DEPENDENCE OF THE GUINEA PIG KIDNEY 17β-HYDROXY-C_{19}-STEROID DEHYDROGENASE ISOENZYME PROFILE ON ANDROGEN

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SUMMARY:

The decrease in the 17β-hydroxy-C_{19}-steroid dehydrogenase activity in the guinea pig kidney cytosol after castration and the restoration after testosterone treatment was due to the disappearance and reappearance of the intermediate band of the isoenzymes obtained on acrylamide gel electrophoresis.

INTRODUCTION:

The activity of the 17β-hydroxy-C_{19}-steroid dehydrogenase of the guinea pig kidney but not that of the liver is decreased by castration and restored by testosterone treatment (1). The enzyme exhibits three strong and two faint bands on acrylamide gel electrophoresis (2). This report demonstrates that the loss in activity after castration and restoration on testosterone treatment is due primarily, if not completely, to changes in one of the strong bands.

METHODS AND MATERIAL:

The guinea pigs were purchased from O'Brien Animal Farm, Madison, Wisconsin, and a local dealer and were maintained as previously described (1). Castration was performed by the scrotal route under ether anesthesia and the testosterone was implanted subcutaneously over the shoulder as two 15 mg cylindrical pellets (3). The guinea pigs were killed by a blow at the base of the skull and bleeding by cutting the blood vessles of the neck. The kidneys were immediately removed,

chilled in crushed ice, homogenized, the cytosol separated by centrifugation and then partially purified by treatment with 1% streptomycin sulfate to remove RNA and with 40-80% saturation $(NH_4)_2SO_4$ as previously described (4).

The steroid dehydrogenase activity of the cytosol and the partially purified preparation was determined with NADP as the coenzyme (4). Protein was determined by absorbance (5) in a Cary Model 14 spectrophotometer. Acrylamide gel electrophoresis was performed by the Davis-Ornstein procedure (2,5). The developed gels were incubated for enzyme activity at room temperature in the dark for 15 minutes in a solution containing 5 ml H_2O, 2 ml 0.1 M sodium pyrophosphate pH 9.6, 0.5 ml NADP (5mg/ml), 0.25 ml phenazine methosulfate (0.25 mg/ml), 1.5 ml nitro blue tetrazolium (1.7 mg/ml) and 0.4 ml testosterone (5mg/ml methanol). The usual two faint bands were not apparent because of the short duration of the incubation period (2).

RESULTS:

The preparation from the kidneys of the normal guinea pigs exhibited three distinct bands on gel electrophoresis and staining for 17β-hydroxy-C_{19}-steroid dehydrogenase activity (Fig. 1,N). The intermediate band disappeared after 35 days of castration (Fig. 1C). Castration for 3 and 6 days did not show any difference from normal. Testosterone (Fig. 1T restored the intermediate (#2) band and at an intensity similar to that in the normal animals.

Similar results were obtained when the cytosol of the various preparations were submitted to electrophoresis. The bands, however, were diffuse and less intense because the lower specific activity of the cytosol required a longer incubation period (2).

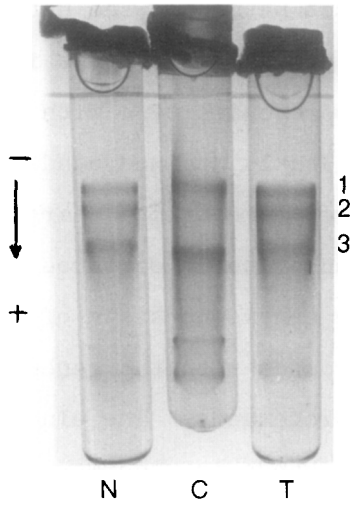

Fig. 1: Comparison of the acrylamide gel electrophoregrams of the partially purified 17β-hydroxy-C_{19}-steroid dehydrogenase activity of the kidneys of normal, castrated and testosterone treated guinea pigs. Castration was at approximately 500 g body weight and of 35 days duration, testosterone was implanted subcutaneously 21 days after castration as two 15 mg pellets and was of 14 days duration. The enzyme activities of the cytosols were 640, 404 and 591 units/gm kidney for the normal, castrated and treated animals respectively. The specific activities of the partially purified preparations (see text) were 22, 19 and 21 units/mg protein. 4.5, 3.4 and 3.4 mg protein from the normal, castrated and treated preparations respectively were used for electrophoresis. The first band is the bromphenol blue tracking dye; the following band is due to a colored impurity (probably hemoglobin).

Two further series of experiments yielded results identical to the above.

DISCUSSION:

The action of testosterone on the 17β-hydroxy-C_{19}-steroid dehydrogenase activity of the guinea pig kidney is selective. The disappearance after castration and the reappearance after testosterone of the enzyme activity is confined to the intermediate of the three strong electrophoretic bands. If the heterogenity of the enzyme is due to isoenzyme formation, then it would appear that testosterone is essential for the formation

of the hybrid. The exact nature of the heterogeneity is not known but our previous data suggests that it is an isoenzyme of the lactic acid dehydrogenase type with the presence of three strongly and two weakly stained bands of the same molecular weight but different electrical charges (2). However, the molecular weight and presence of three strongly stained bands could indicate that the enzyme consists of two subunits of similar dimers in accordance with the general proposal of Whitehead (6) for dehydrogenases. The elucidation of the exact nature of the different bands is underway. The enzyme has been purified and band 1 has been separated from bands 2 and 3 (unpublished).

We have previously suggested (1) that the guinea pig utilizes the 17β-hydroxy-C_{19}-steroid dehydrogenase as a regulator of androgenic stimulation by maintenance of a proper spectrum of androgens. The responsive tissues e.g. muscles and the accessory sex organs, of the guinea pig are stimulated only to the normal level even when excessive doses of testosterone for prolonged periods are administered (1,3,7). The protective mechanism apparently not only utilizes the production of at least one specific C_{-19}-steroid dehydrogenase, 17-β-hydroxy-C_{19}-steroid dehydrogenase, but also a special form of the enzyme.

The changes in the enzyme activity of the kidney also may be for specific local actions. The kidney shows a very small response in not only weight (3) but also certain enzymes e.g. alkaline phosphatase and arginase (8) on administration of androgens. The disappearance after castration and reappearance after testosterone of the apparently hybrid form of the isoenzyme would indicate that the kidney employs

a special mechanism for the regulation of androgen stimulated growth. The other target tissues possibly possess a similar regulatory mechanism.

The extension of these studies to the very responsive kidney of the mouse, the nonresponsive kidney of the hamster and the intermediary responsive kidney of the rat as well as other target tissues (cf 9) becomes of special interest.

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