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Serotonin immunoreactivity in the skin of the porcupine (*Hystrix cristata*)

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ABSTRACT

Immunohistochemistry was applied in the investigation of the possible existence of serotonin in porcupine (*Hystrix cristata*) skin. For this purpose, normal skin from four healthy porcupines were used. Serotonin immunoreactive cells were found at the stratum basale and stratum granulosum of the epidermis. In addition serotonin immunoreactivity was detected in the internal root sheat, medulla and epithelium of hair follicule cells. No immunoreactivity of serotonin occurred in the dermis. These results are the first evidence of serotonin immunoreactivity in epidermis cells, hair follicule cells of porcupines. Thus, serotonin should be considered a cellular modulator in skin.

Key words: immunohistochemistry, serotonin, skin, porcupine

Introduction

Hystrix cristata belongs to part of the family of Hystricidae. Almost the entire body is covered with bristles, which are either dark brown or black and rather coarse. Porcupine quills, or spines, take on various forms, depending on the species, but all are modified hairs coated with thick plates of keratin, and are embedded in the skin musculature (KURU, 1987; DEMIRSOY, 1992).

Serotonin, also known as 5-hydroxytryptamine, is a hormone. It resides in the pineal glands, blood platelets, digestive tract and brain and is responsible for transmitting signals between nerve cells. Serotonin acts as a chemical messenger, causing blood vessels to narrow through chemical interaction with nerve cells. Changes in serotonin levels in the body are responsible for mood alterations and specific skin problems (KEMA et al., 2000).

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Serotonin-like immunoreactivity has been demonstrated in amphibian skin (KRAMER, 1970; PEARSE, 1976), conger-eel skin (ZACCONE, 1986), fish epidermal sacciform glandular cells (ZACCONE et al., 1986), pig snout skin (GARCIA-CABALLERO et al.,1989), frog skin (SENGEZER-INCELI et al., 2004), human skin (JOHANSSON et al.,1998; LUNDEBERG et al., 1999), rat hair follicle (ENGLISH et al., 1991; TACHIBANA et al., 2005), rat glabrous skin (CARLTON and COGGESHALL, 1997) and the vertebrate nervous system (DICKE et al., 1997). However, there is no information related to immunohistochemical localization of serotonin in porcupine skin(*Hystrix cristata*).

The objective of this investigation was, therefore, to improve knowledge of the localization and to create a better understanding of the functional role of serotonin in porcupine skin.

Materials and methods

Four adult porcupines (*Hystrix cristata*) were used in this study. They were captured by villagers in Eastern Anatolia (Turkey). The experimental study was carried out in accordance with ethical considerations. Small pieces of tissue were dissected from skin after death and placed in 10% formalin in phosphate-buffered saline (PBS), pH 7.4, for 18 hours. Tissue samples were routinely processed through a graded series of alcohols, cleared in xylol and embedded in paraffin. 5 μm thick sections were obtained and processed for immunohistochemical staining.

Immunohistochemical staining was carried out using the streptavidin-biotin complex technique (POLAK and VAN NOORDEN, 1997). Blocking of endogenous peroxidase activity was carried out with 0.08% hydrogen peroxide (H_2O_2) in methanol for 5 minutes. In order to block non-specific binding, an incubation with Large Volume Ultra V Blok (Lab Vision co) was performed for 30 min.

Sections were incubated for 16-20 hours at 4 °C in rabbit anti-serotonin (Sigma). The antibody was diluted to 1:500 with PBS containing 0.25% sodium azide and 2.5% bovine serum albumin respectively. Sections were then incubated in biotinlated secondary antiserum (Lab Vision) for 1 hour, followed by rabbit streptavidin-biotin-peroxidase complex (Lab Vision) for 1 hour, at room temperature. Sections were washed in PBS for 30 minutes after each incubation and finally immersed in AEC (Dako) chromogen substrate for 10 minutes. After washing in distilled water, sections were counterstained with Mayer Hematoxylin. Sections were dehydrated and coverslips mounted with squamos mounting medium. Sections were examined with a light microscope and photographs taken (Olympus 13X51, Japan).

Immunoreactivity in the place of the primary antibody was controlled by phosphate buffer saline (PBS), and porcupine intestine was used as a positive tissue control (TIMURKAAN et al., 2005).

Results

This investigation detected immunoreactive endocrine cells to the antisera against serotonin in the porcupine skin(*Hystrix cristata*).

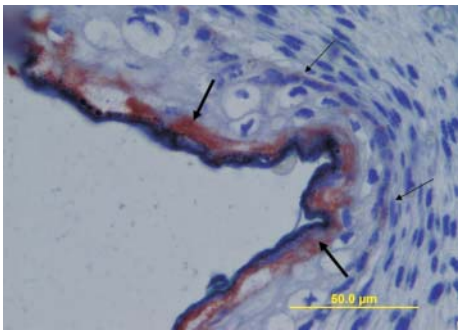

Fig. 1. Serotonin immunoreactive cells were situated exclusively in the stratum basale (thin arrow) and stratum granulosum (thick arrow) of the epidermis. The immunoreactivity of stratum granulosum was stronger than that of the stratum basale, ×100.

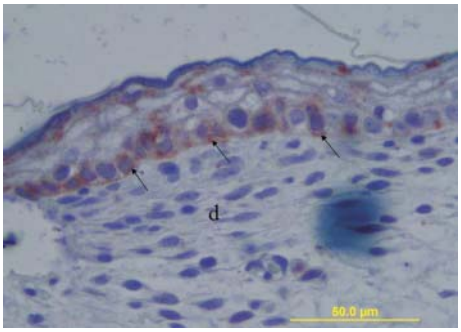

Fig. 2. Serotonin staining cytoplasma of the melanocytes were observed in the stratum basale (thin arrow). No positive reactions were detected in the dermis (d), ×100.

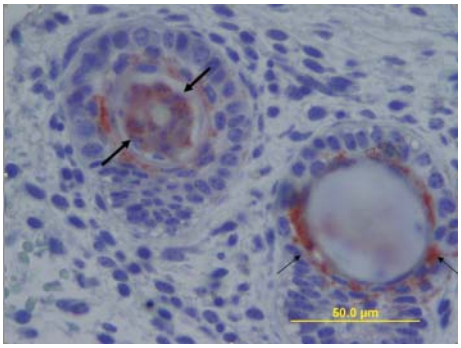

Fig. 3. Serotonin immunoreactivity was found in the medulla of the hair (thick arrow) and in the epithelium of hair follicle (thin arrow), ×100.

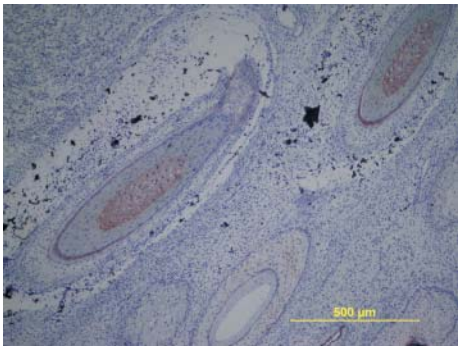

Fig. 4. General view of the dermis. Serotonin immunoreactivity was detected in the medulla of some hair follicles and the epithelium of hair follicles, ×20.

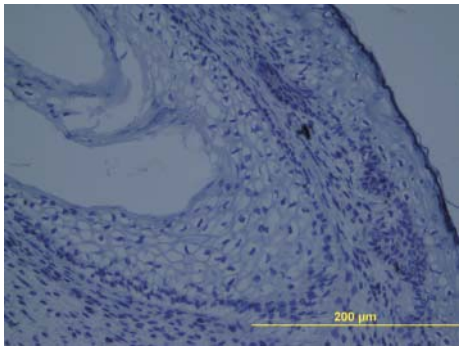

Fig. 5. The negative control sections of the dermis, ×40

Serotonin immunoreactive cells were situated exclusively in the stratum basale and stratum granulosum of the epidermis (Fig. 1, Fig. 2). However, in some areas of the stratum granulosum, immunoreactivity was stronger than in the stratum basale (Fig. 1). The signal was associated with both the cytoplasm and the plasma membrane. The immunoreactive cells were round, oval or spindle in shape. There was intense keratinization in the epidermis. The presence of serotonin-like immunoreactivity in this region indicated that this receptor may be of importance for normal differentiation of keratinocytes. Serotonin staining cytoplasma of the melanocytes were observed in the stratum basale (Fig. 2). It seems to us that serotonin may be a specific and sensitive diagnostic marker for melanocytes. Additionally, serotonin immunoreactive cells were found in the groups located in the internal root sheaths of the hair follicles (Fig. 3). However, immunoreactivity was also determined in the medulla of the hair and the epithelium of some hair follicles (Fig. 4).

No positive reactions were detected in the dermis and the negative control sections (Fig. 2, Fig. 5).

Discussion

It has been previously shown, by immunohistochemistry, that serotonin is present in the skin of amphibians (KRAMER, 1970; PEARSE, 1976), conger-eel (ZACCONE, 1986), pig snouts (GARCIA-CABALLERO et al., 1989), frogs (SENGEZER-INCELI et al., 2004), fish epidermal sacciform glandular cells (ZACCONE et al., 1986) human skin (JOHANSSON et al., 1998; LUNDEBERG et al., 1999), rat hair follicles (ENGLISH et al., 1991; TACHIBANA et al., 2005), earthworm epidermis (LICATA et al., 1998) and the vertebrate nervous system (DICKE et al., 1997). However, no study has been undertaken so far on porcupine skin. Therefore, this study documented the localization of serotonin in porcupine skin.

Serotonin is a monoamine acting as a neuromediator in the central and peripheral nervous systems. ROSS et al. (1995) and JOHANSSON et al. (1998) have stated that serotonin has various functions, such as muscle contraction and the regulation of ion selection in epithelial cells. Serotonin-containing epithelial cells have been found in the gastrointestinal tract's nerve fibres, mucosal endocrine cells of some bony fishes (KILIAAN et al., 1989) and amphibians (MAAKE et al., 1999). We previously also detected serotonin immunolocalization in the gastrointestinal tract of porcupines (*Hystrix cristata*) (TIMURKAAN et al., 2005).

LUNDEBERG et al. (2002) reported that immunoreactivity for 5-HT1AR was predominantly seen in basal epidermal cells in healthy human skin. According to LUNDEBERG et al. (2002), the localization of 5 HT1AR immunoreactivity in the upper epidermis is interesting and this receptor may be of importance for differentiation of keratinocytes. In our study, immunoreactivity for serotonin was observed in both the stratum granulosum and stratum basale of the epidermis. The report that this receptor may be of importance for differentiation of keratinocytes (LUNDEBERG et al., 2002) is in accordance with our study.

HUANG et al. (2004) have suggested that no positive expression of serotonin occurs in normal human skin cells. However, 5-HT immunoreactivity has been found in the epidermis of the earthworm (*Lumbricus terrestris*) (LICATA et al., 1998). In the present study, immunoreactivity to serotonin was observed in normal porcupine skin.

Serotonin-like immunoreactivity has been demonstrated in normal porcupine cutaneous melanocytes. A similar situation has also been observed in human skin by LUNDEBERG et al. (1999) and JOHANSSON et al. (1998).

Cutaneous melanocytes originate from the neural crest and they are associated with nerve fibres (SÉGUÉLA et al., 1989). JOHANSSON et al. (1998) suggested that the serotonin is generated in the cutaneous melanocytes and that serotonin should be considered a cellular modulator rather than a transmitter in skin.

In the current study, the presence of serotonin has been detected in the porcupine (*Hystrix cristata*) skin by using the immunohistochemical method. According to our immunohistochemical results, the widespread detection of serotonin immunoreactivity in skin epidermis indicates that it has a possible neuromodulator effect.

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SAŽETAK

Moguća prisutnost serotonina u koži dikobraza (Hystrix cristata) istražena je imunohistokemijom. Za istraživanje je bila uzeta koža četiriju zdravih dikobraza. Imunoreaktivnost serotonina bila je ustanovljena u stanicama stratum basale i stratum granulosum epiderme. Također je bila dokazana u unutarnjem sloju korijena, srži i epitelnim stanicama dlačnih folikula. Imunoreaktivnost serotonina nije bila dokazana u koži. To je prvi dokaz imunoreaktivnosti serotonina u stanicama epiderme i dlačnih folikula dikobraza. Serotonin se u koži može smatrati staničnim modulatorom.

Ključne riječi: imunohistokemija, serotonin, koža, dikobraz