Effects of Testosterone on the Distribution of Carnitine, Acetylcarnitine, and Carnitine Acetyltransferase in Tissues of the Reproductive System of the Male Rat*

NORMAN R. MARQUIS AND IRVING B. FRITZ

From the Department of Physiology, University of Michigan, Ann Arbor, Michigan

(Received for publication, October 22, 1964)

In an accompanying publication, we have shown that in most tissues there is an approximate correspondence between levels of carnitine acetyltransferase activity and concentrations of carnitine or acetylcarnitine (1). In addition, this correspondence may generally be related to the magnitude of the increase in fatty acid oxidation elicited by added carnitine in particular tissue preparations (1, 2). Thus heart muscle, skeletal muscle, and brown adipose tissue have high levels of transferase, carnitine, and acetylcarnitine, and mitochondria from these tissues show a large increase in long chain fatty acid oxidation in response to carnitine addition (1-3). In contrast, brain and kidney have considerably lower levels of these components and the effect of carnitine on fatty acid oxidation by particulate fractions from these tissues is less pronounced (3). Testis is an exception to both these generalizations in that carnitine acetyltransferase levels are unusually high relative to carnitine concentrations (1), and the magnitude of the metabolic response of testicular particulate fractions to added carnitine is low (3). We therefore decided to investigate testis and accessory sex tissues in more detail in an effort to determine the distribution of the components of the carnitine acetyltransferase system in various cell types. Data to be reported indicate that both transferase levels and carnitine concentrations in testis and in epididymis are under androgenic control, and that spermatozoa have the highest carnitine acetyltransferase activities of any cells thus far investigated.

EXPERIMENTAL PROCEDURE

Materials and Procedures

The same general and analytical procedures were used as those described in the accompanying publication (1). Testosterone propionate (Perandren) was obtained from Ciba Pharmaceutical Products, Inc.

Preparation of Animals and Tissues

Tissues were taken from fed male rats of the Sprague-Dawley strain. Adult rats signify animals 10 to 14 weeks old, weighing between 240 and 350 g; immature rats signify animals 5 weeks old, weighing between 90 and 110 g; and baby rats signify animals 2 weeks old, weighing approximately 30 g.

Castrate and cryptorchid rats were 5 weeks old at the time of operation. Cryptorchid rats were prepared by displacing the testes and epididymides to the abdominal cavity, sectioning the gubernaculum and closing by suturing the inguinal canals. One group of castrate rats received no androgen replacement therapy for a period of 3 weeks following the operation. A second group of castrate animals received daily subcutaneous injections of 0.2 mg of testosterone propionate for a period of 18 days beginning the 3rd day after operation. A third group, designated castrate regenerates, received no treatment for 3 weeks, followed by injections of 0.2 mg of testosterone propionate daily for 18 days. Cryptorchid rats were killed on the 18th day following the operation, at which time it was confirmed that the testes remained within the abdominal cavity.

Rat epididymal seminal fluid was obtained by cutting the epididymis where it joined with the vas deferens, and then milking the epididymis of fluid by scraping and collecting at the exposed surfaces with a glass rod which had been warmed at 37°. The viscous fluid was then dispersed slowly by stirring in 1 ml of 0.9% NaCl maintained at 37°. The epididymal sperm were separated from seminal fluid by centrifugation at 1000 X g for 5 minutes, and the residual sperm were washed several times with 1-ml portions of 0.9% NaCl. The sperm that sedimented after the last wash were then extracted and analyzed for carnitine, acetylcarnitine, and carnitine acetyltransferase as previously described for other tissues (1). The pooled supernatant fractions, termed "sperm-free epididymal fluid," were assayed directly. Epididymal sperm obtained in this way when viewed microscopically showed a high degree of motility. Epididymal fluid was free of sperm or cell debris.

RESULTS

Carnitine, Acetylcarnitine, and Carnitine Acetyltransferase Levels in Reproductive Tissues of Adult Rats

Carnitine concentrations in epididymal fractions (Table I) were exceedingly high; the caudal epididymus showed a concentration approximately 17 times that reported for rat heart (4). Greatest tissue concentrations of acetylcarnitine found thus far were also in caudal epididymal extracts.

Highest carnitine acetyltransferase specific activities were ob-
tained from isolated sperm, and it therefore appears likely that spermatozoa in epididymis and testis account for part of the transferase activities of these two tissues. Spermatozoa, while rich in carnitine and enzyme, had no detectable acetyl carnitine. Epididymal fluid (supernatant fractions) contained both carnitine and acetyl carnitine in relatively large amounts, but were free of enzymatic activity (Table I).

Concentrations of both carnitine and acetyl carnitine increase in the following order: testis, caput epididymis, and caudal epididymis. Carnitine and transferase present in vas deferens extracts may have originated from sperm and epididymal fluid stored in the duct.

**TABLE I**

<table>
<thead>
<tr>
<th>Region</th>
<th>Carnitine</th>
<th>Acetyl carnitine</th>
<th>Carnitine acetyl transferase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis</td>
<td>1.74 ± 0.10</td>
<td>0.54 ± 0.10</td>
<td>146 ± 17</td>
</tr>
<tr>
<td>Caput epididymis</td>
<td>10.8 ± 1.8</td>
<td>0.99 ± 0.15</td>
<td>112 ± 6.4</td>
</tr>
<tr>
<td>Caudal epididymis</td>
<td>83.5 ± 7.5</td>
<td>4.7 ± 0.44</td>
<td>95.2 ± 8.8</td>
</tr>
<tr>
<td>Epididymal fluid*</td>
<td>255 ± 37</td>
<td>13.3 ± 0.81</td>
<td>50.0</td>
</tr>
<tr>
<td>Epididymal sperm</td>
<td>13.0 ± 1.0</td>
<td>0.0</td>
<td>276 ± 14</td>
</tr>
<tr>
<td>Vas deferens</td>
<td>15.0 ± 0.19</td>
<td>0.0</td>
<td>31.4 ± 3.7</td>
</tr>
</tbody>
</table>

* In epididymal supernatant fraction the levels are expressed as micromoles per g dry weight, of sperm sedimented from the total epididymal fluid.

**TABLE II**

Carnitine concentrations and content in testes and epididymides from rats of varying ages and degrees of testicular function

Tissue extracts were prepared as described in "Experimental Procedure." Carnitine concentrations were determined spectrophotometrically at 412 mµ. Values are given as the mean ± the standard deviation, and n represents the number of separate animals analyzed.

**TABLE III**

Carnitine acetyl transferase levels in testes and epididymides of rats

Tissues were treated as previously described in "Experimental Procedure." Carnitine acetyl transferase activity was determined under conditions cited in Table I. Values are given as the mean ± the standard deviation, and n represents the number of separate animals analyzed.
Carnitine could not be detected in extracts from epididymides of 2-week-old rats, or from epididymides of older untreated castrated animals (Table III). Treatment with testosterone restored carnitine concentrations in epididymides to concentrations found in normal adult and cryptorchid rats (Table II). Testosterone treatment increased carnitine acetyltransferase activities of epididymides from castrate rats to levels comparable with those in 5-week-old or cryptorchid rats but below those of tissues from adult animals (Table III). These findings show a considerable increase in both transferase and carnitine concentrations in epididymides during maturation of normal rats, and indicate that the increases are under the influence of testosterone.

**DISCUSSION**

The composition of testis and accessory glands of various mammalian species has been extensively reviewed (6, 7). Since various secretions from glands of the reproductive tract are known to be rich sources of many nitrogenous compounds, such as choline, glycerylphosphorylcholine, and ergothionine, it is perhaps not too surprising that carnitine may be added to the list. No other tissues examined thus far have been found to contain concentrations of free carnitine as high as that found in caudal epididymis or in epididymal fluid free of sperm. Similarly, no tissue begins to approach in carnitine acetyltransferase activity that observed in isolated rat spermatozoa. Such high levels suggest that carnitine, acetylcarnitine, and the transferase may play an important function in spermatozoan metabolism. Aside from a possible role in acetyl group transfer discussed previously (2, 8), no known function for the carnitine acetyltransferase system exists.

The progressive increase in carnitine concentrations of testis and epididymides during maturation of rats may be correlated with the differentiation of these organs. Reid and Cleland (9) have shown that rat epididymides are relatively undifferentiated up to 3 weeks of age. There follows complete differentiation of the head by the 5th week and the tail by the 14th week. Clermont and Pecy (10) have shown that spermatogenesis in the rat proceeds to the primary spermatocyte stage by the 3rd week, and germ cell differentiation increases rapidly thereafter. Sperm are released to the epididymis by the 8th to 9th week. The increasing carnitine and transferase levels of testis during this period; the low levels of enzyme found in epididymides of immature rats; and the high enzyme activity of epididymal sperm in the adult suggest that both carnitine and carnitine acetyltransferase are acquired by the germ cells during some phase of differentiation. Further support for this contention is provided by the significant decrease in levels of these components in the testis after cryptorchidism. It is known that the germinal epithelium of testis tubules degenerates during cryptorchidism (11, 12), with maximal degeneration in the rat occurring after 15 to 21 days (13).

The implantation of testes and epididymides in the abdominal cavity did not appear to alter epididymal carnitine concentrations very much (Table II). This is consonant with findings that sufficient androgens are elaborated by the cryptorchid testis to permit approximately normal function of accessory glands (11, 14, 15). In contrast, castration lowered epididymal carnitine concentrations to nondetectable levels and testosterone administration in large part prevented the decrease (Table II). It may therefore be concluded that androgens permit epididymal tissue, in the absence of a source of spermatocytes, to accumulate carnitine. Other investigators have shown that androgens are required to maintain levels of fructose (16), citric acid (17), and glycerophosphorylethanolamine (18) in various accessory glands.

Carnitine acetyltransferase activities in epididymides were directly correlated with levels of testicular function (Table III). While activities were down in immature and cryptorchid rats, lowest enzyme levels were obtained in epididymal tissue from castrated animals. Testosterone administration to castrated rats increased transferase activities only slightly (Table III). Failure to obtain larger increases indicates that epididymal tissue itself did not synthesize appreciable amounts of enzyme in response to androgen injection. It appears likely that high carnitine acetyltransferase levels in epididymides of adult rats reflect primarily enzyme in spermatozoa stored within the epididymides. It may therefore be concluded that both testosterone and a source of sperm cells are required to maintain high carnitine acetyltransferase levels in epididymal tissue, while only testosterone is required to maintain maximal carnitine concentrations.

Insufficient evidence is available to indicate whether the high carnitine content in epididymides arises from synthesis de novo, or from accumulation of existing carnitine by epididymal epithelial cells. High carnitine concentrations in epididymal fluid suggest that carnitine may be secreted by epithelial cells of the epididymal tubule. It will be of interest to determine whether epididymal tissue is capable of carnitine biosynthesis.

**SUMMARY**

Levels of carnitine, acetylcarnitine, and carnitine acetyltransferase were analyzed in reproductive system tissues obtained from male rats of varying ages. Spermatozoa had highest specific activities of transferase thus far observed in tissues, and it was concluded that at least part of the enzyme found in testes and epididymides was contributed by sperm. Caudal epididymides of adult rats contained 83.5 μmoles of carnitine and 4.7 μmoles of acetylcarnitine per g, dry weight. These concentrations were an order of magnitude greater than those reported previously for heart muscle, which in turn had higher levels than other rat tissues. Androgens were required to permit maintenance of normal carnitine and carnitine acetyltransferase levels in testes and epididymides. Carnitine concentrations and transferase activities in tissues of the reproductive tract increased during a period of growth associated with rapid differentiation of epididymal and germ cells. Effects of cryptorchidism and castration were reported. It was shown that lowered epididymal levels of carnitine in castrated rats could be restored to normal by administration of testosterone. In contrast, lowered epididymal levels of carnitine acetyltransferase in castrated rats were increased only slightly following testosterone injection. It was concluded that both testosterone and a source of sperm cells were required to maintain high carnitine acetyltransferase levels in epididymal tissue, while only androgens were required to maintain maximal carnitine concentrations in this tissue.

**REFERENCES**

Effects of Testosterone on the Distribution of Carnitine, Acetylcarnitine, and Carnitine Acetyltransferase in Tissues of the Reproductive System of the Male Rat

Norman R. Marquis and Irving B. Fritz