Turkish Journal of Zoology

Volume 23 | Number 3 Article 15

1-1-1999

The functional relationships between the neurosecretory material and the adrenal gland of Rana ridibunda (Amphibia-Anura)

SONGÜL SÜREN

Tr. J. of Zoology
23 (1999) 305-311

The functional relationships between the neurosecretory material and the adrenal gland of *Rana ridibunda* (Amphibia-Anura)

Songül SÜREN
Department of Biology, Faculty of Sciences, Istanbul University, Istanbul-TURKEY

Received: 04.09.1997

Abstract: In this research, the structural changes that appear in the adrenal glands and neurosecretory cells of frogs (*Rana ridibunda*) which had been kept under different temperatures (+4˚C, +10˚C, +20˚C) were studied with light microscope and the relation between these structures were discussed.

The pericaryons of Gomori (+) nuerons that form the preoptic nuclei had different sizes. These were big and small neurosecretory cells. The adrenal gland of *Rana ridibunda* had three types of cells, being corticosteroidogenic, catecholaminergic and Stilling cells. Although neurosecretory cells were observed to be generally active at all temperatures administered especially, small neuorosecretory cells were vulnerable to temperature increases according to their sizes and became more active. In other words, the increase in temperature caused small neurosecretory cells to produce secretion and discharge. Temperature induced an increase in synthesis and secretion in corticosteroidogenic cells and Stilling cells of the adrenal gland. On the other hand, materials were stored distinctly in catecholaminergic cells, even if there was the production of secretion in those cells.

As a result, there may be a functional similarity between the small neurosecretory cells and corticosteroidogenic cells of the adrenal gland. In addition, a functional similarity may also be observed between corticosteroidogenic cells and Stilling cells. Catecholaminergic cells may be effective in stimulating the secretion activities of both corticosteroidogenic and Stilling cells.

Key Words: Preoptic nucleus, Adrenal Gland, Corticosteroidogenic cells, Catecholaminergic cells, Stilling cells.

Rana ridibunda (Amphibia-Anura)'nın nörosekresyon materyali ile böbreküstü bezi fonksiyonu arasındaki ilişkiler

Özet: Bu araştırmada, farklı sıcaklıklar (+4˚C, +10˚C, +20˚C)'da tutulan kurbağa (*Rana ridibunda*)'ların nörosekresyon hücreleri ve böbreküstü bezi hücrelerinde oluşan yapısal değişiklikler ışık mikroskobu düzeyinde incelenmiş, her iki yapı arasında ne tür bir ilişkinin olabileceği tartışılmıştır.

Preoptik nukleusu oluşturan Gomori (+) nöronların perikaryonları, büyük ve küçük boyutlular olmak üzere, farklı büyüklüktedir. *Rana ridibunda*'nın böbreküstü bezinin, kortikosteroidojenik hücre, katekolaminerjik hücre ve Stilling hücre olarak adlandırılan, üç tip hücresi vardır. Uygulanan tüm sıcaklık derecelerinde, genelde, nörosekresyon hücrelerinin aktif olduğu gözlenmiş ise de, özellikle küçük nörosekresyon hücreleri, büyüklerine göre sıcaklık artışından daha çok etkilenip, daha aktif hale dönüşmüşlerdir. Diğer bir tanımla, sıcaklık artışı küçük nörosekresyon hücrelerinin salgılarını üretip boşaltmalarına neden olmuştur. Böbreküstü bezi hücrelerinden kortikosteroidojenik hücreler ve Stilling hücrelerinde de sıcaklık, sentez ve salgılamada artışa neden olmuştur. Oysa katekolaminerjik hücrelerde salgı üretimi olsa bile, materyal belirgin olarak hücrelerde sitoplazmada depolanmıştır.

Sonuç olarak, küçük nörosekresyon hücreleri ile böbreküstü bezindeki kortikosteroidojenik hücreler arasında fonksiyonel açıdan bir paralelliğin olduğu söylenebilir. Öte yandan, kortikosteroidojenik hücreler ile Stilling hücreler arasında da, fonksiyonel açıdan bir ilişki olabileceği söylenebilir. Katekolaminerjik hücreler ise, kortikosteroidojenik hücreler ile Stilling hücrelerinin salgı aktivitelerine uyarıcı yönde etkili olabilirler.

Anahtar Sözcükler: Preoptik nukleus, Böbreküstü bezi, Kortikosteroidojenik hücreler, Katekolaminerjik hücreler, Stilling hücreleri.

Introduction

The active form of the neurosecretory substance in blood in mammals is oxytocin and vasopressin (1). However, the active form of the neurosecretory material in amphibians is arginin-vasotocin, mesotocin, and in some species the active form is oxytocin (2).

The pericarya, which produce the neurosecretory substance of Gomori (+) neurons, come together to form a pair of preoptic nuclei (PON) in the brain of amphibians (3). The cells of preoptic nuclei may also produce substances other than the hormones mentioned above. These substances are either synthesized in different neurons of PON, or two different substances may be produced in the same neuron. Cumming et al. (1982), using immunocytochemical methods in the goldfish *Carassius auratus,* showed that different pericarya in PON

contain arginin-vasotocin, isotocin and encephalin (4). While van Vossel-Daeninck et al. (1981) reported that different neurons produce arginin-vasotocin, mesotocin and somatostatin in the preoptic nuclei of *Rana temporaria* (5). The pericarya of the PON of the eel fall into three groups according to their functions. The first group contains only arginin-vasotocin; the second group contains only corticotropin-releasing hormone (CRH); and the third contains both hormones, that is, CRH and arginin-vasotocin together (6).

Usually the adrenal gland in Anura-Amphibians is composed of corticosteroidogenic cells and cotecholaminergic cells, which produce steroid hormones and catecholamines respectively. However, the adrenal glands of the Ranidae family contain a third type of cells, called the Stilling cells. In the Anura-Amphibian samples studied, all the of cells are arranged in a reticular fashion to form the gland (7). Henke and Weber (1965) showed that different zones contain groups of different types of cells in the adrenal gland of *Rana temporaria.* Researchers claim that this grouping of cells, representing the cortex and medulla, is much more apparent in adrenal glands which are in an active state (8).

In the present study, the main aim was to determine effect of Gomori (+) neuron secretion on vertebrate adrenal gland function regulated with CRH by a negative feedback mechanism. Specimens of *Rana ridibunda.* a member of the Ranidae family which has Stilling cells in the adrenal glands, were kept at various temperatures and examined for structural changes in the adrenal glands and preoptic nuclei with light microscope. The kind of functional relationship between these two structures was considered, and the kind of functional relationship of the cells making up the adrenal gland is suggested.

Materials and Methods

Four groups, three animals in each group, were examined in this study. The first group was the control group. They were collected at 20°C and immediately decapitated. The animals in the other three groups were kept for twenty days at +4˚C, +10˚C and +20˚C respectively in aquariums placed in a glass cabinet. The temperature of the cabinet was controlled from the outside and the cabinet was illuminated by daylight. The dissection of all the experimental groups was performed in the cabinet at the same temperature. Both the brain and kidney samples were fixed in Boin's fluid. After routine preparation procedures, 5 μm sections were cut from paraffin blocks. The brain tissue sections were stained with Aldehyde Fuchsin (9) for the study of the preoptic nucleus, and kidney tissue sections were stained with Haematoxylin-Eosin (10) for examination of the adrenal gland. The slides were examined with a light microscope. The measurements were made with an ocular micrometer and the pictures were taken with a Carl-Zeiss photomicroscope.

Results

The preoptic nucleus

The pericarya of the Gomori (+) neurons constituting the preoptic nucleus were found to be of two sizes, big and small. There was no difference between the general shape and the shape of the nucleus of the neurosecretory cells in the control animals and the experimental groups kept at different temperatures. However, heat caused a subtantial change in the nucleus size of both types of cells (Table 1).

It was observed that the cytoplasm of all the neurosecretory cells in the control animals contained slightly stained fine granules. The neurosecretory cells in the experimental animals had both coarse and fine granules. However, the cells which contained fine granules were numerous in number. With the rise in temperature, the number of small neurosecretory cells containing fine granules increased.

It was also observed that all the neurosecretory cells in the control and experimental groups contained vacuoles

CELLS	BIG CELLS	SMALL CELLS 5mm)
GROUPS		
I(+20˚C) control	7.7	5.9
II (+4˚C)	7.6	5.8
III (+10˚C)	*7.9	*5.7
IV (+20˚C)	*8.1	*6.5

Table 1. Average size of nuclei of the neurosecretory cells of the preoptic nucleus in both control and experimental groups.

The difference in the size of the big cells and small cells in the experimental group and control group was analysed statistically. The difference between the control group and the group marked (*) were considered statistically significant ($p<0.05$).

of various sizes in the cytoplasm. Generally, with the rise in temperature, the numbers of vacuoles increased. However, the vacuoles were smaller in the experimental animals that were kept at the highest temperature (20˚C). These variations observed in cytoplasmic vacuoles were more conspicuous in the small neurosecretory cells (Fig. 1: a, b, c, d, e).

The adrenal glands

The cells in the adrenal gland were interwoven with blood vessels. (Fig. 2: a, b).

Most of the corticosteroidogenic cells came together and formed groups. Though the cell walls were not definite, their shapes were polygonal and they had short extensions. The cytoplasm had fine particles and small vesicles. These cells were stained pink with Eosin.

The catecholaminergic cells either formed groups or remained separate among the other cells. They were usually polygonal, but some had triangular shapes. Their cytoplasm, also, seemed to have fine granules which were dispersed homogenically and stained dark red with Haematoxylin. Some catecholaminergic cells contained small cytoplasmic vacuoles.

The Stilling cells were oval in shape and each cell was surrounded by different numbers of corticosteroidogenic cells. Their cytoplasm contained relatively coarse granules which were stained pink with Eosin.

Although all three types of cells seemed to be spread evenly throughout the gland, measurements revealed that the numbers of cells found in a square unit of the peripheral and central parts of the gland differed. It was determined that, while the catecholaminergic cells were greater in number in the central parts, the peripheral parts contained more corticosteroidogenic and Stilling cells. This difference in number was statistically significant ($p<0.05$) (Table 2).

Some structural changes due to heat occurred in the adrenal glands. Blood vessels expanded in specimens, especially those kept at 20°C. No changes were detected in the shapes of the cells, but the size of the nuclei increased with the change in temperature when compared to the control group in both the corticosteroidogenic and catecholaminergic cells. However, there were no significant changes in cell size and nucleus size in the animals that were kept at the same temperature (+20°C) as the control group (Table 3).

The corticosteroidogenic cells located at the periphery of the gland contained nuclei which were bigger in size than those of the cells located in the center, and this difference in size was also statistically significant ($p<0.05$) (Table 4). The cytoplasmic vacuoles of the corticosteroidogenic cells gradually enlarged with increases in temperature compared to the control group, and they were located close to each other.

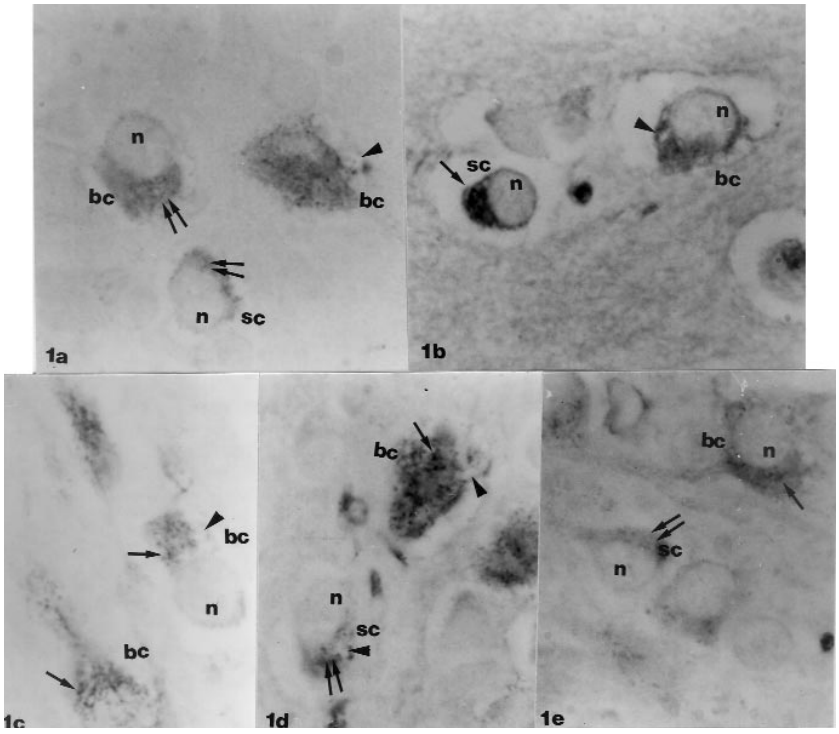

Figure 1. Neurosecretory cells at different temperatures. a: +20˚C (Control group), b: +4˚C, c, d: +10˚C, e: +20˚C. Arrow: big neurosecretory granules; double arrows; small neurosecretory granules; arrow head: vacuoles; n: nucleus, bc: big neurosecretory cells; sc: small neurosecretory cells. Aldehyde Fuchsin-Light Green. x1748.

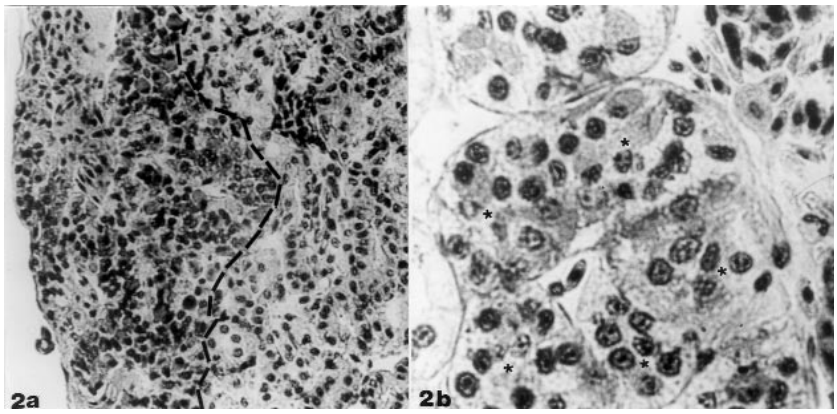

Figure 2. a: General appearance of adrenal gland. The edge of the gland is showen by dashed lines. Haematoxylin-Eosin. x230. b: One of the lobes containing five lobules (*) of control individuals' adrenal gland. Haematoxylin-Eosin. x699.

Table 2. Number of cells per square unit (36.10^{-4} mm^2) at the periphery and the center of the adrenal gland.

CELLS	Corticosteroidogenic Cells		Catecolaminergic Cells		Stilling Cells	
GROUPS	Peripheral	Central	Peripheral	Central	Peripheral	Central
I (+20°C) control	10.65	→ 8.18	2.64	← 3.88	4.93	→ 3.80
II (+4°C)	11.84	→ 10.30	1.94	← 3.94	6.76	→ 5.98
III (+10°C)	8.74	8.08	2.72	← 4.82	4.18	→ 3.56
IV (+20°C)	10.32	10.16	3.34	3.24	3.70	→ 2.40

Significant differences between the peripheral and central cells are indicated with arrows ($p<0.05$). The direction of the arrows indicates a decrease.

The cytoplasmic granules of the catecholaminergic cells in all the experimental groups were greater in size than those in the control group. It was observed that the coarse granules of the catecholaminergic cells were located very close to each other. An increase in temperature also caused the expansion of the cytoplasmic vacuoles of these cells.

In addition, the temperature increase caused the cytoplasmic granules of the Stilling cells to enlarge but these seemed to be further apart from each other in comparison with the control group (Fig. 3: a, b, c, d, e).

Discussion and Conclusion

When the nucleus size (11), the size and the degree of the staining reactions of the cytoplasmic granules (12, 13), and the cytoplasmic vacuoles (14, 15) were taken as criteria, it was observed that, in all animals of the experimental groups all the big and small Gomori (+) neurosecretory cells produced and released secretions. Thus we can say that they were activated. However, there is some deviation from this general statement in terms of big and small cells. The synthesizing and secreting activities in the majority of the small cells showed a greater increase with increasing temperature than in the big cells.

Table 3. Total cell size (TCS) and nucleus size (NS) of the three types of cells in the adrenal gland (in micrometers).

CELLS	Corticosteroidogenic Cells		Catecholaminergic Cells		Stilling Cells	
GROUPS	TCS	NS	TCS	NS	TCS	NS
I (+20°C) control	12.13	6.83	13.35	7.01	11.62	5.31
II (+4°C)	*13.94	*7.13	*15.97	6.88	11.54	5.23
III (+10°C)	*12.77	*7.13	*14.86	*7.48	*13.50	5.27
VI (+20°C)	*11.63	6.98	*12.88	7.07	11.44	5.46

There was a sighificant difference between the control group and the (*) marked groups ($p<0.05$).

CELLS	CSC				CC				SC			
	TSC		NS		TSC		NS		TCS		NS	
	P	C	P	C	P	C	P	C	P	C	P	C
I(+20°C) control	12.46	11.69	7.00	6.60	13.66	13.65	7.04	7.20	11.95	11.37	5.30	5.28
II (+4°C)	15.10	13.12	7.32	6.91	16.50	16.43	6.96	6.96	11.48	11.48	5.19	5.26
III (+10°C)	13.62	12.30	7.28	6.90	16.05	14.41	7.73	7.31	13.35	13.59	5.31	5.37
IV (+20°C)	12.02	11.51	6.97	6.92	12.95	12.74	7.17	6.86	11.64	11.29	5.54	5.37

The groups exhibiting a significant difference between the peripheral (P) and central (C) cells are indicated with an arrow ($p<0.05$). The direction of the arrow indicates a decrease.

Table 4. The total cell size (TCS) and nucleus size (NS) of cells located at the periphery (P) and the center (C) of the adrenal gland (in micrometers).

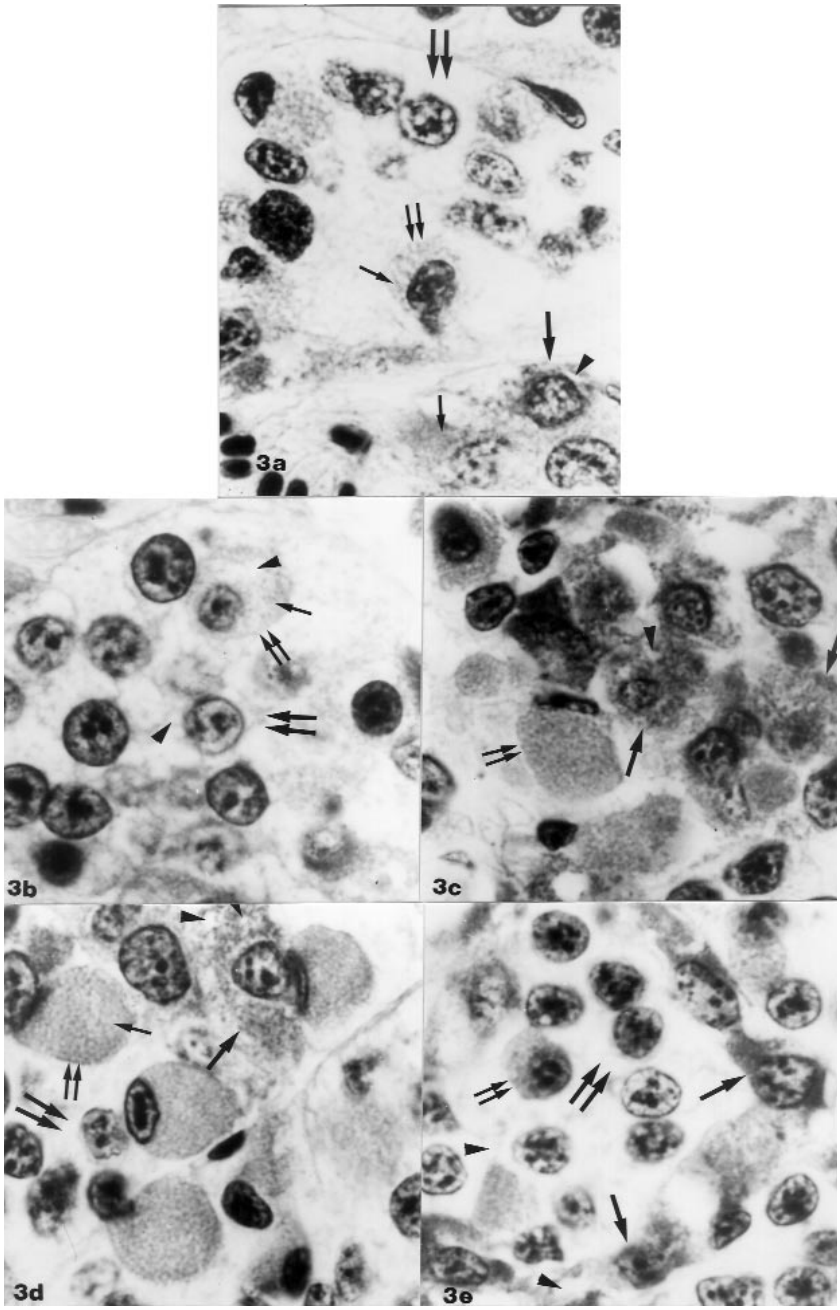

Figure 3. Adrenal gland at different temperatures. a: +20°C (Control group), b, c: +4°C, d: +10°C, e: +20°C. Thick arrow: Catecholaminergic cell; thick double arrows: Corticosteroidogenic cell; thin double arrows: Stilling cell; arrow head: vacuoles; thin arrow: cytoplasmic granules. Haematoxylin-Eosin. x1748.

Although it was observed that all three types of cells were spread evenly throughout the adrenal gland, different numbers of each type of cell per square unit were determined in the peripheral and central zones. When these measurements, are considered the high concentration of corticosteroidogenic cells and Stilling cells in the peripheral zone, and the high concentration of catecholaminergic cells in the central zone in *Rana ridibunda* may be interpreted as being at least a preliminary step in structural development although the typical zoning found in mammals was not present. It can also be concluded that the peripheral corticosteroidogenic cells were more active because their nuclei were bigger than those in the center. Nucleus size (11) is believed to be the best criterion for cell activity.

The expansion of the lipid vacuoles of the corticosteroidogenic cells with increasing heat indicates that these cells may contain excessive amounts of substance (16). Cells that make steroid-type secretions produce and secrete hormones only in the limited amounts that are necessary, so the hormones are not stored inside the cell. Thus, the increase in the size of the vacuoles with increasing heat indicates the activation of the cells in both producing and secreting cells. The fact that the plasma concentration of corticosterone is known to increase with increasing temperature (17) also supports this proposal.

The operation of the corticosteroidogenic cells of the adrenal gland is regulated by a negative feedback mechanism. The main control is achieved through the corticotropic releasing hormone (CRH), which is produced in the specific neurons of the hypothalamus (18). It is known that vasopressin produced by the Gomori (+) neurons is a neurohormone which has similar activity to that of CRH (19). Most probably, the neurosecretory substance having CRH activity, which is secreted by the neurosecretory cells that are activated by increasing temperature, causes the pituitary gland to secrete adrenocorticotropic hormone (ACTH). This, in turn, activates the corticosteroidogenic cells.

An increase in temperature causes the production and accumulation of secretory material in the catecholaminergic cells. On the other hand, adrenalin and noradrenalin levels in blood are known to rise at low temperatures (20). In various amphibian specimens, an immunoreactive substance similar to vasotocin and arginin-vasotocin is produced together with catecholamins in catecholaminergic cells (21). Arginin-vasotocin may act as a paracrine factor that is produced by catecholaminergic cells and regulates the secretion activity of the corticosteroidogenic cells. Thereby, corticosteroidogenic cells can be stimulated in two ways and they may also produce and secrete their hormones in the same way as with the neurosecretory substance in the bloodstream, with the local effect of the secretion of the catecholaminergic cells. As a result, these hormones hep the individual to withstand the stress of temperature changes, emotional factors, tension, and trauma. In the present study, we can say that the subject endured the stress caused by long term exposure to heat with the help of the catecholamines that were produced.

In this experiment, the third type of cells in the adrenal gland of *Rana ridibunda,* the Stilling cells, were located close to the corticosteroidogenic cells. Their close positioning led us to think that they might also be related functionally. The growth in size and the scarcity of granules in the Stilling cells with the increase in temperature led us to the conclusion that these cells also became active and produced their secretory substance in the same way as the corticosteroidogenic cells did.

With the increase in temperature, the structural and functional changes in the small neurosecretory cells in the brain and the corticosteroidogenic cells of the adrenal gland supported each other. In addition, parallel changes modified each other in the corticosteroidogenic cells and Stilling cells. This indicates that there may be functional relationships between the neurosecretory substance and the adrenal gland of *Rana ridibunda* and also between the cells that constitute the adrenal gland.

References

1. Norman, A. W., Liktwack, G., Hormones, New York, 1987 Academic Press.
2. Gorbman, A., Dickoff, W. W., Vigna, S.R., Clarck, N. B. and Ralph, C. L, Comparative Endocrinology. John Wiley and Sons, New York, 1983.
3. Kawamura, K. and Kikuyama, S., Atlas of Endocrine Orgnas, Akira Matsumoto, Susumu Ishii, New York, 1992, 109-122.
4. Cumming, R., Reaves, Jr., T. A. and Hayward, J.N., Ultrastructural Immunocytochemical Characterization of Isotocin, Vasotocin and Neurophysin Neurons in the Magnocellalur Preoptic Nucleus of the Goldfish: Cell Tissue Res. 223: 685-694, 1982.

5. van Vossel-Daeninck, J., Dierickx, K., Vandesande, F. and van Vossel, A., Electron Microscopic Immunocytochemical Demonstration of Separate Vasotocinergic, Mesotocinergic and Somatostatinergic Neurons in the Hypothalamic Magnocellular Preoptic Nucleus of the Frog: Cell Tissue Des. 218: 7-12, 1981.

6. Olivereau, M., Moons, L., Olivereau, J. and Vandesande, F. Coexistence of Corticotropin Releasing Factor-Like Immunoreactivity and Vasotocin in Perikarya of the Preoptic Nucleus in the Eel: Gen. and Comp. Endocr. 70: 41-48, 1988.

7. Chester Jones, I. and Phillips, J.G., Vertebrate Endocrinology, Peter K.T. Pang and Martin P. Schreibman, New York, 319-350, 1986.

8. Hanke, W. and Weber, K., Histophysiological Investigation on the Zonation, Activity, and Mode of Secretion of the Adrenal Gland of the Frog, Rana temporaria Linnaeus: Gen. and Comp. Endocr. 5: 444-455, 1965.

9. Cameron, M. L. and Steele, J. E., Simplified Aldehyde Fuchsin Staining of Neurosecretory Cells. Stain. Technol., 34: 265-266, 1959.

10. Humansol, G. L., Animal Tissue Techniques, H. W. Freeman and Company. San Fransisco, 1972.

11. Troyer, J. R., Neurosecretory Material in the Supraoptic-Hypophyseal Tract of the Bat Throughout the Hibernating and Summer Periods: Anat. Rec., 162(4): 407-415, 1968.

12. Imai, K., Studies in Neurosecretion IV. Occurrence of two Kinds of Neurosecretory Material in the Cells of the Preoptic Nucleus in the Toad (Bufo vulgaris formosus): Endocrinol. Japon., 2(1): 41-46, 1955.

13. Vulling, H.G.B., Influence of Light and Darkness on the Hypothalamo-Hypophyseal Systeam of Rana temporaria L.: Z. Zellforsch., 113(2): 174-187, 1971.

14. Borg, B., Anderson, E., Mayer, I., Zanbergen, M. A. and Peute, J., Effects of Castration on Pituitary Gonadotropic Cells of the Male Three-Spined Stickleback, Gastrosteus aculeatus L., under Long Photoperiod in Winter: Indications for a Positive Feed-back: Gen. and Comp. Endocr., 76: 12-18, 1989.

15. Uemura, H., Kobayashi, H., The Hypothalamo-Hypophyseal Neurosecretory System of the Neotenous Salamander, Necturus maculosus: Acta Anat. Nipponica, 37(3): 218-229, 1962.

16. Van Oordt, P.G.W.J. and Peute, J., Fish Physiology, Hoar, W.S., Randal, D.J., and Donaldson, E.M., Vol. IX A, New York, 1983, 137-186.

17. Jurani, M., Murgas, K., Mikulaj, L. and Babusikova, F., Effect of Stress and Environmental Temperature on Adrenal Function in Rana esculenta: Journal of Endocrinology, 57(3), 385-391, 1973.

18. Nurnberger, F., The Neuroendocrine System in Hibernating Mammals: Present Knowledge and Open Questions: Cell Tissue Res., 391-412, 1995.

19. Gillies, G., Van Wimersma Greidanus, T.B. and Lowry, P.J., Characterization of Rat Stalk Median Eminence Vasopressin and Its Involvement in Adrenocorticotropin Release: Endocrinology, 103, 528-534, 1978.

20. Saakov, B. A., Eremina, S. A. and Gul'yants, E. S., Secretory Activity of the Hypothalamus-Pituitary-Adrenal System in Hypotermia: Bull. Exp. Biol. Med. (USSR), 67(1), 22-25, 1969.

21. Larcher, A., Delarue, C., Idres, S., Lefebvre, H., Feuilloley, M., Vandesande, F., Pelletier, G. and Vaudry, H., Identification of Vasotocin-Like Immunoreactivity in Chromaffin Cells of the Frog Adrenal Gland: Effect of Vasotocin on Corticosteroid Secretion. Endocrinology. 125, 2691-2700, 1989.