells: Key cells of neuroendocrine regulation. Expert Opinion on Biological Therapy 7:939-949.

Bodey B, Bodey BJr, Siegel SE and Kaiser HE (2000). The role of the reticulo-epithelial (RE) cell network in the immune-neuroendocrine regulation of the intrathymiclymphopoiesis. Anticancer Research 20:1871-1888.

Dardenne M, Kelly PA, Bach JF and Savino W (1991). Identification and functional activity of prolactin receptors in thymic epithelial cells. Proceedings of the National Academy of Sciences 88:9700-9704.

Ericsson A, Geenen V, Robert F, Legros JJ, Vrindts-Gevaert Y, Franchimont P, Bene S and Persson H (1990). Expression of preprotachinin-A and neuropeptide Y messenger RNA in the thymus. Molecular Endocrinology 4:1211-1216.

Ferone D, van Hagen PM, Colao A, Annunziato L, Lamberts SW and Hofland LJ (1999). Somatostatin receptors in the thymus. Annals of Medicine 31:28-33.

Gomoriz RP, Lorenzo ML, Cacicedo Z, Vincente A and Zapata AG (1990). Demonstration of immunoreactive vasoactive intestinal peptide (IR-VIP) and somatostatin (IR-SOM) in rat thymus. Brain, Behaviour and Immunity 4:151-161.

Gomoriz RP, DelgradoM, Naranjo, JR Melstrom B, Tormo A, Mata F and Laceta J (1993). VIP gene expression in the rat thymus and spleen. Brain, Behaviour and Immunity 7:271-278.

Kawai K (1993). Immunocytochemical and ultrstructural studies on the endocrine cells in the chick thymus. Japanese Journal of Veterinary Research 41:26-26.

Leposavic G, Todorovic V, Nikolic I and Perisik M (2011). Immunoreactive neuropeptides in the cells of the human thymus. Archives of Biological Science Belgrade 63:971-977.

Moustafa MS, Berg R and Taher el-S (1969). Prenatal growth of some organs in the camel (*Camelus dromedarius*). Relationship between body weight and brain, thymus, stomach and oesophagus weights. Zentralbl Veterinar med 16:536-542.

Robert F, Geenen V, Schoenen J, De Groote D, De Legros JJ and Franchmont P (1991). Colocalisation of immunoreactive oxytocin, vasopressin and interleukin-1 in human thymic epithelial neuroendocrine cells. Brain, Behaviour and Immunity 5:102-115.

Screpanti I, Modesti A and Gulino A (1993). Heterogeneity of thymic stromal cells and thymocyte differentiation: a cell culture approach. Journal of Cell Science 105:601-606.

Silva AB, AwD and Palmer DB (2006). Evolutionary conservation of neuropeptide expression in the thymus of different species. Immunology 118:131-140.

Soder O and Hellstrom P (1989). The tachykininsneurokinin A and physaelaemin stimulate murine thymocyte proliferation. International Archives of Allergy and Immunology 90:91-96.

Weihe E, Muller S, Fink F and Zentel HJ (1989). Tachikinins, calcitonin-gene related peptide and neuropeptide Y in nerves of mammalian thymus: interaction with mast cells in autonomic and sensory neuroimmunomodulation. Neuroscience Letters 100:77-82.

Zhang X, Zhu J, Loh YP and Bergham LR (2009). Carboxypeptidase E, an essential element of the regulated secretory pathway, is expressed and partially co-localized with chromogranin A in chicken thymus. Cell and Tissue Research 337:371-379.