SPECIAL LECTURE

Biosynthesis of Steroid Hormones in the Testis

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During the past few years we have established what appears to be the principal biosynthetic pathways to pregnenolone in various steroid-producing tissues. Cholesterol is an important precursor which may be successively hydroxylated at positions 20 α and 22R or, alternatively, the order of hydroxylation may be 22R followed by 20 α . Either pathway leads to 20 α , 22R-dihydroxycholesterol which is converted to isocaproic aldehyde and pregnenolone by an enzyme designated as pregnenolone synthetase. The present communication deals specifically with these pathways and their control in rat testicular tissue.

MATERIALS AND METHODS

Enzyme Preparations. Mature Sprague-Dawley rats were sacrificed, the testes removed and decapsulated, and placed in ice-cold 0.25 M sucrose. The following operations were carried out in a cold room at 4°; the tissue was homogenized with 3 times its weight of 0.25 M sucrose in an all glass Potter-Elvehjem type homogenizer. The homogenate was centrifuged at 700 $\times$ g for 15 minutes at 0° and the resultant supernatant fluid was centrifuged at 6,500 $\times$ g for 25 minutes to obtain the mitochondrial pellet. When indicated, this mitochondrial pellet was washed by resuspending it in 4 times its weight of 0.25 M sucrose solution and centrifuged for 15 minutes at 20,000 $\times$ g. This procedure was repeated 3 times to obtain the washed mitochondrial preparation.

The supernatant fluid obtained after the 6,500 $\times$ g centrifugation was centrifuged for 90 minutes at 105,000 $\times$ g to obtain the microsomal pellet. This pellet was washed twice by resuspending it in 4 times its weight of 0.25 M sucrose followed by centrifugation at 105,000 $\times$ g for 30 minutes.

The soluble preparation was obtained by homogenizing decapsulated tissue and centrifuging at 105,000 $\times$ g for 90 minutes.

Incubations. Unless otherwise stated, incubations were carried out at 37.5° for 2 hours under the following conditions: the enzyme preparation, equivalent to 1 gram of fresh tissue, was suspended in 2 ml of 0.033 M phosphate buffer, pH 7.2 containing 20 μ moles MgCl₂, 124 μ moles KCl, and the respective pyridine nucleotides in the quantities indicated. This suspension was added to 20 ml beakers containing substrate in 0.1 ml propylene glycol.

Determination of Isocaproic Acid Liberated. The incubations were terminated by the addition of 0.5 ml 3 N H_2SO_4 and the mixture was steam distilled. The distillate was acidified to pH 1-2 with 3 N H_2SO_4 and extracted twice with ether, and the combined ether extracts were then extracted with 0.1 N NaHCO_3 . The NaHCO_3 was acidified with 1 ml 3 N H_2SO_4 and extracted with ether.

The ether soluble-acid fraction was made basic with ethanolic KOH and transferred to scintillation vials and concentrated to dryness under a stream of nitrogen. After addition of 10 ml of scintillation media which was composed of 4 g of 2,5-diphenyl oxazole and 100 mg [1,4-bis-2-(5-phenyl oxazole) benzene] dissolved in 1 liter of toluene, one drop of glacial acid was added and the contents thoroughly shaken, and the radioactivity determined in a Packard-Tri Carb liquid scintillation counter.

RESULTS AND CONCLUSIONS

The cholesterol side-chain cleavage enzyme system of rat testis has been detected in the mitochondrial fraction of the homogenate. Pyridine nucleotides are required for the over-all reaction. Reduced triphosphopyridine nucleotide (TPNH) was the most effective co-factor and DPNH and TPN were less effective. The activity of the mitochondrial fraction appears to be related, at least in part, to the concentration of free cholesterol. Higher concentrations of free cholesterol appear to inhibit the over-all reaction.

The stimulatory effect of TPNH is inhibited by DPNH and DPN probably by inhibiting the enzymatic oxidation of pregnenolone to progesterone and the accumulated pregnenolone then inhibiting the cleavage of its immediate precursor, 20α , 22R -dihydroxycholesterol.

The rate limiting step in the conversion of cholesterol to pregnenolone by rat testis appears to be 20α -hydroxylation and it is precisely this reaction which may be influenced by gonadotrophins. For the studies leading to these conclusions 25-day old male rats were treated daily for one week with 100 I.U. HCG, after which they were sacrificed, the testes removed, and a washed mitochondrial fraction prepared from the testes homogenate. 26-C^{14} -Cholesterol and 22-C^{14} - 20α -hydroxycholesterol were incubated separately with the mitochondrial fraction and the rate of side-chain cleavage of the respective substrates determined by measuring the amount of radioactivity liberated as isocaproic acid. The testis mitochondrial fraction obtained from HCG-pretreated animals cleaved the cholesterol side-chain at a rate 3 to 6 times greater than the preparations obtained from untreated animals, but no effect of HCG pretreatment on the rate of side-chain cleavage of 22-C^{14} - 20α -hydroxycholesterol was observed. However, the rate of cleavage of 20α -hydroxycholesterol by the testes preparation of untreated animals was almost 40 times greater than observed with 26-C^{14} -cholesterol.

In a typical experiment 1.15×10^6 cpm of cholesterol- 4-C^{14} ($22 \mu\text{C}/\mu\text{M}$) was incubated in the presence of a rat testis homogenate derived from 4 g of tissue. Pregnenolone

(0.35%), progesterone (1.31%), 17 α -hydroxyprogesterone (1.31%), and testosterone (0.48%) were isolated.

Testosterone was found to be an efficient inhibitor of isocaproic acid formation from either cholesterol or 20 α -hydrocholesterol. With the former substrate 20 μ g of added testosterone produced 63% inhibition and 40 μ g of testosterone inhibited at the level of 79%. With 20 α -hydroxycholesterol as the substrate, testosterone at 20 and 40 μ g inhibited to the extent of 57 and 69%, respectively.

As little as 5 μ g of 20 α -cholesterol inhibited the formation of isocaproic aldehyde to the extent of 30% and at 40 μ g this inhibition was of the order of 90%.