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Anatomical and Clinical Perspective of APUD Cell Series: An Overview

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ABSTRACT

APUD system (Amine Precursor Uptake and Decarboxylation) encompasses a wide range of endocrine cell types scattered throughout the body like respiratory system, gastro-enteropancreatic system, reproductive system, thyroid gland, pancreatic islets, adrenal medulla, carotid body, kidney, pituitary gland etc. The APUD cell family now includes around 40 members. The APUD concept has more popularly been replaced by 'diffuse neuroendocrine system (DNES)' in recent past. These cells release their secretion into blood (endocrine secretion) or have paracrine effect on the neighboring cells. The diffuse neuroendocrine system can regulate the activities of immune system also. Clinical importance of these diffuse populations of endocrine cells is well established. Neural crest origin of the endocrine polypeptide (APUD) cells was earlier proposed. However, endodermal, mesodermal and even epidermal origin of many such cells are also suggested. Several histological methods are employed for demonstration of neuroendocrine (APUD) cells. Many special light microscopic staining techniques are still useful for identification of these cells. Electron microscopic (TEM) and immunohistochemical techniques, specially the latter ones are by far the best in this regard. Thorough knowledge of APUD cells would be of paramount importance to better understand their functional significance specifically in respect to growth of broiler chicken and neuro-endocrinological pathology.

Key words: *APUD cells, Distribution, Identification, Importance, Origin*

INTRODUCTION

APUD system (Amine Precursor Uptake and Decarboxylation) is a collective term for a diffuse spectrum of endocrine cell types scattered throughout the body like respiratory system, gastro-enteropancreatic system and reproductive system[1]. The term 'APUD system' was coined to encompass adrenal chromaffin cells, enterochromaffin cells, mast cells, pancreatic B cells, pituitary corticotrophs and melanotrophs, and thyroid C cells[2]. The APUD cell family has rapidly enlarged to a span of around 40 members including in the mucosa of the gastrointestinal tract, in the respiratory epithelium, thyroid gland, pancreatic islets, adrenal medulla, carotid body, kidney, pituitary gland[3].

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APUD cells in the digestive tract have attracted worldwide attention. Gastrointestinal tract is considered the biggest and most complex endocrine organ in animals[4]. There are many hormonal secretory cells releasing polypeptides and amines like 5-hydroxytryptamine in the vertebrate digestive system. The secretion of these cells can be released into blood (endocrine secretion) or by having an effect on neighbouring cells (paracrine secretion). These cells are part of the APUD system, although at present this term has been replaced by 'diffuse neuroendocrine system' (DNES)[5,6]. The presence of these cells in the pancreatic-gastrointestinal tract (PGIT) from different species of vertebrates has been corroborated by several surveys[7]. Products of these cells (peptides/ amines) suppress, amplify or modulate the activities of the other two divisions of the nervous system[8]. The diffuse neuroendocrine system can regulate the immune system at all levels: innate immunity, adaptive immunity, and maintenance of immune tolerance[9]. The neuroendocrine system and the immune system are considered as two autonomously acting networks[10]. The clinical importance of these diffuse populations of cells can be well evident from the fact that when these cells undergo unopposed proliferation they result in production of a number of neoplastic syndrome and tumors[11] such as neuroendocrine tumor in lung of dog[12]. In view of the aforesaid fact, the present review is an earnest attempt to give an insight to detailed anatomical perspective of APUD cell series.

APUD CELL SERIES IN DIFFERENT LOCATIONS

a) Gastrointestinal tract -

The APUD system is the most wide spread in the gut specially stomach and small intestine and a part of the large intestine. In the stomach they predominate in pyloric region and in the intestine they occur both in villi and crypts. Most frequently these cells are wedged between the basement membrane and the chief cells of gastric glands or in the intestinal villous epithelium. Usually these endocrine cells do not reach the surface of the epithelium (Closed type). But few endocrine cells may extend across the glandular or surface epithelium (Open type). At least a dozen types of such endocrine cells exist[13]. Common enteroendocrine cells reported in stomach of different mammalian species are gastrin (G), somatostatin (D), serotonin/5-HT(EC), histamine (ECL), pancreatic polypeptide (PP) and those of intestine are-gastrin (G), secretin (S), cholecystokinin (I), somatostatin (D), serotonin/5-HT(EC), pancreatic polypeptide (PP), gastric inhibitory peptide (K),glucagon – like peptide (L), motilin (Mo), neurotensin (N)[14,3].

Somatostatin-, pancreatic polypeptide (PP)-, polypeptide YY-, glucagon-, secretin-, vasoactive intestinal peptide (VIP)-, gastrin-, cholecystokinin-, neurotensin-, bombesin-, substance P-, enkephalin-, motilin-, and FMRFamide-like immunoreactive endocrine cells were described in avian gastrointestinal tract. Most endocrine cells are located in the antrum; many in the proventriculus and small intestine but few in the gizzard, caecum, and rectum[15]. EG (glucagon – like peptide/ GLP - 1), APP (avian pancreatic polypeptide), ECL (enterochromaffin – like cells), chromogranin A, NSE (neuron specific enolase), CGRP (calcitonin gene related peptide), gastric releasing peptide (GRP), glicentin were also reported in different avian species[16,17,18, 19]. Besides, six types (I to VI) [20] and nine morphological types (I to IX) [21] of gut endocrine cells were identified in broiler chicken.

a) Pancreas –

A or Alpha cells - Form the second most numerous type of endocrine cell within the islet. The glucagon secreting cells represent approximately 3-5% of the islet population. In horse the A cells are located in the centre whereas in cattle they tend to arrange at the periphery. The pancreatic islets of the uncinate process of dogs are devoid of A cells[3,13].

B or Beta cells - Are the most numerous cells in the pancreatic islets comprising approximately 60- 80% of the total islet cell

population (upto 98% in sheep). The insulin secreting cells predominate in the periphery of the islet of horse and in the centre in cattle[3,13].

D or Delta cells - Are of relatively rare occurrence (approximately 5% in dogs) and are located mainly in the periphery of the islets. They synthesize somatostatin[3,13].

C cells – Are nongranulated or sparsely granulated cells that are considered to be precursor cells for other cell types. These cells comprise relatively small population of islet cells[3,13].

PP or F cells - Very rare population of pancreatic polypeptide secreting cells may occur either among the pancreatic acini or in the islets[14,22].

In avian pancreas, endocrine cells producing glucagon, somatostatin, PPA (Avian pancreatic polypeptide), and PYY (YY polypeptide) were shown[23]. Insulin secreting cells (B cells) were most numerous, preferably occupying the central place in the pancreatic islets of a Brazilian sparrow sp. Somatostatin, PPA, PYY and glucagon immunoreactive cells occurred in a lower frequency in the periphery of pancreatic islets[7].

Functional aspects of pancreatic endocrine cells are detailed by aforesaid workers as well as in available texts[14,3,13,24].

b) **Respiratory Epithelium–**

The lining respiratory epithelium of the trachea and bronchial tree contains neuroendocrine cells or APUD cells/ Kulchitsly Cells (K)[3]. Neuroendocrine cells are common in the form of solitary cells in the bronchial tree of sheep but are much less in the yak and its inter breeding with the cattle[25]. Lung endocrine cells secreting pneumokinin were earlier described[26]. Pulmonary neuroendocrine (NE) cells and neuroepithelial bodies (NEB) were studied in details in human and animal lungs[27,28]. The study revealed many scattered APUD cells secreting bombesin, calcitonin, leu-enkephalin, serotonin peptide hormones in pulmonary tissues. Calcitonin (CT), synaptophysin (SY), somatostatin (ST), and neuron-specific enolase (NSE) immuno reactive neuroendocrine cells were reported in respiratory system[29,30]. APUD cells were recently studied in ovine lungs[31]. Detailed account on functional facets of pulmonary neuroendocrine system and pulmonary neuroendocrine cells (PNECs) was earlier given[30].

c) Urogenital System -

The juxtaglomerular cells (JG) form the juxtaglomerular apparatus along with the macula densa and extra glomerular mesengial cells. The Renin secreting JG cells are a group of modified smooth muscle cells located at the distal end of mainly afferent arteriole (and partly efferent arteriole), close to the macula densa of the distal straight tubule[3]. Renin acts to restore blood pressure, stimulates secretion of aldosterone and ADH via renin – agiotensin mechanism[13,24].

In urogenital system, few APUD cells were reported earlier[32,33]. In the testis, epididymis, ductus deferens and vesicular gland no endocrine cells were found in any of the animals (boars, bulls, horses and donkeys) studied. Chromogranin-A, serotonin, somatostatin and enkephalins were present in endocrine/paracrine cells in the surface or glandular epithelia. In the prostatic complex and the urethral epithelium, the most consistent number of endocrine cells was serotonin secreting cells. Few argentaffin, argyrophil and chromogranin-A immunoreactive cells were also present. Somatostatin- and enkephalin-immunoreactive cells were rare in the bull and boar, absent in stallions[34].

The endocrine (APUD) cells in the oviduct of sheep were localized as paraneurons in the lamina propria layer sandwiched between this layer and tunica muscularis[35]. The occurrence, distribution and immunohistochemical character of NE cells (paraneurons) were studied in the vestibule, vagina, uterus, oviduct, ovary, urethra, urinary bladder and ureter of pig[36] Paraneurons (Chromogranin A- and somatostatin – positive) in the vestibular glands were found to be the most numerous, moderate in the uterine horn and urethra, few in the oviduct and occasional in the urinary bladder.

Regulatory and modulating effects of secretions (hormones) of different endocrine/ neuroendocrine (APUD) cells on muscular contraction and secretions in genital organs are well established [37,34].

d) Thyroid Gland -

The parafollicular (C) cells usually occurs as single cell enclosed within the basal lamina of the follicles but may also form groups in the same location or outside the follicles especially in dog. They do not border directly the lumen but are separated from it by overarching processes of neighboring follicular cells[38,3,13]. Parafollicular cells in the dog thyroid gland occurs as single cells but frequently form relatively large cluster. These cells secrete calcitonin hormone that lowers plasma calcium level[3,13,24].

e) **Adrenal Medulla -** The endocrine cells of the adrenal medulla are modified postganglionic sympathetic neurons, the secretory activity of which is regulated by postganglionic sympathetic innervation. When treated with fixatives containing chromium salts, the large cells stain dark brown, consequently they are often referred to as chromaffin cells[3]. In horse, cow, sheep and pigs the adrenal medulla is subdivided into 2 distinct zones, an outer zone made of large epinephrine secreting cells and an inner zone of clusters of small polyhedral norepinephrine secreting cells[3,13].

f) Pituitary Gland –

Corticotropes in pars distalis - They are dispersed throughout the pars distalis and are usually difficult to identify. The cells may be spherical, ovoid or stellate depending on the species.

Melanotropes in the pars intermedia - Melanotropes are present in the pars intermedia of pituitary gland. The pars intermedia is well developed in domestic mammalian species enveloping the neural lobe of the neurohypophysis in many instances including the carnivores, the pig and the horse. The parenchyma of the pars intermedia is arranged in clusters, cords and small follicles[13].

g) **Carotid Body -** The carotid bodies are inconspicuous flattened structure at the bifurcation / trifurcation of each common carotid artery. It is composed of irregular groups of pale staining epithelioid cells in intimate relation to capillary sinusoids lined with fenestrated endothelium[14]. The cells contain typical neurosecretory granules[22]. These are capable of responding to change in blood levels of carbon dioxide, oxygen and pH levels[39]. Almost all of the serotonin cells in the wall of the common carotid artery in chicken are intensely immunoreactive to the neuropeptide Y, met- and leu-enkephalin. Densely populated Calcitonin gene-related peptide (CGRP)-and substance P-immunoreactive varicose nerve fibers, moderate somatostatin-immunoreactive fibers, and sparse Galanin-and vasoactive intestinal peptide (VIP)-immunoreactive fibers are distributed in the serotonin cell groups[40].

h) Skin – Melanocytes and Merkel cells in the skin were reported to possess APUD characteristics[41]. Merkel cells are located at the basal layer of the epidermis in intimate contact with dermal neurites, and are thought to act as slowly adapting mechanoreceptors and neuromodulator[42,22]. Merkel cell – neurite complexes are thought to respond to tactile stimuli in mammals[43]. Detection of vasoactive intestinal peptide (VIP) and metencephalin has been reported in the Merkel cells[42,14].

ORIGIN OF APUD CELLS

Neural crest origin of the endocrine polypeptide (APUD) cells of the gastrointestinal tract, pancreas and 'C' cells of thyroid gland in chick embryo was proposed[44,45]. However, the majority of gut endocrine cells are of endodermal origin and are not derived from the neural crest or neuroectoderm as earlier proposed[15]. Endodermal origin of the APUD cells was also suggested later[46,47]. APUD cells of the respiratory tract arise from the same precursor cells as the other epithelial cells[48]. The

parafollicular 'C'cells of thyroid are derived from the neural crest[3] with the exception in dogs where they originate from the ultimobranchial bodies[49].

The pancreatic islets originate from the same endoderm that gives rise to the rest of the exocrine pancreas[13]. The neural crest derived cells migrate and become gland cells (non nervous) of adrenal medulla, active in the production of a specific hormone[50]. Metanephric mesenchyme derived juxtaglomerular cells (JG cells) are highly specialized myoepitheloid granulated cells[51,52]. The chemoreceptor cells of carotid artery are derived from mesodermal corticotrophs on the wall of each internal carotid artery and belong to chromaffin category[53]. The adenohypophysis is of neuroectodermal origin[53], whereas the melanotropes of pars intermedia are ectodermal in origin. Conclusive evidence for the epidermal origin of mammalian Merkel cells is given[54].

IDENTIFICATION OF APUD CELLS

Several methods are employed for demonstration of neuroendocrine (APUD) cells.

Tinctorial methods – In routine haematoxylin and eosin (H & E) staining, gut endocrine cells appear as 'clear cells' without any distinct morphology[14,22,3]. Some special light microscopic techniques like Argentaffin reaction (Singh's modification), Argyrophilia (Grimelius Silver method), Lead haematoxylin method, Ferric ferrycyanide reduction test, Gomori's Alkaline Diazo reaction, Masked metachromasia are used for identification of different APUD / neuro endocrine cells[55,22]. Selective argentaffin and argyrophil reactions are more commonly used for light microscopic study of different enteroendocrine cells in avian species[20,56]. However, Ferric ferrycyanide reduction test (after due standardization of reaction time) appeared one of the most convenient light microscopic methods for study of avian gut endocrine cells[21]. Few peptide secreting endocrine cells can be localized by Ninhydrin – Schiff method[57,22,20].

Formaldehyde induced fluorescence (FIF) – APUD cells in GI tract and pancreas were demonstrated by fluorescence microscopy (using APUD – FIF method)[44]. Amine (catecholamines, 5 HT/ serotonin) secreting endocrine cells and nerve fibers produce bright yellow fluorescence in FIF method[22].

Ultrastructural /TEM study – Transmission electron microscopy can be used for detailed morphological study of the APUD cells and their secretory granules[58,22].

Immunohistochemical methods – Sophisticated, very sensitive immunohistochemical techniques have mostly replaced the conventional staining methods for identification of neuroendocrine cells[22]. A wide range of specific antibodies (markers) are now available commercially for this purpose[22,59,30]. Immunological techniques were employed for identification of different APUD cells and their secretions in different locations[40,28, 60,36,37,29,34,54].

Brazilian scientists studied endocrine cells in the pancreas of the Brazilian sparrow species by immunocytochemical method[7]. A detailed account on identification of APUD cells by histological, histochemical and ultrastructural methods was given in recent past[61]. Fe (Iron)-ECR (Eriochrome cyanine R) as a substitute for routine H & E, stains selectively intestinal cells of the diffuse neuroendocrine system (DNES)[62].

CONCLUSION

APUD cell series still continues to expand and attract the attention of histochemists and histopathologists not only for their histophysiological role in the respective organs but also for their clinico - pathological correlation. Better the understanding on these cells, better will be the strategy to improve digestion and in turn to augment growth specifically in broiler chicken, as well as to combat the neuroendocrinological pathology, namely the tumors of APUD cell origin.

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