ENDOCRINE (APUD) CELLS IN THE OVIDUCT OF THE SHEEP

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Summary

APUD cells in the oviduct of the sheep at standing estrus were localized as paraneurons in the lamina propria sandwiched between this structure and the tunica muscularis by the method of masked metachromasia to toluidine blue after hot mineral acid hydrolysis. These were also confirmed by lead haematoxylin stain and argyrophilia. The oviduct was serialised into 66 zones. Cells were absent in the first and last 2 zones, and most parts of the isthmus. There was however abundant number of APUD cells in the ampulla which were fusiform shaped and were about 5 μm in width and also in the junctura, where the cells were of a smaller width (3 μm) and were quite numerous reaching 180-200 in some zones. It is concluded that peptide secreting cells are numerous in the oviduct and that this may qualify the oviduct as an endocrine organ.
(**Key Words**: APUD Cells, Oviduct, Sheep)

Introduction

After Pearse introduced his concept of Amine Precursor Uptake and Decarboxylation (APUD) in 1966 (Pearse, 1966), it became quite clear that the concept was a significant one in relation to the occurence and description of peptide secreting (or argyrophil-argentaffin) cells of the vertebrate body from the cytochemical point of view of amine uptake and its decarboxylation within the cells (Scully, Aguirre and DeLellis, 1984). Fujita followed up with his suggestion of the paraneuron family (Fujita, 1977) and his concept found favour amongst morphologists since paraneurons were described as endocrine brothers of neurons whose ultrastructural features were similar to those of neurons. These cells also belong to the diffuse neuroendocrine system of Pearse (1977) and Pearse and Polak (1978) and were earlier grouped together under the clear cell system of Feyrter (Feyrter, 1938, 1952).

Recent reports have shown that neuropeptides seem to repeat their presence in most of these APUD or paraneuronal cells in the body and considerable numbers of these peptide hormones or regulatory peptides are being increasingly identified in the vertebrate body (Yanaihara et al., 1988; Ida, 1991). In the urogenital system a few APUD cells have been identified although no specific role has been yet assigned to them (Pearse, 1983; Williams et al., 1989). No investigation has yet studied the occurrence of these peptide cells as APUD cells or paraneurons in the oviduct. The present study uses histological-histochemical marker stains for both paraneurons and APUD cells to determine existence of the cells in the oviduct of the sheep.

Materials and Methods

Ten of sheep oviducts breed West African sheep and Merino of 1½ to 2 years were obtained at standing estrus and zoned after the method of Ogunranti (1991; 1992) as follows:

Tissues were fixed *en bloc* in 10% formol-saline and Bouin's fluid and glutaraldehyde-picric acid and passed through graded series of alcohol for dehydration. They were then cleared in xylene. Prior to embedding for sectioning, they were divided into 66 zones as follows,

1. The whole cleared oviduct is separated into segments-preampulla and fimbriae, ampulla, isthmus and junctura.

2. The segments are divided into three equal subsegments as proximal, middle and distal subsegments.

3. The subsegments are divided as follows,

a) preampullary subsegments are divided into four zones each, running from zone 1 (the most

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Received December 7, 1993
Accepted June 30, 1994

distal) to zone 12.

b) ampullary-isthmic junctural subsegments are divided into six zones each, running from 13-66.

66 blocks were therefore obtained all together for each oviduct. They were then embedded separately. Sections were cut from separate blocks and labelled as separate zones. 3-4 sections were cut randomly from each block to represent a zone. The sections were stained for the following techniques.

a) Technique of unmasking metachromasia for the demonstration of peptide secreting cells (APUD and paraneurons) following the method of Solcia (Vassallo and Capella, 1968). Histochemical validity of the masked metachromatic technique for staining endocrine peptide cells was discussed by Pearse (1969).

b) Lead haematoxylin stain after the method of Solcia, Capella and Vassallo, 1969).

c) Grimelius silver nitrate argyrophilia method after Grimelius, (1968). This method was compared with argyrophilia by the method of Marsland et al. (1954).

d) Control slides: Some of picric acid-glutaraldehyde fixed tissues were cut on the cryostat and treated directly with aqueous toluidine blue without prior hydrolysis with hydrochloric acid. These served as control slides for masked metachromasia technique. In lead haematoxylin reaction, the staining solution of control slides did not have lead nitrate. With argyrophilic reactions, the control slides were stained with solution which did not contain silver nitrate.

Slides were examined under microscope for existence of APUD cells (or paraneurons) for each zone of the sheep oviduct. But only one oviduct was used to count the distribution of APUD cells in the various zones, using representative section for each zone.

Results

The technique of unmasking metachromasia in the sheep oviduct was successful in demonstrating metachromatic cells which were visualised as deeply metachromatic (β) under the microscope (figure 1). These cells were also positive for lead haematoxylin stain and argyrophilia (figure 2). They were localized as closed paraneurons in the lamina propria sandwiched between this structure and the tunica muscularis. They were absent in the first 2 zones of the preampulla and most of the isthmic zones. They were large and abundant in number in the ampulla and preampulla, most especially in the proximal preampulla and the distal half of the ampulla (figure 3). They were large, reaching up to 5-10 μm in diameter and fusiform shaped with numbers reaching up to 180 in some zones. See distribution as counted for one oviduct in figure 3. The ampullary isthmic junctional zone 3 3 had the highest recorded value of peptide cells in most sections examined. These cells have different morphology from the preampullary-ampullary cells. The cells in the ampullary-isthmic junction were round and small (3 μm in diameter). Small cells were also found in the junctural zones but

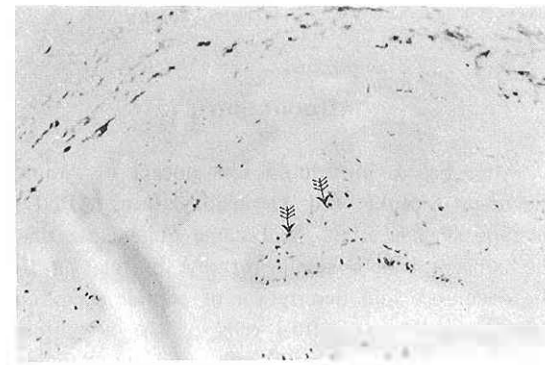

Figure 1. Masked metachromatic reaction to toluidine blue after HCl hydrolysis at 60°C. Note metachromatic cells (arrows) in sheep oviduct at estrus. Zone 58 of sheep oviduct. Glutaradehyde-picric acid fixed. Magnification × 150.

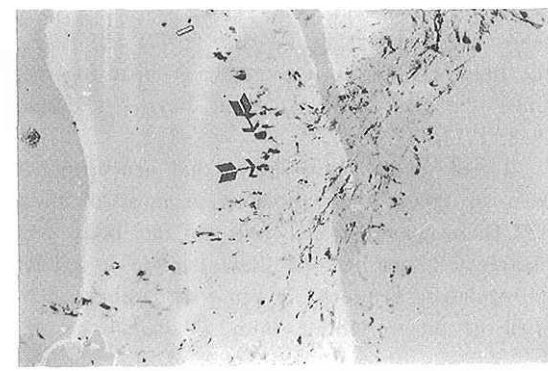

Figure 2. Argyrophilic reaction of Marsland et al on zone 58 of sheep oviduct. Note reactive cells (arrows). Formol-saline fixed. Magnification × 150.

were absent in most of the other zones of the isthmus. The ampullary cells can be labelled discoid paraneurons while the isthmic-junctural cells can be called round paraneurons in view of their observed morphology (figure 2).

The reaction for peptide cells does seem to be higher in the ampullary segment and less in the isthmus. They were numerous and small in the junctura, while the cells in the ampulla tend to be large and spindle shaped.

All reactive cells are seen in the concentric lamina propria and they tend to occupy the position between the muscularis and the concentric lamina propria (figure 2). A few cells occur in the basal lamina propria only in the preampulla.

All control slides stained were negative.

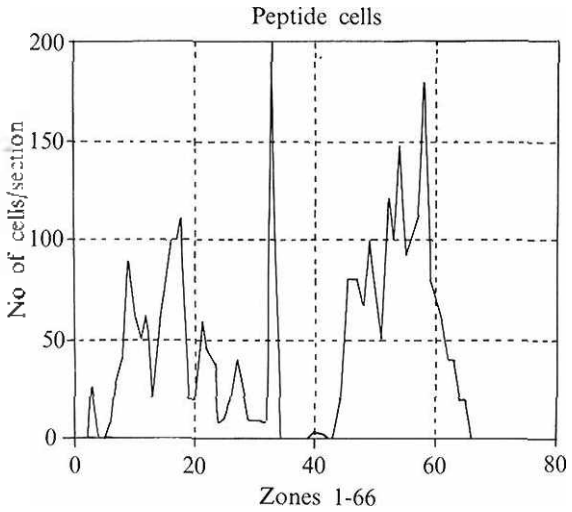

Figure 3. Graph of number of APUD cells per zone in one estrous sheep oviduct to demonstate distibution.

Discussion

According to the classification of Fujita (1980), closed paraneurons reside buried from the luminal surface in the mucosa and are mainly stimulated by pressure changes in the tube. Examples in the gut include the deep residing gastrin (G) cells in the gut. The cells of the APUD series colocalised as paraneurons in the sheep oviduct were all closed since none could be found in the luminal surface. They were all sandwiched between the lamina propria and the muscularis presumably to be under the direct stimulatory influence of the muscularis tone mainly and also probably increase oedema of the lamina propria (Hodgson, 1978; Spilman, 1980) and dilatation of subserosal venous plexus (Verco, Gannon and Jone, 1982) all of which are accompaniment of estrus in most mammalian oviducts. It is also noteworthy that most gut closed paraneurons are also fusiform shaped and are therefore discoid paraneurons according to the classification of Fujita (Fujita, 1977, 1983). Example of round paraneurons are mast cells (Fujita, 1977).

It therefore seems easy, based on morphological considerations to suggest that the peptide cells observed in the oviduct of the sheep are of two major types- the discoid paraneurons and the round, probably secreting different peptides and subserving different functions. Their absence in the isthmus is significant since the isthmus seems quite different in the phenomenon of secretion, and egg transport from other parts of the oviduct (Ogunranti, 1992).

It is quite interesting to conjecture on the types of hormones secreted by these oviductal APUD cells.

Gonadotropin theory of oviduct secretion

Reports on the existence of gonadotropin cells in the reproductive tract continue to increase in the literature and it is a already common knowledge that the follicular fluid of most mammals including man, does contain abundant gonadotropins, the origin of which is obscure (McNatty and Sawers, 1975; McNatty, 1978). Hypothalamic gonadotropin release factor stimulates a large increase in estradiol secretion when perfused through the ovarian artery to the ovary (Scaramuzzi et al., 1986). It is possible that the increased estradiol secretion could be performed via the peripheral stimulation of gonadotropins in the ovarian and oviductal stroma which then causes the stimulation of steroidogenic cells for the output of steroids (Ida, 1991).

Oxytocin secretion theory

Oxytocin is already known to stimulate contractile activity of the oviduct (Zetler Monkemeier and Wiechell, 1969) and also has been localized not only in the oviduct (Schaeffer et al., 1984) but also in the ovary (Clements et al., 1988). Schaeffer et al. (1984) reported the existence of abundant oxytocin reactivity in the ovarian tissue (most especially the corpus luteum) and also the

follicular fluid but none in the uterus (Shaha, 1988).

Opioid peptide theory

It is possible to postulate the existence of opioid peptides in oviduct paraneurons. They have no doubt been examined and found to occur in autonomic neurons (Huang et al., 1984). Since they seem to stimulate contractility in the oviduct (Zetler, Monkemeier and Wiechell, 1969), it seems again quite attractive to postulate that some paraneurons of the oviduct produce opioid peptides.

Other neuropeptides

The existence of other neuropeptides like galanin, bombesin, porcine heptacosapeptide and vasoactive intestinal peptide cannot be ruled out. Indeed their presence in autonomic nerves of the oviduct have already been confirmed (Samuelson and Dalsgaard, 1984; Yeats et al., 1984; Huang et al, 1984). Other peptides may include those derived from the proopiomelanocortin (POMC) molecule (Clements et al., 1988).

Without division of the oviduct into zones and the report of the reactions in a zonal fashion, the description of cellular endocrine apparatus of the peptide and steroid series (Ogunranti, 1992), will not have been made. Indeed Fox, Kazzaz and Langley, (1964) found no argyrophil cells in the human oviduct, but they did not serialise the oviduct into zones.

Clements et al. (1988) in reviewing the functions of gonadal peptides which have been identified and characterized in the ovary, said '...they would appear to play a paracrine (perhaps autocrine) rather than the classical endocrine role, as part of complex intragonadal regulation...' This may also apply to the oviduct.

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