HISTOCHEMICAL PROFILE OF HYDROXYSTEROID DEHYDROGENASE ENZYMES IN THE LEYDIG CELLS OF A MICROCHIROPTERAN BAT HIPPOSIDEROS SPEORIS (SCHNEIDER) DURING THE ANNUAL TESTICULAR CYCLE

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KEYWORDS

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INTRODUCTION

Leydig cells are prime cells in the interstitium of testis as they have exclusive role in steroidogenesis. The Leydig cells have two generations, the foetal and adult. Fetal Leydig cells produce testosterone required for male sexual differentiation and neuroendocrine functions. Later, testosterone secreted by adult Leydig cells stimulate male sexual characteristics and spermatogenesis (Akingbemi et al., 1998; Lejeune et al., 1998).

Hydroxysteroid dehydrogenases function in a specific manner at different steps in the biosynthesis of steroid hormones (Christensen and Gillim, 1969; Blackshaw, 1970; Neaves, 1975; Christensen, 1975). The qualitative pattern of the steroidogenic pathways in the Leydig cells resembles those of other steroid-producing tissues i.e. the adrenals and ovaries. The conversion of cholesterol to testosterone involves three hydroxylations, two cleavages, two hydrogenations and one isomerization (Rebois, 1982; Aquillano and Dufau, 1984; Simpson and Waterman, 1988; Ascoli, 1991; Hanukoglu, 1992, Labrie et al., 1992). Histochemical studies on hydroxysteroid dehydrogenases (HSDH) have been considered helpful in determining the site of steroid hormone formation and in investigating the role of steroidogenesis in sex differentiation and development in mammals (Bailie et al., 1966). Of all the steroid dehydrogenases, 3β-HSDH is the most extensively investigated enzyme in the steroid gland cells. 3β-HSDH are membrane bound enzymes and are distributed to both mitochondrial and microsomal membranes depending on the type of cell in which they are expressed. The enzymatic actions of the 3β-HSDH are essential for the production of all active steroid hormones. Like 3β-HSDH, the 17β-HSDH play essential role in steroidogenesis. This enzyme catalyzes the final step in the biosynthesis of active gonadal steroid hormones, estradiol and testosterone (Dumont et al., 1992; Labrie et al., 1997; Adamski and Jacob, 2001; Mindnich et al., 2004; Payne and Hales, 2004). Both the enzymes belong to the same phylogenetic protein family, namely the short-chain alcohol dehydrogenase reductase superfamily (Penning, 1997). The present study describes the histochemical changes of the hydroxysteroid dehydrogenase enzymes in the Leydig cells of H.speoris throughout the annual testicular cycle.

MATERIALS AND METHODS

All experiments were conducted in accordance with the principles and procedures approved by the departmental research committee at RTM University, Nagpur, Maharashtra, India. Three bats were trapped in each calendar month with the help of a mist net from the natural population inhabiting abandoned mines in Khapa, Nagpur, Maharashtra (28°923' N 78°953' E). The reproductive cycle of H. speoris is divided in the following five phases: (1) Quiescence (April-August): The resting stage. (2) Recrudescence (September-October): Preparation of testis for breeding. (3) Breeding (November-Early December): Peak of spermatogenesis. (4) Post mating (Mid-December): Decline in spermatogenesis. (5) Regression (January-March): Spent testis. The sucrose fixed tissues were cut on freezing microtome at -20°C and stained.
were histochemically stained by the method of Wattenberg (1958). The incubation medium for 3bHSDH contained a-steroid substrate (with hydroxyl groups) at a final concentration of 1mg/mL dissolved in dimethyl formamide (DMF), b-NAD (1mg/ml) and Nitroblue tetrazolium salt (NBT) (1mg/ml) in phosphate buffer at pH 7.3. For demonstration of D5- 3b - HSD the following steroids are used, 3 b - hydropregnen - 5 - ene - 2 - one (Pregnenolone) and 3b-hydroxyandrost–5 ene – 17 one (Dehydroepiandrostenedione). Similar incubation medium was used for 17bHSDH, only the substrate employed was testosterone and the reaction was carried out at pH 8.1 and NADP was substituted for NAD. Histochemical reaction and reaction intensity was graded visually on an arbitrary scale (from + = low, ++ = moderate, +++ = high, ++++ = intense). Control incubations were done without the substrate.

RESULTS AND DISCUSSION

The present study describes two hydroxysteroid dehydrogenases that function in a specific manner at different steps in the biosynthesis of steroid hormones (Christensen and Gillim, 1969; Blackshaw, 1970; Neaves, 1975; Christensen, 1975). The qualitative pattern of the steroidogenic pathways in the Leydig cells resembles those of other steroid-producing tissues i.e the adrenals and ovaries. The conversion of cholesterol to testosterone involves three hydroxylations, two cleavages, two hydrogenations and one isomerization (Rebois, 1982; Aquillano and Dufau, 1984; Simpson and Waterman, 1988; Ascoli, 1991; Hanukoglu, 1992, Labrie et al., 1992). Histochemical studies on hydroxysteroid dehydrogenases (HSDH) have been considered helpful in determining the site of steroid hormone formation and in investigating the role of steroidogenesis in sex differentiation and development in mammals (Baillie et al., 1966). Of all the steroid dehydrogenases, 3β-HSD is the most extensively investigated enzyme in the steroid gland cells. 3β-HSDs are membrane bound enzymes and are distributed to both mitochondrial and microsomal membranes depending on the type of cell in which they are expressed. The enzymatic actions of the 3β-HSD are essential for the production of all active steroid hormones.

The variations recorded in the reaction intensity of the enzymes in H. speoris along with Leydig cell development during different reproductive stages are depicted in Table-1. Thus

Figure 1: Less proliferated Leydig cells are characterized by fine and moderately sized granules (arrow) of 3β-HSD randomly distributed in the interstitium during early quiescence X400.

Figure 2: Comparable increase in 3β-HSD reactivity as well as number of Leydig cells when visualized histochemically using pregnenolone as a substrate showing moderate to high staining profile (arrow) during late quiescence X450

Figure 3: Less proliferated Leydig cells characterized by fine moderately sized granules (arrow) of 17β-HSD in the interstitium during early-quiescence X450

Figure 4: Comparable increase in 17β-HSD reactivity as well as number of Leydig cells when visualized histochemically using testosterone as a substrate showing moderate to high staining profile (arrow) during late quiescence X 450

Figure 5: An increase in the number of Leydig cells elicited a strong but constant reaction product for 3β-HSD which is widely dispersed (arrow) X450

Figure 6: An increase in the number of Leydig cells elicited a strong but constant reaction product which is NAD or NADP dependent for 17β-HSD which is widely dispersed (arrow) X 450
Table 1: β-HSDH and 17β-HSDH profile in the Leydig cells in different reproductive stages

<table>
<thead>
<tr>
<th>Reproductive stages</th>
<th>3β-HSDH</th>
<th>17β-HSDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quiescence</td>
<td>+ to ++</td>
<td>+ to ++</td>
</tr>
<tr>
<td>Recrudescence</td>
<td>++ to +++</td>
<td>++ to +++</td>
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<tr>
<td>Breeding</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Regression</td>
<td>+</td>
<td>+</td>
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</tbody>
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Figure 7: Leydig cells (arrow) from breeding phase (November) photographed to show remarkable reactivity as evident by the presence of dark blue formazan granules after utilization of tetrazolium salt X450.

Figure 8: Remarkable growth of Leydig cells during breeding phase (November): Note presence of dark blue formazan granules of 17β-HSD after utilization of tetrazolium salt (arrow) X450.

Figure 9: Note depletion in the granulations of 3β-HSD (arrow) as well as number of Leydig cells during post mating phase due to their utilization in steroidogenesis X400.

Figure 10: 3β-HSD reactivity is almost negligible and gives little NAD-linked colour in the late regression phase in the Leydig cells (arrow) X400.

Figure 11: Note depletion in the granulations of 17β-HSD (arrow) in the Leydig cells during post-mating phase due to their utilization in steroidogenesis X400.

Figure 12: The Leydig cells illustrates regression in number and size, correlative to this the 17β-HSD reactivity is almost negligible and gives little NAD-linked colour (arrow) X 400.

Like 3β-HSDHs, the 17β-HSDHs play essential role in steroidogenesis. This enzyme catalyzes the final step in the biosynthesis of active gonadal steroid hormones, estradiol and testosterone (Dumont et al., 1992; Labrie et al., 1997; Adamski and Jacob, 2001; Payne and Hales, 2004; Mindnich et al., 2004). Both the enzymes belong to the same phylogenetic protein family, namely the short-chain alcohol dehydrogenase reductase superfamily (Penning, 1997). They are involved in the reduction and oxidation of steroid hormones requiring NAD+/NADP+ as acceptors and their reduced forms as donors of reducing equivalents and they do not exhibit any substrate specificity (Bogovich and Payne, 1980; Payne and Saughnessy, 1996; Saez, 1994; Payne and Hales, 2004). One of the major differences between the P₄₅₀ enzymes and the hydroxysteroid dehydrogenases is that each of the P₄₅₀ enzymes is a product of a single gene, whereas there are several isoforms for the 3β-HSDHs and 17β-HSDHs, each a product of a distinct gene. The number of isoforms or isozymes varies in different species, in tissue distribution, catalytic activity (whether they function predominantly as dehydrogenases or reductases), substrate and co-factor specificity, and subcellular localization. 3β-HSDs/isomerases are membrane-bound enzymes and are distributed to both mitochondrial and microsomal membranes depending on the type of cell in which they are expressed (Samuels et al., 1951; Tamaoki, 1973; Sulmouici et al., 1973).

The presence of Δ5-3β-HSD and 17β-HSD has been demonstrated histochemically in the human, mouse, and pig.
fetal testis (Hitzemen, 1962; Baillie, 1965; Baillie and Griffiths, 1964b; 1965b; Baillie et al., 1964 a, b; Hart et al., 1966; Dufour, 1967; Moon and Raeside, 1972; Orth and Weisz, 1980). Specific studies on testis has also been reported in various mammals such as mouse, human, guinea pig, gerbil, rat, goat, pig, stallion, boar, ram, bull (Wattenberg, 1958; Pearson and Grose, 1959; Baillie, 1961; Niemi and Ikonen, 1961; 1963; Baillie and Griffiths, 1964a; Baillie and Griffiths, 1965 a,b; Baillie and Mack, 1966; Mear, 1965; Hay and Deane, 1966; Bilaspuri, 1978; Bara, 1979; Chandrakala and Sarkar, 1980; 1981; Bilaspuri and Guraya, 1984a; Kurosumi et al., 1986; Slavsky and Clavey, 1992; Neumann et al., 1993; Shan et al., 1993; Haider and Servos, 1998; Sakurai et al., 2006) but studies related to bats are limited and were reported only in *M. lucifugus* (Baillie et al., 1966b; Gustafson, 1975; 1976; 1979) and *V. pipistrellus* (Saidapur, 1976). Furthermore, as there is discernible difference in the intensity of the enzymes throughout the year suggestive of their exclusive role in the complex process of steroidogenesis indicated by heavy formazan deposits at the time of breeding and sudden depletion at mating phase.

REFERENCES


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