0013-7227/91/1282-1167$03.00/0
Endocrinology

Vol. 128, No. 2
Printed in U.S.A.

Basement Membrane Increases G-Protein Levels and Follicle-Stimulating Hormone Responsiveness of Sertoli Cell Adenylyl Cyclase Activity*

MARTIN DYM, SUPANYA LAMSAM-CASALOTTI, MENG-CHUN JIA†, HYNDA K. KLEINMAN, AND VASSILIOS PAPADOPOULOS

Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research, National Institutes of Health (M.D., H.K.K.), Bethesda, Maryland 20892; and the Department of Anatomy and Cell Biology, Georgetown University Medical Center (M.D., S.L.-C., M.-C.J., V.P.), Washington, D.C. 20007

ABSTRACT. On a basement membrane substrate, Sertoli cells in culture have been shown to assume a phenotype similar to that of the *in vivo* differentiated cells. Sertoli cells from 10-day-old rats were cultured on plastic and on different extracellular matrix substrates [laminin, a reconstituted basement membrane (Matrigel), and a synthetic laminin peptide containing the arginine-glycine-aspartic acid (RGD) tripeptide sequence] to investigate the effects of the extracellular matrix on FSH responsiveness. Both laminin and Matrigel markedly enhanced the cAMP response to FSH and cholera toxin, indicating modifications at the level of guanine nucleotide-binding regulatory (G) proteins. Furthermore, Sertoli cells grown on either of these two substrates responded to physiological levels of FSH (25–50 ng/ml), whereas pharmacological levels of FSH (500 ng/ml) were required for cells grown on either plastic or on the RGD-containing laminin peptide. Immunoblotting of Sertoli cell plasma membranes with antibodies directed against the α-subunit of the stimulatory G-protein (Gsα) of adenylyl cyclase indicated that Sertoli cell culture on either laminin or Matrigel increased the amounts of Gsα. These results were further confirmed by immunoprecipitating the Gsα protein from the particulate fraction of [^{35}S]methionine metabolically labeled Sertoli cells. However, Northern blot analysis using a cDNA probe for Gsα did not demonstrate changes in gene expression when Sertoli cells were grown on the various substrates. Immunofluorescent studies revealed that the Gs complex of adenylyl cyclase was preferentially located at the base of the Sertoli cells at the site of contact with the extracellular matrix. These data suggest that culture of epithelial Sertoli cells on basement membrane substrates enhances the Gs complex of adenylyl cyclase and the cAMP response to FSH, consistent with the more differentiated morphology and function of the cells. (*Endocrinology* **128**: 1167–1176, 1991)

INCREASING evidence demonstrates that extracellular matrix can influence cell adhesion, shape, polarity, growth, migration, metabolism, and differentiation *in vitro* (1, 2). In the 10-day-old developing testis, the seminiferous cords consist of early germ cells and Sertoli cells (3), which are tall columnar cells with a highly polarized cytoplasm (Fig. 1). Sertoli cells are in contact at their basal surface with a basement membrane matrix composed of laminin, type IV collagen, heparan sulfate proteoglycan, and possibly entactin (4). When Sertoli cells from 10-day-old rats are cultured on plastic, they exhibit a squamous morphology and generally lack the polarity observed *in vivo*. On a laminin substrate, the Sertoli cells become somewhat more differentiated and assume a cuboidal to low columnar shape (Fig. 1). However, when cultured on a Matrigel substrate, the Sertoli cells adopt a phenotype very similar to that of the *in vivo* 10-day-old Sertoli cells. They appear tall and columnar and are highly polarized with basally located nuclei and abundant supranuclear cytoplasm (5) (Fig. 1). Sertoli cells cultured on Matrigel also secrete more total protein and more androgen-binding protein and transferrin than cells grown on plastic (5). Little is known about the intracellular signals involved in the Sertoli cell response to basement membrane. In fact, the signal mechanisms for cellular responses to extracellular matrix components in most other cell systems have not been adequately studied.

Sertoli cell function is under the control of the gonadotropin FSH (6, 7). FSH binding to plasma membrane receptors stimulates adenylyl cyclase and, subsequently, cAMP production, the postulated second messenger of FSH action in Sertoli cells (6, 7). Activation of adenylyl cyclase by the FSH-receptor complex is mediated by the Gs protein (8). The exchange of GDP to GTP at the

Received August 30, 1990.
Address correspondence to: Dr. Vassilios Papadopoulos, Department of Anatomy and Cell Biology, Georgetown University School of Medicine, 3900 Reservoir Road NW, Washington, DC 20007.
* This work was supported by Grant HD-16260 from the NIH and a grant from the Mellon Foundation.
† WHO research training grant recipient.