HISTOCHEMISTRY OF PHOSPHATASES IN THE EPIDIDYMIS OF RAM DURING POSTNATAL DEVELOPMENT*

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ABSTRACT

The localization of phosphatases in the epididymis of Madras Red ram during postnatal development was studied. The alkaline phosphatase activity was weak in the pre-weaning and pre-pubertal periods but with the onset of puberty it was better observed, especially in the epididymal epithelium. In the ductus epididymidis, the activity in the epididymal tubules was greater than that in the inter-tubular area. Regional variations in the enzyme activity were observed in the different segments. The acid phosphatase activity changed during different stages of postnatal development. The weak activity in the pre-weaning and pre-pubertal periods changed to moderate and strong in the different regions, with the onset of puberty. The reaction in the different segments of the epididymis was varied with respect to the basal and apical areas of the epithelium. The adenosine triphosphatase activity in the pre-weaning and pre-pubertal periods was weak. It changed to moderate and strong in the different regions, with onset of puberty. The activity in the epididymis was greater in the epididymal tubules than in the inter-tubular area. The activity in the cauda region of the epididymis was stronger compared to the moderate activity in the corpus and caput regions.

Key words: Histochemistry, Phosphatases, Postnatal development, Ram

INTRODUCTION

Phosphatases are enzymes that catalyze the hydrolysis of phosphoric acid esters with the liberation of phosphate ions. Depending on the pH optima at which these enzymes act, two major groups were recognized and these have been designated as acid and alkaline phosphatase (Burstone, 1962). Phosphatases are of interest since they are thought to be influenced by steroid hormones (Goyal and Vig, 1984). Moreover, it has been suggested that the activity and location of these enzymes change during growth and puberty (Mayorga and Bertini, 1985).

The male animal passes through certain androgenic phases depending on the functional activity of the reproductive organs at various ages.

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The development of testis, epididymis and other accessory reproductive structures is closely dependent on the optimum level of testosterone secreted by the Leydig cells of the testis. The characterization of puberty with the associated changes in the reproductive organs is the available tool for selection of a male for breeding (Verma and Sahni, 1997). Information on the histochemical changes that occur in the postnatal development of epididymis is very valuable to determine the onset of puberty and maturity in breeding stock. Reports are available on the histochemistry of the epididymis in the adults of different species but very few reports are available during postnatal development from birth till maturity. Hence, the present work was undertaken to detail the postnatal localization of phosphatases in the epididymis of Madras Red ram.

MATERIALS AND METHODS

The present study was undertaken using tissue pieces of epididymis from thirty two Madras Red rams in the Chennai Corporation abattoir immediately after slaughter. The rams used were divided into four age groups viz; pre-weaning (birth to 3 months), pre-pubertal (4 to 6 months), pubertal (7 to 9 months) and post-pubertal (10 to 12 months), each of which consisted of eight animals. The determination of age was ascertained based on the eruption of teeth (Noden and de Lahunta, 1985). The tissue pieces collected were fixed in 4°C chilled formol-calcium or 10% neutral buffered formalin. Frozen sections of 15-20 µm thickness were cut using a cryostat. The following methods were used for localization of the enzymes viz. Gomoris’ calcium method for alkaline phosphatase activity (Bancroft and Gamble, 2003), Gomoris’ lead method for acid phosphatase activity (Bancroft and Gamble, 2003).

Unfixed fresh frozen tissues were used for Lead method for adenosine triphosphatase activity (Bancroft and Gamble, 2003).

RESULTS AND DISCUSSION

Alkaline phosphatase

The alkaline phosphatase activity was weak in the pre-weaning and pre-pubertal periods but with the onset of puberty it was better observed, especially in the epididymal epithelium. This is in accordance with the report of Goyal and Hrudka (1980) in the bull calf. They stated that the activity was absent in the early stages but developed at later stages, being evident with the onset of spermatogenesis and luminization of seminiferous tubules. A weak activity was observed in the tunica albuginea. However, Karmore et al. (1999) reported that in the cauda epididymidis of goat, the tunica albuginea showed a mild activity. They also observed that the activity in the epithelium was strong, compared to the mild activity in the inter-tubular area.

In the present study, the activity was seen both in the ductuli efferentes and ductus epididymidis. The activity in the epithelium of the ductuli efferentes was weak and mostly seen towards the luminal side. These findings concur with the observation of Singh et al. (1991a) who reported that in the ductuli efferentes of horses, the activity was weakly positive with coarse granules which were sparsely distributed throughout the cytoplasm. At places, the reactive granules were relatively more concentrated in the supra-nuclear region, presumably in the areas with patches of non-ciliated cells indicating the alkaline phosphatase associated absorptive function of the epithelium. The activity was localized in the sub-epithelial capillaries as well. However, Ariyaratna et al. (1996) described that in the swamp buffalo, the efferent ducts were free of this enzyme.

In the present study, the activity in the epididymal tubules was greater than that in the inter-tubular area. In the inter-tubular area, the peri-tubular smooth muscle and the endothelial lining of the blood vessels showed a moderate to strong
reaction whereas the connective tissue showed a weak reaction. In the epididymal tubules, the reaction varied in the different regions. The epithelium showed moderate to strong reaction, in both the basal and apical regions. The basement membrane showed a strong reaction. The stereocilia and the clumps of spermatozoa in the lumen showed the reaction product. In the caput region, which comprised the segments I to III and in the corpus region, which comprised the segment IV, the activity was very strong along the basal region of the epithelium compared to the apical region (Fig. 1). In the cauda region, which comprised the segments V and VI, the activity in the apical region of the epididymal epithelium was strong compared to that in the basal region and basement membrane (Fig. 2).

On the contrary, Ariyaratna et al. (1996) described that in the swamp buffalo, a moderate activity occurred along the basal region of the epithelium in zones I, III and IV of the ductus epididymidis and in the stereocilia of zones I and II. The report of Veerabrahmaiah et al. (1997) is also in contrast to the finding in the present study. They cited that in the pig, the cytoplasm and apical portions of the epithelial cells and the sub-epithelial connective tissue were moderately reactive. However, Karmore et al. (1999) reported that in the cauda epididymidis of goat, the ductular epithelium showed an intense activity.

The epididymis provides a milieu for sperm maturation by its absorptive and secretory activity and it has been suggested that the epithelial cells may be involved in spermoiphagy (Robaire and Hermo, 1988). Testosterone is transferred to the duct system of the testis, as it is essential for the development of the epididymis and also for the maintenance of the function of accessory glands. The maturation and storage of spermatozoa in the epididymis is also dependent on the threshold value of testosterone. The activity was observed both in the luminal and basal borders of the epithelium in the epididymis. This was because the epididymis received a dual supply of testosterone, from the luminal fluid as well as from the blood vessels located outside the ductus epididymidis.

The regional variations in the enzyme activity, observed in the different segments were probably due to the minor differences in the dual supply of testosterone through the luminal and basal borders. The varied activity was seen in all the segments but the cauda region showed an overall lesser intensity. This was probably due to the fact that maturation of spermatozoa takes place in the caput and corpus regions and hence the increased absorptive activity and increased localization. As the cauda region acts more as a storage space, the enzyme activity was less and hence the lesser intensity.

**Acid phosphatase**

In the present study, the acid phosphatase activity was weak in the pre-weaning and pre-pubertal periods but with the onset of puberty, it was better observed, especially in the epididymal epithelium. A weak activity was observed in the tunica albuginea and the luminal border of the ductuli efferentes. The observation that the activity in the ductuli efferentes was in the luminal border concurred with the report of Ariyaratna et al. (1996) who described that in the swamp buffalo, the distribution of acid phosphatase was granular in the luminal region of the epithelium of the efferent ductules.

In the present study, the activity in the epididymal tubules was more compared to that in the inter-tubular area. There was moderate to weak activity in the peri-tubular smooth muscle layers. The reaction in the different segments of the epididymidis varied with respect to the basal and apical areas of the epithelium. The activity ranging from weak to strong was also observed in the clumps of spermatozoa seen in the lumen of the tubules. In the caput and corpus regions, the activity was similar. It was strong in the basement membrane.
around the tubules, moderate to strong in the epididymal epithelium and strong in the apical border. The clumps of spermatozoa in the lumen showed moderate to strong reaction in the different tubules. The cauda region showed a mild activity in all the areas including the clumps of spermatozoa in the lumen. The apical region of the epididymal epithelium showed more reaction compared to the basal region (Fig. 3).

However, Chandrapal and Bharadwaj (1986) stated that in the Indian buffalo, the activity was mild to moderate in the cytoplasm of the columnar cells of ductus epididymidis. Nakai et al. (1989) reported that in the domestic fowl, intense activity was observed on the luminal and lateral surfaces of the epithelial cells of the connecting ductules and in the epididymal duct. The findings in the present study concur with the report of Singh et al. (1991a). They reported that in the ductuli efferentes of horse, the activity was weakly positive with uniform distribution of non-granular reaction product throughout the cytoplasm.

Our findings also confirm with the observation of Delhon and Lawzewitsch (1994) who reported the regional differences in the various segments of the ductus epididymidis in llama. They mentioned that the epithelial cells in segment V of the ductus epididymidis showed strong activity whereas in segment VI they showed moderate activity. Ariyaratna et al. (1996) also described that in the swamp buffalo, the distribution of acid phosphatase was granular in the luminal region of the epithelium of the efferent ductules. In the ductus epididymidis, four zones were identified, all showing varying degrees of activity in the epithelium, with the most pronounced activity in the apical region and stereocilia of zone II.

Our findings also conform to the report of Veerabrahmaiah et al. (1997) who cited that in the pig, the cytoplasm of the epithelial cells in the epididymis showed the activity and it was marked in the region of the corpus epididymidis. Our findings are also in accordance with the report of Karmore et al. (1999) who observed that in the cauda epididymidis of goat, the tunica albuginea and the basal lamina showed a weak activity. The epithelium of the ductules showed a moderate activity.

The enzyme activity changed during different stages of postnatal development. The weak activity in the pre-weaning and pre-pubertal periods changed to moderate and strong in the different regions, with the onset of puberty. In the epididymis, the activity observed was strong in the caput and corpus regions that were concerned with sperm maturation whereas in the cauda region, the activity was mild, as the function of this region was only sperm storage.

**Adenosine triphosphatase**

The adenosine triphosphatase activity in the pre-weaning and pre-pubertal periods was weak. It changed to moderate and strong in the different regions, with onset of puberty. The activity in the epididymis was greater in the epididymal tubules than in the inter-tubular area. In the ductus epididymidis, the apical portion of the epithelium in the caput and corpus regions showed a moderate activity. The cauda region showed a slightly stronger activity. The activity in the peri-tubular smooth muscle increased proximo-distally. The luminal contents in all the regions showed the moderate activity (Fig. 4).

However, Tingari and Moniem (1979) reported that in camel, the activity was localized in the sub-epithelial tissue, blood vessels, stereocilia and luminal contents and the strongest activity occurred in the middle segment of the epididymis. Singh et al. (1991b) reported that the ductuli efferentes of goat were strongly positive and the activity was present along the apical borders of many cells. Karmore et al. (1999) reported that in the cauda epididymidis of goat, the tunica albuginea and the basal lamina showed mild and moderate activities respectively. The epithelium of the ductules showed a moderate to intense activity.
Adenosine triphosphatase is associated with mitochondrial activity in the tissues. The enzyme activity changed during different stages of postnatal development. The weak activity in the pre-weaning and pre-pubertal periods changed to moderate and strong in the different regions, with the onset of puberty.

The activity in the cauda region of the epididymis was stronger compared to the moderate activity in the corpus and caput regions. This might probably be due to the maximal spermatozoal packaging in the cauda region, which was primarily the sperm storage space. The peri-tubular smooth muscle in this region was also the thickest and the myoid adenosine triphosphatase might also be a cause for the increased activity.

REFERENCES


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Fig. 1

Photomicrograph of the corpus epididymidis in nine month-old ram showing alkaline phosphatase activity

Ep - Epithelium
Ms - Peri-tubular smooth muscle
CT - Connective tissue
Sp - Spermatozoa
BV - Blood vessel

Gomori's Alkaline Phosphatase x 100
Fig. 2
Photomicrograph of the cauda epididymidis in nine month-old ram showing alkaline phosphatase activity

Ep - Epithelium  CT - Connective tissue  Ms - Peri-tubular smooth muscle  Sp - Spermatozoa
Gomori’s Alkaline Phosphatase x 100

Fig. 3
Photomicrograph of the caput epididymidis in nine month-old ram showing acid phosphatase activity

TA - Tunica albuginea  Sp - Spermatozoa  Ms - Peri-tubular smooth muscle  CT - Connective tissue  Ep - Epithelium  BM - Basement membrane
Gomori’s Acid phosphatase x 100
Fig. 4
Photomicrograph of the caput epididymidis in three month-old ram showing adenosine triphosphatase activity

BV - Blood vessel
CT - Connective tissue
Ep - Epithelium
Lu - Lumen
Ms - Peri-tubular smooth muscle

Gomori's Adenosine triphosphatase x 40