Sertoli Cells Produce Vitamin-Binding Proteins

PETER M. FETTEROLF AND MICHAEL K. SKINNER

Department of Pharmacology
Vanderbilt University School of Medicine
Nashville, Tennessee 37232

The seminiferous tubule in the rat testis is composed primarily of three cell types, Sertoli cells, peritubular myoid cells, and germinal cells in various stages of development. Tight junctions near the basal surfaces of adjacent Sertoli cells contribute to a blood-testis barrier, which creates a unique microenvironment within the tubule. The barrier effectively blocks passage of small and large molecules from plasma into the seminiferous tubule compartment formed by Sertoli cells.[1] Consequently, plasma constituents required for most stages of germinal cell development apparently must first pass through Sertoli cells. One approach to understanding the role of Sertoli cells in the maintenance and control of spermatogenesis is to determine the function of proteins secreted by the cell and then to monitor or to manipulate their function under various conditions. For example, Sertoli cells produce testicular transferrin[2] and testicular ceruloplasmin,[3] which specifically bind components (iron and copper, respectively) that are believed essential for germinal cell development.

Vitamins are required for proper metabolic function and consequently germinal cell development. Vitamin deficiencies can have dramatic effects on the morphology of the seminiferous tubule and a negative effect on spermatogenesis.[4] Vitamins that reach the inside of cells from plasma are quickly bound to proteins. Free vitamins would therefore be unlikely to exist in high concentrations in Sertoli cell cytosol and could not readily diffuse through the plasma membrane to developing germ cells. Further, the blood-testis barrier probably prevents diffusion of vitamins directly from the bloodstream to developing germinal cells. A logical, testable, hypothesis is that Sertoli cells synthesize and secrete proteins that bind and transport vitamins to germinal cells. Preliminary results are reported that suggest that Sertoli cells in serum-free primary monoculture secrete a substance that binds riboflavin with a high affinity.

Twenty-day-old rats were killed by cervical dislocation, the testes were removed, and Sertoli cells were prepared.[5] Serum-free medium was collected every 48 or 72 hours and concentrated by ultrafiltration with membranes having a molecular weight exclusion limit of 10,000. The medium (Hams F12, Gibco) contained substantial riboflavin that was removed using dextran-coated charcoal to permit competitive binding assays.

Riboflavin-binding activity was detected using the described competitive assay (TABLE 1). In conditioned medium, the activity was saturable and exhibited a dissociation constant (K_d) of approximately 6.8×10^{-8} M. Charcoal-treated conditioned medium applied to 3-*N*-carboxymethylriboflavin affinity gel[8] contained a substance that bound to the column. The substance was at least 95% pure by the criterion of a silver-stained electrophoretic gel and had an approximate molecular mass of 14,000 daltons (data not shown). Binding activity was retained after elution from the affinity column (TABLE 1).

TABLE 1. Results of Competitive Assays for Riboflavin-Binding Protein in Crude Preparations and in 3-Carboxymethylriboflavin-Affinity Column Eluate from Conditioned Medium and Chicken Egg White (Sigma)[a]

	Conditioned Medium		Chicken Egg White	
	Crude	Affinity Eluate	Crude	Affinity Eluate
Mean	.38	1428	.76	964
SE[b]	.04	211	.12	186
N[b]	9	4	3	3

[a] Data are specific activity expressed as a percent of control and standardized by dividing by the amount of protein (μg) in the sample. Protein in samples was estimated using a Bradford[6] assay in crude samples and from semiquantitative estimates with silver-stained electrophoresis gels on the affinity column eluate. Chicken egg white contains a riboflavin-binding protein and was used to test the efficacy of the competitive assay and the affinity column.[7] [^{14}C]riboflavin (Amersham) was used to detect binding activity in conditioned medium by incubating samples in 96-well nitrocellulose plates (Millipore) with or without the nonradioactive ligand in 100-fold excess. Parallel controls used the same procedure but with fresh medium substituted for conditioned medium. After equilibration, the binding activity (putative protein) was separated from unbound ligand by absorption onto the nitrocellulose. After washing with ice-cold saline, the radioactivity on the nitrocellulose was quantified by liquid scintillation counting. Assays were optimized for equilibration time and temperature.

[b] Binding activity/μg total protein; SE = standard error of the mean; N = sample size.

In summary, the results suggest that Sertoli cells may synthesize and secrete a riboflavin-binding protein that may be involved in the delivery of riboflavin to developing Sertoli cells. Preliminary evidence not presented here suggests that a biotin-binding protein is also synthesized and secreted by these cells.

REFERENCES

1. SETCHELL, B. P. & G. M. H. WAITES. 1975. *In* Handbook of Physiology. D. W. Hamilton & R. O. Greep, Editors. Vol. V(7): 143–172. American Physiological Society. Washington, D.C.
2. SKINNER, M. K. & M. D. GRISWOLD. 1980. J. Biol. Chem. **255:** 9523–9525.
3. SKINNER, M. K. & M. D. GRISWOLD. 1983. Biol. Reprod. **28:** 1225–1229.
4. HAMMER, R. E. & E. S. E. HAFEZ. 1981. *In* CRC Handbook of Nutritional Requirements in a Functional Context. M. Rechcigl, Ed., Vol 1: 397–425. CRC Press. Boca Raton, FL.
5. TUNG, P. S., M. K. SKINNER & I. B. FRITZ. 1984. Biol. Reprod. **30:**199–221.
6. WHITE, H. B. 1986. Methods Enzymol. **122:**221–227.
7. BRADFORD, M. 1976. Anal. Biochem. **72:** 248–252.
8. MERRILL, A. H. & D. B. MCCORMICK. 1978. Anal. Biochem. **89:**87–102.