THE EFFECT OF AGE ON THE ACTIVITY OF CHOLESTEROL SIDE-CHAIN CLEAVAGE IN RAT TESTIS

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SYNOPSIS

The enzymatic activity of side-chain cleavage of cholesterol and 20α-hydroxycholesterol in rat testes aged 3 week to 20 month old was determined. The activity of cholesterol side-chain cleavage was low in testes of the 3 week old rats. The activity then increased rapidly as animals matured and reached the highest level at 4 months. Thereafter, the activity remained constant throughout the experimental period. Changes in the activity of 20α-hydroxycholesterol side-chain cleavage exhibited nearly the identical pattern as that of cholesterol side-chain cleavage.

Cleavage of the cholesterol side-chain to yield pregnenolone seems to be an important step in the biosynthesis of androgens by testicular tissue, since it has been reported that gonadotrophic hormones regulate this step. The histological examination of the 20 month old testes revealed very little spermatogenesis in these tissues. Nevertheless the activity of cholesterol side-chain cleavage remained at the level of the 4 month old rats. These results suggest that the availability of pregnenolone for androgen biosynthesis is almost constant between young matured rats and aged ones. Axelrod and Snipes have reported a decline in activities of C-17, 20-desmolase and 17β-hydroxysteroid dehydrogenase in testes of aged subjects. Thus, it may be possible that the regulation of steroid hormone biosynthesis in testes of aged animals is controlled by mechanisms other than the side-chain cleavage of cholesterol.

There have been many investigations regarding urinary excretions of 17-ketosteroids, testosterone and gonadotrophic hormones, in humans, to evaluate the effect of aging on the functional state of the testis (Dirgemanse et al., 1934; Goldzieher and Goldzieher, 1953; Hamberger et al., 1945; Kubos, 1934; Robinson, 1947). However, few studies have been concerned with the direct measurement of the activity of the enzymes involved in steroid hormone biosynthesis in testes of young and old subjects. Axelrod (1965) reported decreased desmolase activity of 17α-hydroxyprogesterone in the testes of old human subjects. Linder (1959) reported that the concentration of androstenedione greatly exceeded that of testosterone in testes of calves while the reverse relationship was found in testes of matured bulls. Snipes et al. (1965) observed the inconsistent difference in progesterone disappearance and 17α-hydroxyprogesterone appearance in testes from guinea pigs of different ages.

Cleavage of the cholesterol side-chain to yield pregnenolone seems to be an important step in the biosynthesis of androgens by testicular tissues, since it has been reported

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that gonadotrophic hormones regulate the side-chain cleavage of cholesterol in testes of rats (Menon et al., 1965). Thus, it is of interest to determine the activity of cholesterol side-chain cleavage in testes of rats of various ages.

MATERIALS AND METHODS

Male rats of Wistar strain were maintained on a standard rat laboratory chow *1 (Funabashi Farm Co., Chiba) in a room of constant temperature (27°C) and humidity (60%). Water was given ad libitum. At scheduled intervals several rats were sacrificed by exsanguination. Testes were quickly excised, decapsulated and homogenized in 6 volumes of ice-cold 0.154 M KCl. The enzymatic activities related to the side-chain cleavage of cholesterol and 20α-hydroxycholesterol, in the homogenates, were assayed by measurements of 14C-radioactivity in isocaproic acid liberated during the incubation period. The incubation mixture for the assay of the activity of cholesterol side-chain cleavage consisted of 1.0 ml of homogenate, 400,000 cpm of 26-14C-cholesterol (specific activity, 14 mCi/mmole, New England Nuclear Corp., Boston, Mass.), 1.0 mole of reduced nicotine-adenine dinucleotide phosphate (NADPH, Sigma Chemical Co., St. Louis, Mo.), 20 μmoles of freshly neutralized KCN and 125 μmoles of phosphate buffer (pH 7.4) in a final volume of 2.5 ml. Incubations were carried out at 37°C in air for 1.5 hrs. To determine the activity of 20α-hydroxycholesterol side-chain cleavage 15,000 cpm of 22-14C-20α-hydroxycholesterol (specific activity, 1.0 mCi/mmole, generously supplied by Dr. K. Shimizu, Tottori University), 1.0 mmole NADPH, 20 μmoles of neutralized KCN and 125 μmoles of phosphate buffer (pH 7.4) were added to 0.7 ml of the homogenate to a final volume of 2.5 ml. Incubations were carried out at 37°C in air for 1 hr. Measurements of 14C-isocaproic acid, liberated during the incubation period, were carried out by the method described previously (Ichii et al., 1963). In preliminary experiments, the above concentration of NADPH was found to saturate the requirement of the homogenate for the full activities of the side-chain cleavage reactions.

The radioactive materials, 26-14C-cholesterol, 22-14C-20α-hydroxycholesterol and 7α-3H-cholesterol, were purified, before use, by paper chromatography in ligroin:propylene glycol and Bush A system.

Mitochondrial cholesterol was determined by the method of Mann (1961) with 7α-3H-cholesterol (specific activity 5 Ci/mmole, New England Nuclear Corp., Boston, Mass.) used for recovery correction.

RESULTS AND DISCUSSION

The activity of side-chain cleavage of 26-14C-cholesterol and 22-14C-20α-hydroxycholesterol was determined in testes of rats, 3 weeks to 20 months of age. The results are depicted in Figures 1 and 2, respectively. The average activity of side-chain cleavage of cholesterol in testes of the 3 week old rats was approximately 30% of the maximum level. The activity subsequently increased to 60% of the maximum by 7 weeks. Testes of the 4 month old rats exhibited the maximum activity in side-chain cleavage. This maximum activity was maintained throughout the experimental period. The 20 month old rats exhibited the maximum activity in side-chain cleavage. This maximum activity was maintained throughout the experimental period. The 20 month old rats exhibited some external features of senile decay such as loss of hairs. Approximately one fourth of these animals died between 18 and 20 months probably from senile weakness. The histological examination of these testes revealed very little spermatogenesis, while the activity

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* The laboratory chow has been used in the authors’ institute for the past 7 years and has proved to be suitable for growth and breeding.

Trivial names used; pregnenolone, 3β-hydroxy-preg-5-en-20-one; androstenedione, androst-4-en-3, 17-dione; testosterone, 17β-hydroxy-androst-4-en-3-one; progesterone, pregn-4-en-3, 20-dione.
Fig. 1. Side-chain cleaving activity of cholesterol in rat testes of various ages.

Horizontal bars in the figure indicate mean values of the activity. Statistically significant differences (p<0.05) were observed between the activity of the 3 week old testes and those of all others, and between the activity of the 7 week old and those of 4 and 10 month old. No statistically significant differences (p>0.05) were observed between the activity of the 5 week old testes and those of 7 and 11 weekold, and between the activity of 11 week old and those of 4, 10, 15 and 20 month old.

of cholesterol side-chain cleavage remained at the level of the 4 month old rats. A similar tendency was observed when the activity of side-chain cleavage was determined using 22-14C-20α-hydroxycholesterol as the substrate (Fig. 2).

The method for the determination of cholesterol side-chain cleaving activity used here may be influenced by the presence, in mitochondria, of endogenous substrate which dilutes the added radioactive external sub-

strate. Mitochondrial free cholesterol, if not all, has been considered the substrate pool for the steroid hormone biosynthesis (Ichii et al., 1965). In the present study the concentration of mitochondrial free cholesterol was not noticeably different in rat testes of various ages (Table 1). Therefore, it would be conceivable that the endogenous substrate could be labeled by the added radioactive material to give almost the same specific activities in mitochondria obtained from rats throughout

Table 1. Amount of free cholesterol in mitochondrial fraction of rat testis of various ages

<table>
<thead>
<tr>
<th>Age (month)</th>
<th>Cholesterol (mean±S.E.)</th>
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<tbody>
<tr>
<td></td>
<td>µg/g wet tissue equivalent</td>
</tr>
<tr>
<td>1.2</td>
<td>622±49.8</td>
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<tr>
<td>2.2</td>
<td>617±27.5</td>
</tr>
<tr>
<td>14</td>
<td>714±18.6</td>
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The testicular tissue was homogenized with 2 volumes of 0.25 M sucrose. The fraction isolated by the differential centrifugation between 600×g and 8000×g for 20 mins. is referred to as “Mitochondrial fraction”. Protein was determined by the method of Lowry et al. (1951).
the experimental period. In this context, the method used in the present study for the assay of enzymatic activities could be considered to reflect the true activities of the enzyme system.

The present results suggest that the availability of pregnenolone for androgen biosynthesis would be almost constant when comparing testes in young-matured and aged rats if the endogenous NADPH level is adequate for the side-chain cleaving system of cholesterol. During the early maturation period, up to 4 months-old, the side-chain cleaving activity in rat testes increased rapidly. This paralleled the results obtained with 20α-hydroxycholesterol. The hydroxylation of cholesterol at position C-20α seems to be particularly significant in the biosynthesis of steroid hormones by endocrine organs (Shimizu et al., 1961), since it has been reported that the regulatory mechanism(s) of trophic hormones in the steroidogenesis are primarily related to this step (Ichii et al., 1964; Koritz and Hall, 1964; Menon et al., 1965). In these studies, there have been no observed effects of the trophic hormones on enzymatic processes in the biosynthetic pathway after 20α-hydroxycholesterol. Furthermore, evidence presented by Menon et al. (1965) states that the 20α-hydroxylation of cholesterol is likely to be a rate-limiting step in the over-all sequence between cholesterol and pregnenolone in rat testes. However, the nearly identical changes in the ability of rat testes to cleave the side-chain of cholesterol and 20α-hydroxycholesterol throughout the lifetime of the animals may strongly suggest that the steroid hormone biosynthesis is not regulated through the synthesis of cholesterol 20α-hydroxylase in that tissue. The authors previously reported that ACTH administered in vivo does not stimulate the activity of cholesterol side-chain cleavage in rat adrenals when the activity was estimated in vitro, in the presence of the saturated amount of NADPH (Ichii et al., 1965).

Axelrod (1965) and Snipes et al. (1965) have reported a decline in activities of C-17, 20-desmolase and 17β-hydroxysteroid dehydrogenase in testes of aged subjects. Thus, possibly, the regulation of steroid hormone biosynthesis, in testes of aged animals, is controlled by mechanisms other than that of side-chain cleavage of cholesterol. However, in both of the above cited papers, minced tissues were used for the determination of the activity without supplementing necessary co-factors, therefore, it may be necessary to carefully evaluate these results quantitatively.

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REFERENCES