Sertoli Cell Adenylyl Cyclase Is Stimulated by a Factor Associated with Germ Cells

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It is not yet apparent how Sertoli cell functions are regulated to coordinate and support differentiation of the adjacent germ cells (GC) in the seminiferous tubules of the testis. Studies have shown that FSH binding and response by cells of the seminiferous tubule,[1,2] as well as activities of specific enzymes,[3] can be correlated with the stage of spermatogenesis existing within isolated tubule segments. Because the hormonal milieu of the testis is essentially constant, these observations support the hypothesis of a mechanism within the seminiferous tubule for localized regulation of morphological and biochemical events.[3] GC may directly affect Sertoli cell function by essentially the same series of biochemical events as the FSH response mechanism.[1,3] To examine the possibility of direct GC effects on Sertoli function, we have studied the effects of GC and GC fractions of adenylyl cyclase (AC) activity in membrane preparations from highly enriched populations of rat Sertoli cells. We observe stimulation of AC by GC and GC fractions.

Basal AC activity of membranes from Sertoli cells cultured for three days in hormone and serum-free medium was found to be low, ranging from 2 to 14 pmoles cAMP/min/mg protein. When the Sertoli membranes were treated with FSH or isoproterenol in the presence of 1 μM GTP, conditions that elicit maximal AC responses, AC was stimulated to average rates of 104 and 76 pmoles cAMP/min/mg, respectively (FIGURE 1a). If freshly prepared GC (50–150 μg protein/assay) instead of hormone were added to Sertoli membranes, AC activity was measured to average 62 pmoles cAMP/min/mg Sertoli protein. When GC alone were assayed, they were found to exhibit no AC activity under the conditions employed. GC were also separated into membrane and cytosol fractions. Both fractions were able to stimulate AC activity of Sertoli membranes to levels significantly higher than basal (FIGURE 1b). The results did not offer any clear evidence concerning the subcellular localization of the GC factor that was activating Sertoli AC.

The possibility of the activation being due to calmodulin was considered. Because calmodulin has been shown to be heat stable with a half-life of 7 min at 100°C,[4] the effect of heat on the GC fractions' ability to activate Sertoli AC was examined (FIGURE 1c). Treatment at 60°C did not diminish the capacity of GC preparations to activate AC. However, 100°C for 1 min abolished completely the ability of GC preparations to activate AC. The results indicate that the stimulatory activity associated with the GC possesses limited heat stability and cannot be attributed to the heat-stable activator calmodulin.

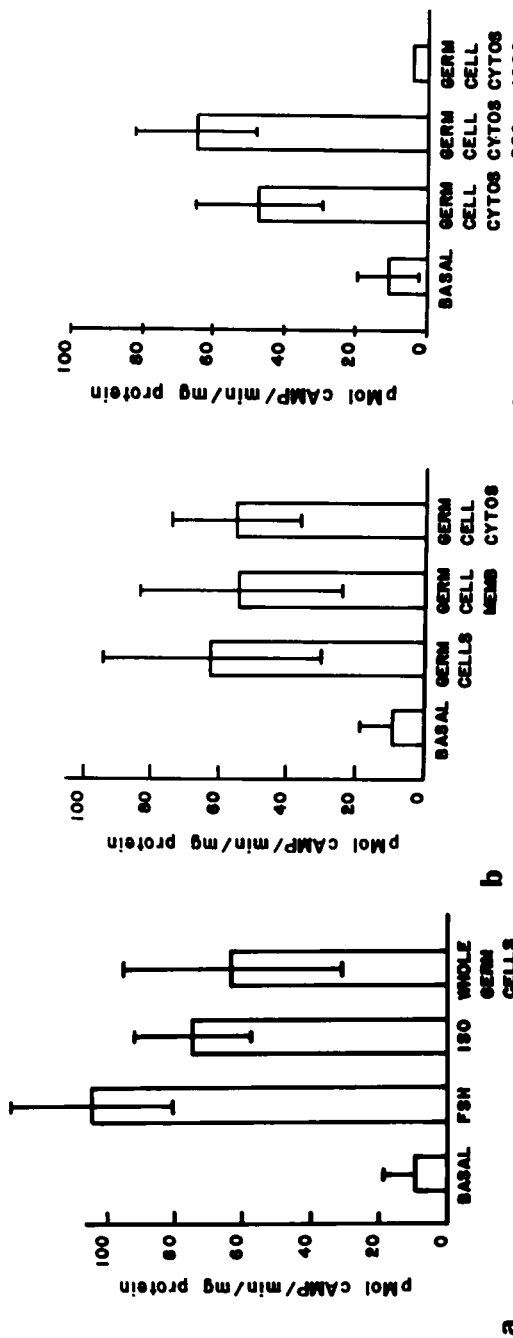

FIGURE 1. (a) Stimulation of Sertoli AC by hormones or whole GC. With all treatments AC activities were significantly higher than basal. (b) Stimulation of Sertoli AC by whole GC or GC fractions. Germ cells, 30,000 × *g* pellet or supernatant fractions stimulated Sertoli AC significantly. (c) Heat stability of GC stimulation of Sertoli AC. Unheated and 60°C-treated GC cytosol fractions significantly stimulated Sertoli AC.

TABLE 1. Response of TRST Adenylyl Cyclase to Various Treatments

Treatment	FSH (5 μg/assay)	Isoproterenol (10 μM)	Whole Germ Cells
Average stimulation (above basal)	Twofold	Fivefold	Threefold

The specific germ cell developmental stage that possesses the most potent ability to stimulate Sertoli AC is not yet evident. The GC preparations used for the experiments contained spermatocytes and virtually all stages of spermatids. The GC stimulator of AC is not limited to AC from Sertoli cells, however. GC and GC fractions have been found to be able to stimulate AC in membrane fractions of the Sertoli-derived cell line TRST (TABLE 1).

Taken together, our results indicate that a factor associated with GC is able to stimulate Sertoli cell AC. The factor is heat labile and does not require calcium for activation. Therefore, the observed stimulatory activity cannot be attributed to calmodulin. The GC factor may be similar in nature to the heat-labile cytoplasmic factors that have recently been reported to be present in lung,[5] or may be similar to the AC-stimulating factor associated with a particulate fraction from bovine epididymal sperm,[6] as reported while this work was in progress.

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