Research Journal of Biological Sciences 6 (9): 432-435, 2011
ISSN: 1815-8846

Histomorphometry of Vomeronasal Organ in the Snake, *Eirenis collaris*

[1]Mohsen Abbasi, [2]Ahmad Gharzi and [2]Parisa Usefi
[1]Department of Basic Sciences, Faculty of Veterinary Medicine,
[2]Department of Biology, Faculty of Basic Sciences, Lorestan University,
Khorramabad, Lorestan, Iran

Abstract: Vomeronasal Organ (VNO) in snakes is engaged in chemical communications and hunting. This organ is located in the roof of the mouth, below the nasal cavity. In contrast to mammals, in snakes, the VNO opens only into oral cavity. Environmental stimuli are collected by the forked tongue and transported into to the VNO ducts where their sensory epithelium produced nervous impulses and send to neural centers in brain. In this survey, 10 snakes of *Eirenis collaris* were collected from their natural habitats and transferred to the laboratory where they were killed and their head were cut and detached from the rest of the body. The heads were then processed for histological examinations. Based on the anatomical and histological investigations, it was found that like other snakes in *Eirenis collaris*, the VNO is located on the roof oral cavity and beneath the nasal cavity. This organ possesses two ducts that open into oral cavity. Sensory and non-sensory epithelium is found in this organ but secretary component is absent in this species.

Key words: Vomeronasal, snake, *Eirenis collaris*, histology, stimuli, hunting, oral cavity

INTRODUCTION

The Vomeronasal or Jacobson's Organ (VNO) is a pair of blind-ended cavities or tubules present in many vertebrates including mammals (Gillingham and Clark, 1981; Parker *et al*., 2008). During embryological development, it forms from the nasal (olfactory) placode at the anterior edge of the neural plate (Doving and Trotier, 1998). In mammals, this organ is located anteriorly on the septum dividing the two nostrils (Grzimek, 2003). Each VNO consists of an olfactory or sensory epithelium innervated by a branch of the olfactory nerve and probably plays a role in the recognition of food and is used to test substances held in the mouth. The presence of a vomeronasal system is often an indication of the use of olfactory signals or pheromones for intraspecific communication.

These organs became highly specialized in the lineage leading to lizards and snakes (Alving and Kardong, 1996; Cinelli *et al*., 2002). In these reptiles, the organs are tubular and well developed but lost their connection with the nasal canal and open into the anterior roof of the oral cavity. Here, the odoriferous chemicals odor molecules hat accumulate on the tongue from the air are transferred to the sensory epithelium of the organ containing chemoreceptors (Linzey, 2001). The information from these chemoreceptors goes to an accessory olfactory bulb in the brain and information from there goes to brain regions involved in sexual and other instinctive behaviors (Parker *et al*., 2008; Schwenk, 1996). In the present study, the researchers examined the gross morphology and histology this organ in a species of colubrid snake, *Eirenis collaris* which occurs in West of Iran.

MATERIALS AND METHODS

To perform this study 5 individuals of the snake, *Eirenis collaris* were collected from their natural habitat in Dorood region, Lorestan province, Iran. This taxon is usually found in rocky places, semi-desert areas and mountainous region (Latifi, 1991). The collected specimens were then transferred to the laboratory where they were systematically identified according their biometric and morphological characteristics with respect to the present keys. After that, the snakes were killed and their head were cut and detached from the rest of the body.

The heads were then fixed in 4% formalin saline for at least a week and following that they decalcified by Goding and Stuart solution. The samples were then processed for histological examinations. About 7 μm serial sections were then cut by a rotary microtome and stained with either Haematoxylin-Eosin, Alcian blue or PAS. Then stained sections were scrutinized in details by the aid of a light microscope.

Corresponding Author: Mohsen Abbasi, Department of Basic Sciences, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Lorestan, Iran

RESULTS AND DISCUSSION

In *Eirenis collaris* studied here, each vomeronasal organ is observed as a dome-shaped structure in cross sections. This organ is situated at baso-lateral side of nasal cavity and surrounded by a cartilago-osseous capsule. In cross sections this capsule completely encases a lumen which is C-shaped as result of a mushroom body bulged into the lumen from the ventral or ventro-lateral side of the organ (Fig. 1).

The VNO begins at the first one third of nasal cavity but its shape varies at the length of the organ become oval, pear-shaped or even triangular in some parts. The osseous capsule enclosing the organ is not complete at the all length of the organ and in some areas mostly dorsal and ventral sides this capsule is not observed and its place is occupied by connective tissue. In fact, the VNO capsule is made of two arches, one medial and the other is lateral one. The lateral arch is the continuation of vomer bone but the medial arch is dorsally extended part of palatine. The space between these arches at basal side is filled by the cartilage. In some areas along the length of the organ, this space is considerably wide (Fig. 2).

The lumen of VNO is lined by three types of epithelium in different regions. The dorsal wall of the lumen is covered by a non-ciliated multilayered sensory epithelium containing apical columnar cells. At the back of this epithelium a lamina properia is observed. The mushroom body is encased by a ciliated bilayered non-sensory epithelium consisting of cuboidal cells. Under this covering, a lamina properia with blood sinuses is present. A hyaline cartilage with many mitotic cells forms the inner pillar or core of the mushroom body (Fig. 3). At the interlude between the mushroom and dorsal wall of the lumen an intermediary non-sensory epithelium lines the lumen. This epithelium is multilayered and contains cuboidal cells. In one case that the researchers measured the length of the VNO it was 644 μm. Here in the examinations, the researchers did not find any connection between this the VNO and nasal cavity whereas the organ opens to the anterior roof of the oral cavity through a narrow canal which connects the lumen with mouth. Lacrimal canal is also opens to the VNO lumen and thus, the secretions of lacrimal gland reach to the oral cavity via the VNO lumen. The VNO opening canal and lacrimal canal are both lined with a stratified 21-squamous epithelium.

At the mid part of the VNO is lacking the mushroom body and here the lumen is cup-shaped and enclosed by a sensory stratified columnar covering. At the terminal end, the lumen become increasingly narrower and finally is lined by a neural multilayered epithelium. Here, the VNO is circular in cross sections. The presence of the VNO in

Fig. 1: Cross section of VNO: Oral cavity (Oc); Nasal cavity (Nc); Mushroom body (Mb); Sensory epithelium (Se) and Lumen (L). H and E, ×4

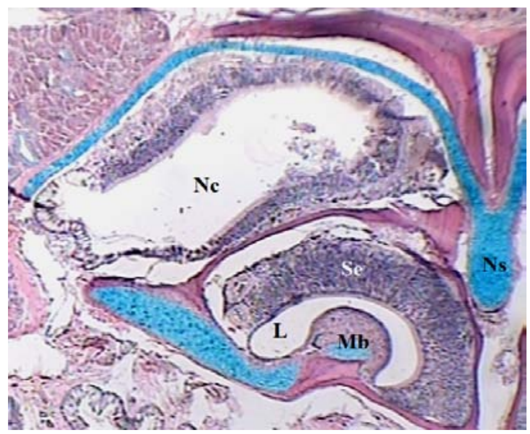

Fig. 2: Cross section of VNO: Mushroom body (Mb) supported by cartilage; sensory epithelium lining the Lumen (L) of VNO; Nasal septum (Ns) and Nasal cavity (Nc); Alcian blue, ×4

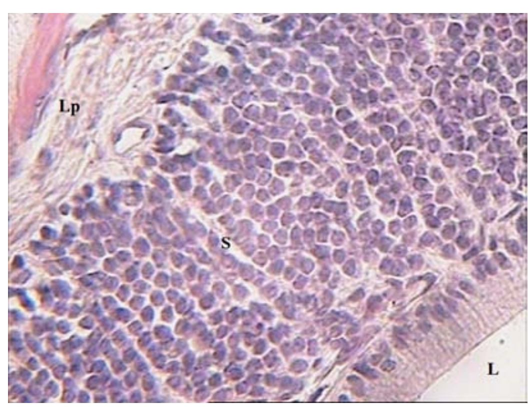

Fig. 3: Sensory epithelium (S) that rest on Lamina properia of VNO lining the Lumen of organ (L); H and E, ×40

many animals has been widely studied and the importance of the vomeronasal system to the role of reproduction and social behavior (through influence on anterior hypothalamus) has been shown in many studies (Abbasi and Khosravinia, 2006; Johns, 1986). Its existence and functionality in humans is widely controversial though most studies agree the organ regresses during fetal development (Meredith, 2001; Wysocki and Preti, 2004). Snakes have long been model organisms for understanding vomeronasal function and studies conducted at the behavioral, electrophysiological and molecular levels demonstrate that the vomeronasal system is crucial in mediating responses to foraging cues (Halpern and Martinez-Marcos, 2003).

The characteristic tongue-flicking behavior of snakes brings molecules into the vomeronasal organ (Schwenk, 1996) and the prevalence of tongue-flicking during hunting suggests that responses to prey odorants are mediated by the vomeronasal system (Burghardt and Pruitt, 1975). Earthworms, a common prey item of garter snake produce a 20-k Daproteinthatis sensed by the garter snake vomeronasal organ (Wang *et al*., 1998). When the nerve is sectioned, the snakes stop following prey odor trails and eventually stop eating prey (Halpern *et al*., 1997). Similarly without a functional vomeronasal organ, rattlesnakes (*Crotalus viridis*) show a decreased rate of striking at prey and fail to eat prey after striking (Alving and Kardong, 1996).

Electrophysiological studies have further confirmed the role of the vomeronasal system in prey discrimination in snakes (Halpern and Martinez-Marcos, 2003). Anatomically, researchers here demonstrated that in snake, *Eirenis collaris*, the VNO is a tubular organ located at the roof of oral cavity and opens to anterior part of oral cavity by a narrow opening. The length of this organ was measured 644 mm and the widest diameter of the organ was found in the middle segment. From this segment, the diameter of the organ was reduced anteriorly as well as posteriorly. These measurements showed that like other species in this taxon the length of the organ is dependent on the snout length too.

In other words with the increase of nasal cavity length, this organ becomes also longer. In contrast to other groups (for example, amphibians and mammals) in reptiles no connection is observed between the VNO and nasal cavity (Altner *et al*., 1970). In this context, the results provided here is in agreement with previous finding as the researchers were not able to find any spatial relation between these two parts. Here, the researchers also demonstrated that the lacrimal canal opens into the lumen of VNO a finding which has not been reported in studied mammals (Doving and Trotier, 1998). The presence of this connecting canal has been documented in other reptiles but their merging site varies among different reptiles. In lizards, this joining takes place in vicinity of the mushroom body while in snakes occurs at opposite side of the structure (Rehork *et al*., 2000). In accordance with other snakes, researchers found that in the *Eirenis collaris* the lacrimal canal joins to The VNO lumen at the side faced to the mushroom body. The epithelium of mucous membrane of the VNO in many laboratory animals, amphibian, reptiles and domestic mammals has been studied. In mammals, the epithelium which lines the VNO near the nasal passageway is a non-keratinizing stratified squamous epithelium. Towards the medial segment, this epithelium turns into stratified columnar then into stratified cuboidal and finally to olfactory epithelium at lateral sides. The lamina properia and submucosa of this organ in all studied mammals are composed of loose connective tissue which is rich in blood vessels and contains seromucous glands (Abbasi and Khosravinia, 2006; Ardalani *et al*., 2001). In reptile, three kinds of epitheliums found in different points of the VNO a sensory epithelium on the dorsal side, a non-sensory epithelium on the mushroom body and another non-sensory epithelium at the intermediary region (Cinelli *et al*., 2002; Rehork *et al*., 2000) sensory epithelium consists of receptor neurons, supporting cells and basal cells which are organized in a covering form. In comparison to mammals, this epithelium is thicker in reptiles and supported by a distinct connective tissue containing blood sinuses (Altner *et al*., 1970).

CONCLUSION

In the species studied here, researchers observed these characters too. The epithelium which covers surface of the mushroom body has been examined in some reptiles and classified as a ciliated cuboidal epithelium. In agreement with the previous study, researchers observed this specific epithelium over this body. Moreover, researchers found that this epithelium thinner than the sensory ones. An intermediary epithelium which is seen at the interval between in the mushroom body and lateral walls has been documented in other species by investigators (Rehork *et al*., 2000). In summary, researchers showed here that in *Eirenis collaris*, the VNO illustrate the same anatomy and structure as found in other snakes and the researchers did not observe any difference in this respect between this and other species.

REFERENCES

Abbasi, M. and H. Khosravinia, 2006. Anatomy and histology of vomeronasal organ in an Iranian native breed of sheep. Indian Vet. J., 83: 200-204.

Altner, H., W. Muller and I. Brachner, 1970. The ultrastructure of the vomeronasal organ in reptilia. Z. Zellforsch Mikrosk Anat J., 105: 107-122.

Alving, W.R and K.V. Kardong, 1996. The role of the vomeronasal organ in rattlesnake (*Crotalus viridis oreganus*) predatory behavior. Brain Behav. Evol., 48: 165-172.

Ardalani, G.H., R. Sadrkhanloo and M. Abbasi, 2001. Anatomy and histology of the vomeronasal organ in buffalo. J. Vet. Res., 55: 5-10.

Burghardt, G.M and C.H. Pruitt, 1975. Role of tongue and senses in feeding of naive and experienced garter snakes. Physiol. Behav., 14: 185-194.

Cinelli, A.R., D. Wang, P. Chen, W. Liu and M. Halpern, 2002. Calcium transients in the garter snake vomeronasal organ. J. Neurophysiol., 87: 1449-1472.

Doving, K. and D. Trotier, 1998. Structure and function of the vomeronasal organ. J. Exp. Biol., 201: 2913-2925.

Gillingham, J.C. and D.L. Clark, 1981. Snake tongue-flicking: Transfer mechanics to Jacobson's organ. Can. J. Zool., 59: 1651-1657.

Grzimek, B., 2003. Grzimek's Animal Life Encyclopedia. Volume: 7 Reptiles. Schlager Group Inc., USA.

Halpern, M. and A. Martinez-Marcos, 2003. Structure and function of the vomeronasal system: An update. Prog. Neurobiol., 70: 245-318.

Halpern, M., J. Halpem, E. Erichsen and S. Broghijd, 1997. The role of nasal chemical senses in garter snake response to airborne odor cues from prey. J. Comp. Psychol., 111: 251-260.

Johns, M.A., 1986. The role of the vomeronasal organ in behavioral control of reproduction. Ann. New York Acad. Sci., 474: 148-157.

Latifi, M., 1991. Snakes of Iran. 2nd Edn., Department of Environment Co., Iran, pp: 174-175.

Linzey, D.W., 2001. Vertebrate Biology. McGraw-Hills, USA., ISBN-13: 9780697363879, Pages: 530.

Meredith, M., 2001. Human vomeronasal organ function: A critical review of best and worst cases. Chem. Senses, 26: 433-445.

Parker, M.R., B.A. Young and K.V. Kardong, 2008. The forked tongue and edge detection in snakes (*Crotalus oreganus*): An experimental test. J. Comp. Physiol., 122: 35-40.

Rehork, S., T.B. Firth and M. Hutchinson, 2000. The structure of the nasal chemosensory system in squamate reptiles. J. Biosci., 1: 181-190.

Schwenk, K., 1996. Why snakes flick their tongues? Am. Zool., 36: 84-84.

Wang, D., P. Chen., X.C. Jiang and M. Halpern, 1998. Isolation from earthworms of a proteinaceous chemoattractant to garter snake. Arch. Biochem. Biophys., 267: 459-466.

Wysocki, C.J. and G. Preti, 2004. Facts, fallacies, fears and frustrations with human pheromones. Anatomical Rec. A Disc. Mol. Cell. Evol. Biol., 281: 1201-1211.