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Neuroendocrine cells in the vestibular glands of the genital tract of cows and pigs

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Summary

The presence of neuroendocrine (NE) cells producing biogenic amines and hormonal peptides has been investigated in the vestibular glands of the genital tracts of cows and pigs using immunohistochemistry. NE cells containing chromogranin A-, serotonin-, cholecystokinin- and somatostatin-immunoreactive material were found in both major and minor vestibular glands. Such cells were numerous, scattered in the acini and excretory duct epithelium, small in size and rounded, triangular or bipolar in shape. The function of the NE vestibular cells has been related to the secretory activity of the glands and to a sexual climax induction mechanism involving the stimulation of $5HT_3$ receptors of vestibular nociceptor nerve fibers. The role of NE cells in small cell carcinomas of the vestibular glands is a topic for further investigation owing to possible parallelism between this type of tumor and the small cell carcinoma of the human prostate.

Introduction

Endocrine cells scattered in the mucosa of many organs that produce biogenic amines and hormonal peptides were defined, three decades ago, as APUD or neuroendocrine (NE) cells (Pearse, 1977) or paraneurons (Fujita, 1980), and are now considered to be part of the diffuse NE system. They contain chromogranin A (chr A), a structural protein of the secretory granules, which has been regarded as a marker of this cellular population (Deftos, 1991). NE cells in the female genital tract have been described in the oviduct, uterus and vestibular glands, but their function still remains poorly understood (Fetissof et al., 1986; Vittoria et al., 1989; Czaja et al., 1996). In particular, the NE cells of vestibular glands have been studied in female humans and pigs and were found to produce, in both health and in some non-neoplastic diseases, the amine serotonin and the peptides bombesin,

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calcitonin, somatostatin, katacalcin, α hCG, synaptophysin and CXCR2 (Fetissof et al., 1989; Czaja et al., 1996; Slone et al., 1999).

The function of the NE vestibular cells has been related to the regulation of mucus and electrolyte secretion from the exocrine epithelium of the glands (Warner et al., 1996). Moreover, in vulvovestibular syndrome, in humans the serotonin produced by a local NE cytotype is thought to stimulate a nociceptive afferent neuron, which induces the central pain sensation typical of such diseases (Warner et al., 1996; Slone et al., 1999).

The role played by NE cells in the development of small cell carcinomas (SCC) of the vestibular glands is a matter of interest for the modern pathologist. SCC or oat cell carcinomas are tumors showing features of NE differentiation and are characterized, generally, by aggressive biological behavior and poor prognosis. In the female genital tract, primary neoplasia of this type were described for the first time in the uterine cervix (Albores-Saavedra et al., 1972) and successively in the ovary, endometrium, cervix, vagina, vestibular glands and vulva (Mirhashemi et al., 1998; Obermair et al., 2001; Varras et al., 2002). The cervix is most frequently affected.

The aim of this research was to investigate the presence of NE cells in the vestibular glands of healthy cows and pigs by means of immunohistochemistry.

Material and methods

Major vestibular glands are present in cows but absent in pigs, while minor vestibular glands are present in both species (Barone, 1993). Samples were taken from five cows and five pigs in a local slaughterhouse soon after the death of the animals, which were healthy and sexually mature at the time of slaughter. The tissues were fixed in Bouin's fluid for 48 h, dehydrated through an ascending series of ethanol and processed to embedding in Paraplast Plus® paraffin wax (Carlo Erba Reagenti s.r.l., Milan, Italy) using routine protocols. 5 μm-thick sections were cut using a microtome, collected on slides and then labeled using the immunohistochemical peroxidase–antiperoxidase technique first described by Sternberger (1986) which was performed as follows. The sections were dewaxed in xylene and brought to distilled water through descending grades of ethanol. Washes between steps and all dilutions were performed using 0.01 M phosphate buffer saline (PBS), pH 7.2–7.4. All steps were performed at room temperature, unless otherwise stated. The activity of endogenous peroxidases was abolished by incubating sections with 3% hydrogen peroxide (Farmac-Zabban SPA, A907181236, Bologna, Italy) for 20 min. Non-specific labeling was blocked by incubation of the sections in a 1:5 dilution of normal goat serum (Jackson ImmunoResearch Lab. Inc., 005-000-121, PA, USA) for 30 min. Sections were then incubated with polyclonal rabbit antisera raised against chr A (ImmunoStar Inc., 20086, Hudson, WI, USA), serotonin (ImmunoStar Inc., 20080), cholecystokinin (ImmunoStar Inc., 20078) and somatostatin (ImmunoStar Inc., 20067). All antisera were diluted 1:800 and incubated on sections overnight at 6–7 °C. Sections were then incubated with goat anti-rabbit IgG (Jackson ImmunoResearch Lab. Inc., 111-005-003), then PAP complex (Jackson ImmunoResearch Lab. Inc., 323-005-024), diluted, 1:50 and 1:100 respectively, and both were applied for 30 min. Labeling was revealed by incubation with 10 μg 3-3′ diaminobenzidine (Sigma-Aldrich, D5905, Milano, Italy) dissolved in 15 ml of Tris buffer (0.5 M, pH 7.6) and 1.5 ml of 0.03% hydrogen peroxide for 15–35 min. Finally, the sections were dehydrated through a graded ethanol series, cleared in xylene and mounted in Eukitt (Kindler & Co., 2442, Freiburg, Germany). Negative controls were performed by substituting the specific antisera with PBS and by absorbing each primary antiserum with an excess of the relative peptides (100 μg of peptide/ml of diluted antiserum). Positive controls were achieved performed using 6 μm-thick sections of Paraplast-embedded fragments from the female urethral mucosa, which is known to contain NE cells producing the substances of interest (Vittoria et al., 1992).

In order to obtain quantitative data, the major and minor vestibular glands from two cows and two pigs were cut entirely into small pieces of equal length (4 mm) and, after embedding, a series of 20 consecutive sections was obtained from each of them. Four non-adjacent sections from each series of sections were immunolabeled as described above. These slides were observed using a Nikon E 600 light microscope and photomicrographs were taken using a Coolpix 8400 Nikon digital camera. The cellular NE subgroups identified by immunohistochemistry were analyzed in relation to the shape and localization of their components.

Results

No substantial differences were found in this study between glandular types and the animal