Localization and characterization of an orphan receptor, guanylyl cyclase-G, in mouse testis

and spermatozoa

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摘要.

We recently identified a novel testis-enriched receptor guanylyl cyclase (GC) in the mouse, designated mGC-G. To further investigate its protein expression and function, we generated a neutralizing antibody specifically against the extracellular domain of this receptor. RT-PCR and immunohistochemical analyses show that mGC-G is predominantly expressed from round spermatids to spermatozoa in mouse testis at both the mRNA and protein levels. Flow cytometry and confocal immunofluorescence reveal that mGC-G is a cell surface protein restricted to the plasma membrane overlying the acrosome and midpiece of the flagellum in mature sperm. Interestingly, Western blot analysis demonstrates that testicular mGC-G is approximately 180 kDa but is subject to limited proteolysis during epididymal sperm transport, resulting in a smaller fragment tethered on the mature sperm surface. On Fluo-3 cytometrical analysis and computer-assisted sperm assay, we found that serum albumin-induced elevation of sperm intracellular Ca(2+) concentration, protein tyrosine phosphorylation, and progressive motility associated with capacitation are markedly reduced by preincubation of the anti-mGC-G neutralizing antibody. Together, these results indicate that mGC-G is proteolytically modified in mature sperm membrane and suggest that mGC-G-mediated signaling may play a critical role in gamete/reproductive biology.