

Short Paper

The distribution of serotonin-immunoreactive cells in larynx, trachea and bronchi of goat (*Capra aegagrus*) and bovine (*Bos taurus*)

Demirbağ, E.* and Çınar, K.

Department of Biology, Faculty of Arts and Sciences, Suleyman Demirel University, Isparta, Turkey

*Correspondence: E. Demirbağ, Department of Biology, Faculty of Arts and Sciences, Suleyman Demirel University, Isparta, Turkey. E-mail: emeldemirbag@sdu.edu.tr

(Received 3 Sept 2011; revised version 30 Jun 2012; accepted 14 Jul 2012)

Summary

The respiratory tract contains numerous peptides secreted from special pulmonary epithelial cells which are called endocrine cells. The function of neuroendocrine cells is modulated by amines. Serotonin has strong vasomotor and bronchomotor effects in the airway mucosa. The objective of this study was to determine presence and distribution of serotonin-positive endocrine cells in respiratory tract of goat and bovine via PAP (peroxidase anti-peroxidase) technique. As a result, while serotonin-immunoreactive cells were found in glands of larynx and bronchial epithelium in bovine, no positive cells were detected in goat. The present study suggests that the distribution of serotonin-immunoreactive cells may show differences between both respiratory tract regions and mammalian species.

Key words: Immunohistochemistry, Respiratory tract, Ruminant, Serotonin

Introduction

The respiratory tract contains numerous peptides produced by and released from specialised epithelial cells. These “endocrine/paracrine” cells in pulmonary epithelium are members of APUD (amine precursor uptake and decarboxylation) system cells that are widely found in the organisms (Seldeslagh and Lauweryns, 1993) and classified as the “pulmonary neuroendocrine cells” (PNECs) (Pan *et al.*, 2004). The PNECs are located within the respiratory epithelium singly or in clusters known as “neuroepithelial bodies” (NEBs) (Shimosegawa and Said, 1991; Scheuermann, 1997) and are shown to be widely distributed in airway mucosa of mammalian (Van Lommel *et al.*, 1999; Pan *et al.*, 2004).

The function of PNECs/NEBs is modulated via amines (Fu *et al.*, 2001). Serotonin is one of these amines synthesized and released by PNECs/NEBs in the airway

mucosa (Dodson *et al.*, 2004) and has strong vaso- and broncho-motor effects (Seldeslagh and Lauweryns, 1993) and also regulates the contraction of smooth muscles by stimulating cholinergic neurons through the release of acetylcholine (Ku *et al.*, 2004; Lee *et al.*, 2004). Serotonin induces hypertrophy and proliferation and stimulates migration of pulmonary arterial smooth muscle cells (Day *et al.*, 2006).

The presence of endocrine cells can be determined by fluorometry (Lauweryns *et al.*, 1977), silver impregnation (Inokuchi *et al.*, 1984), immunohistochemistry (Çınar *et al.*, 2006; Çınar and Demirbağ, 2007; Kuru *et al.*, 2010) and immunoassay (Ebrahimi, 2006; Sakhaee *et al.*, 2009).

The presence, distribution and function of serotonin in PNECs in some mammals are known, but in goat and bovine these cells are not reported. Thus, the purpose of the present study is to determine the distribution and density of serotonin in PNECs of epithelium and glands of the larynx, trachea

and bronchi of goat and bovine by immunohistochemistry.

Materials and Methods

In this study, larynx, trachea and primary bronchi from 10 adult male goat (*Capra aegagrus*) and 10 adult male bovine (*Bos taurus*) were used. All tissue samples were taken from Isparta Integrated Meat Foundation (Isparta/Turkey). The tissue specimens were fixed in 10% formaldehyde about 24 h at room temperature. Then they were dehydrated through graded ethanol and embedded in paraffin. Five μm thick sections were obtained and immunolocalization of serotonin examined using the peroxidase anti-peroxidase (PAP) technique (Sternberger, 1986). In this technique tissue sections were deparaffinized with xylene and alcohols, washed three times with 0.1 M phosphate-buffered saline (PBS) and endogen peroxidase was blocked by 3.0% hydrogen peroxide for 20 min. After that, slides were washed with 0.01 M PBS (pH = 7.4, containing 0.2% Triton X-100 and 0.1% bovine serum albumin). In order to block unspecific binding, sections were preincubated with 10% normal goat serum (Sigma G9023) for 30 min and incubated with rabbit polyclonal antibody against serotonin (Sigma S5545) at a dilution of 1/200 for 20 h at 4°C. Sections were then incubated in goat anti-rabbit IgG (1/50) (Sigma A0545), followed by rabbit PAP complex (1/100) (Sigma P1291) about 20-30 min at room temperature. After each incubation, sections were washed in PBS (pH = 7.4) for 30 min, and finally, immersed in DAB (3,3'-diaminobenzidine tetrahydrochloride) (Sigma D4418). Sections were dehydrated and then mounted with entellan. Serotonin-immunoreactive cells were counted in the epithelium and glands of the larynx, trachea and bronchi of goat and bovine. The relative frequencies of endocrine cells were subjectively graded into three groups: negative (-), a few (+) and numerous (+++).

Results

Results showed that there was no

serotonin-positive endocrine cell in the epithelium and glands of the larynx, trachea and bronchi of goat (Table 1), but in bovine, serotonin immunoreactivity was detected in glands of the larynx and epithelium of bronchi (Fig. 1). The immunoreaction was not recorded in the glands of trachea and bronchi, and epithelium of larynx and trachea. It was also found near the capillaries and in connective tissue layer in trachea (Fig. 2) and bronchi (Fig. 3). The serotonin-positive cells were few in number in larynx and numerous in bronchi. The serotonin-positive cells were also usually located solitarily in the larynx, although they sometimes formed clusters to become the so-called neuroepithelial bodies (NEBs) in epithelium of bronchi (Fig. 4).



Fig. 1: Serotonin-positive cell (arrow) in epithelium of bovine's bronchi (scale bar: 50 μm)

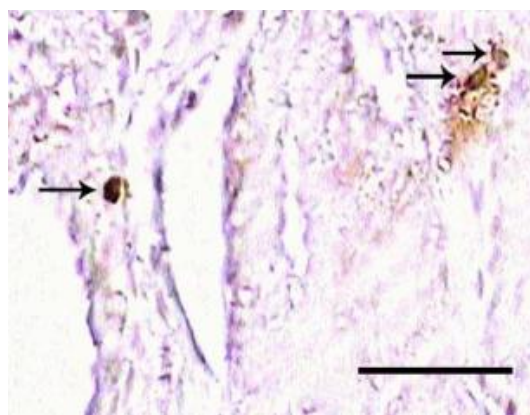


Fig. 2: Serotonin-positive cells (arrows) in connective tissue layer of bovine's trachea (scale bar: 50 μm)

Table 1: The distribution and density of serotonin-immunoreactive cells in larynx, trachea and bronchi of goat and bovine

	Larynx		Trachea		Bronchi	
	<i>L. epithelialis</i>	Glands	<i>L. epithelialis</i>	Glands	<i>L. epithelialis</i>	Glands
Goat	-	-	-	-	-	-
Bovine	-	+	-	-	+++	-

-: negative, +: a few, +++: numerous

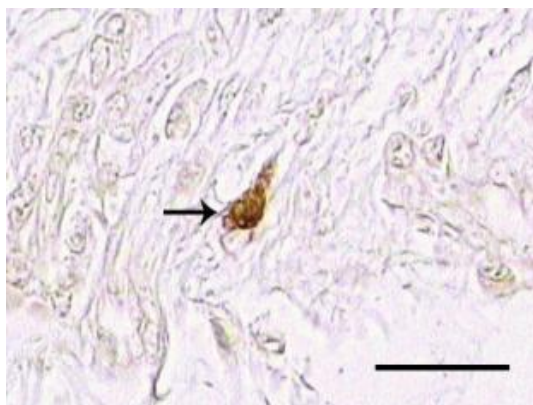


Fig. 3: Serotonin-positive cell (arrow) in connective tissue layer of bovine's bronchi (scale bar: 50 µm)

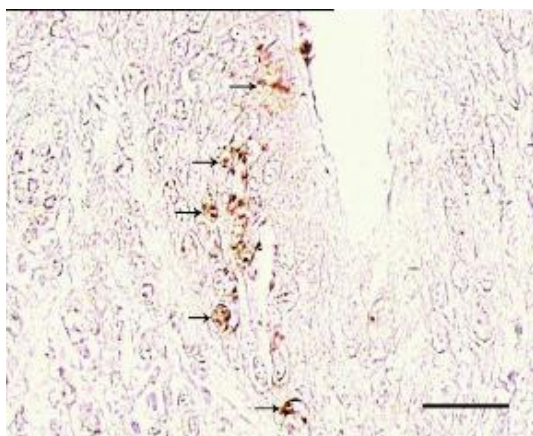


Fig. 4: Serotonin-positive cells formed clusters (NEBs) (arrows) in epithelium of bovine's bronchi (scale bar: 50 µm)

Discussion

Presence of serotonin immunoreactive cells in the larynx, trachea and bronchi of mouse, rat, hamster, guinea pig, pig, sheep and squirrel monkey had been reported (Luts *et al.*, 1991). The serotonin immunoreactive cells were found in the epithelium of trachea and bronchi in the mouse (Çınar and Demirbağ, 2007). The findings of the authors (Luts *et al.*, 1991; Çınar and

Demirbağ, 2007) are in contrast to our findings, where serotonin-positive cells were not detected in trachea of goat and bovine and bronchi of goat.

It was reported that while the solitary PNECs are found throughout the tracheobronchial epithelium, the NEBs are found only in the intrapulmonary airways (Van Lommel, 2001). Balaguer *et al.* (1992) also reported that serotonin-containing cells were found within lower respiratory tract mucosa of sheep as solitary and NEBs. These findings (Balaguer *et al.*, 1992; Van Lommel, 2001) are similar to our findings in the bovine. Keith and Ekman (1988) reported that a few of both solitary and clustered serotonin-containing neuroendocrine cells were localized in the trachea and bronchi of hamster. However, in the present study, although the number of serotonin immunoreactive cells is low in the larynx of bovine, this is numerous in the bronchi. The present study showed that serotonin immunoreactive cells are distributed around blood vessels and in the connective tissue layer of trachea and bronchi. This distribution is similar to evidence obtained from a study on the mouse (Çınar and Demirbağ, 2007).

There are only a few studies about distribution of serotonin immunoreactivity in ruminant respiratory tract in literature. Therefore, this study will be required to determine distribution of this hormone in the larynx, trachea and bronchi of goat and bovine.

As a result, while serotonin-immunoreactive cells were found in glands of the larynx and bronchial epithelium in bovine, no positive cells were detected in goat. In conclusion, the present study has revealed that presence and distribution of serotonin-immunoreactive cells may show differences between both respiratory tract regions and mammalian species.

References

- Balaguer, L; Romano, J and Ruiz-Pesini, P (1992). Localization of serotonin, cholecystokinin and somatostatin immunoreactivity in the lower respiratory tract of embryonic, foetal and postnatal sheep. *Histol. Histopathol.*, 7: 703-708.
- Çınar, K and Demirbağ, E (2007). The immunohistochemical localizations of some peptides in mucosa of trachea, bronchus and bronchulus in adult Albino mouse (*Mus musculus*). *Med. J. Sdu.*, 14: 21-25.
- Çınar, K; Şenol, N and Özen, MR (2006). Immunohistochemical study on the distribution of endocrine cells in the gastrointestinal tract of the flower fish (*Pseudophoxinus antalyae*). *World J. Gastroenterol.*, 12: 6874-6878.
- Day, RM; Agyeman, AS; Segel, MJ; Chevere, RD; Angelosanto, JM; Suzuki, YJ and Fanburg, BL (2006). Serotonin induces pulmonary artery smooth muscle cell migration. *Biochem. Pharmacol.*, 71: 386-397.
- Dodson, AM; Anderson, GM and Rhodon, KJ (2004). Serotonin uptake and metabolism by cultured guinea pig airway smooth muscle cells. *Pulm. Pharmacol. Ther.*, 17: 19-25.
- Ebrahimi, M (2006). Vitollegenin assay by enzyme-linked immunosorbent assay (ELISA) as a biomarker of endocrine disruptor chemicals (EDCs) pollution. *Iranian J. Vet. Res.*, 7: 6-12.
- Fu, XW; Wang, D; Pan, J; Farragher, SM; Wong, V and Cutz, E (2001). Neuroepithelial bodies in mammalian lung express functional serotonin type 3 receptor. *Am. J. Physiol. Lung Cell Mol. Physiol.*, 281: 931-940.
- Inokuchi, H; Azuma, T; Kawai, K; Yakeuchi, Y and Sano, Y (1984). Serotonin immunohistochemistry reveals immature EC cells. *Histochemistry*. 80: 117-118.
- Keith, IM and Ekman, R (1988). Calcitonin gene-related peptide in hamster lung and its coexistence with serotonin: a chemical and immunocytochemical study. *Regul. Pept.*, 22: 315-323.
- Ku, SK; Lee, JH and Lee, HS (2004). Immunohistochemical study on the endocrine cells in gut of the stomachless Teleost, *Zacco platypus* (Cyprinidae). *Anat. Histol. Embryol.*, 33: 212-219.
- Kuru, N; Çınar, K; Şenol, N; Demirbağ, E and Diler, D (2010). Endocrine cells in the gastrointestinal tract of *Garra rufa*. *Kafkas Univ. Vet. Fak.*, 16: 235-241.
- Lauweryns, JM; Cokelaere, M; Deleersnyder, M and Liebens, M (1977). Intrapulmonary neuroepithelial bodies in newborn rabbits. *Cell Tissue Res.*, 182: 425-440.
- Lee, JH; Ku, SK; Park, KD and Lee, HS (2004). Immunohistochemical study of the gastrointestinal endocrine cells in the Korean aucha perch. *J. Fish Biol.*, 65: 170-181.
- Luts, A; Uddman, R; Absood, A; Hakanson, R and Sundler, F (1991). Chemical coding of endocrine cells of the airways: presence of helodermin-like peptides. *Cell Tissue Res.*, 265: 425-433.
- Pan, J; Yeager, H and Cutz, E (2004). Innervation of pulmonary neuroendocrine cells and neuroepithelial bodies in developing rabbit lung. *J. Histochem. Cytochem.*, 52: 379-389.
- Sakhaee, E; Khalili, M and Kazemi Nia, S (2009). Serological study of bovine viral respiratory diseases in dairy herds in Kerman province, Iran. *Iranian J. Vet. Res.*, 10: 49-53.
- Scheuermann, DW (1997). Comparative histology of pulmonary neuroendocrine cell system in mammalian lung. *Microsc. Res. Tech.*, 37: 31-42.
- Seldeslagh, KA and Lauweryns, JM (1993). Endothelin in normal lung tissue of newborn mammals: immunocytochemical distribution and co-localization with serotonin and calcitonin gene-related peptide. *J. Histochem. Cytochem.*, 41: 1495-1502.
- Shimosegawa, T and Said, SI (1991). Co-occurrence of immunoreactive calcitonin and calcitonin gene-related peptide in neuroendocrine cells of rat lungs. *Cell Tissue Res.*, 264: 555-561.
- Sternberger, LA (1986). *Immunocytochemistry*. 3rd Edn., Wiley, New York.
- Van Lommel, A (2001). Pulmonary neuroendocrine cells (PNEC) and neuroepithelial bodies (NEB): chemoreceptors and regulators of lung development. *Pediatr. Respir. Rev.*, 2: 171-176.
- Van Lommel, A; Bollet, T; Fannes, W and Lauweryns, JM (1999). The pulmonary neuroendocrine system: the past decade. *Arch. Histol. Cytol.*, 62: 1-16.