NEW ASPECTS OF LEYDIG CELL FUNCTION

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1. SUMMARY

Previous studies indicated that the Leydig cells of the human testes show similarities to neuroendocrine cells. In this context, the local synthesis of two neuroactive signaling molecules, namely nitric oxide (NO) and C-type natriuretic peptide (CNP), both acting via the second messenger, cyclic guanosine monophosphate (cGMP), might be of physiological relevance. By immunoblotting, immunohistochemical analyses and affinity crosslinking experiments, respectively, the presence of soluble guanylate cyclase (sGC), the NO receptor, and of guanylate cyclase B (GC-B), representing the CNP receptor, was demonstrated in Leydig cells, seminiferous tubules and blood vessels of the human testis. Moreover, cGMP and its binding protein cGMP-dependent protein kinase type I (GK I) were found in these structures. The functional activity of the two receptors was proved by generation of cGMP in response to treatments with the NO donor, sodium nitroprusside (SNP), and with CNP, respectively. As indicated by immunohistochemical analyses and by treatments of cells with either SNP or CNP, human Leydig tumour cells and MA10 cells, representing a mouse Leydig tumour cell line, were found to be distinguished by a reduced expression of the receptors for NO and CNP. Furthermore, expression levels of the components of the two cGMP-generating systems were found to be widely unchanged in Leydig cells during different ontogenetic stages. Though cGMP has been shown to influence testosterone release, the constant developmental expression patterns of NO and CNP apparently independent of differences in androgen production, the down-regulation of their receptors in tumorous cells, and the presence of GK I, may point to additional autocrine functions of these factors and of cGMP in Leydig cells. Moreover, possible paracrine actions of NO and CNP may include relaxation of seminiferous tubules and blood vessels in order to modulate sperm transport and testicular blood flow, respectively. These findings suggest that Leydig cell-derived factors may exert activities different from or in addition to those involved in the regulation of testosterone production.
2. INTRODUCTION

Androgen production is the primary function of testicular Leydig cells. In this context, the presence of Leydig cell-specific organelles such as smooth endoplasmic reticulum is well established (Schulze, 1984). Recently, Leydig cells of the human testis have been shown to possess cytoplasmic vesicles and storage granules similar to those found in neuroendocrine and nerve cells (Davidoff et al., 1993). Moreover, a series of nerve cell-specific substances has been detected in Leydig cells (for review see: Saez, 1994; Davidoff et al., 1997a). Two of the agents recently demonstrated in these cells (Davidoff et al., 1995; Middendorff et al., 1996), namely nitric oxide (NO) and C-type natriuretic peptide (CNP), bind to and activate guanylate cyclases, resulting in elevated intracellular levels of the second messenger cyclic guanosine monophosphate (cGMP). This factor regulates a variety of complex and hitherto not completely understood cellular functions acting through binding to different molecular targets (Lincoln & Cornwell, 1993).

CNP belongs to the family of natriuretic peptides, which also includes atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). Whereas ANP and BNP are mainly secreted by cardiac cells to act as hormones in the regulation of blood pressure and fluid volume homeostasis (for review see: Drewett & Garbers, 1994), CNP is produced and of particular physiological relevance in the brain (Komatsu et al., 1991; Minamino et al., 1993; Langub et al., 1995). NO, on the other hand, serves as a neurotransmitter in the nervous system, as a mediator of endothelium-dependent relaxation of blood vessels and mediates the tumoricidal and bactericidal actions of macrophages (Moncada et al., 1991; Bredt & Snyder, 1994; Schmidt & Walter, 1994). NO can be produced by either a neuronal (nNOS), an endothelial (eNOS) or an inducible (iNOS) isoform (Forstermann et al., 1994).

Whereas NO, which diffuses freely across membranes (Moncada et al., 1991), binds to and activates a soluble guanylate cyclase (sGC), CNP is the specific ligand of a plasma membrane receptor, designated as GC-B (Drewett & Garbers, 1994).

The question, which processes are influenced by cGMP-dependent mechanisms in the human testis, has not yet been elucidated. The data presented here, show the presence and activity of receptors for NO and CNP in the human testis. We also provide evidence that cGMP may act by interaction with cGMP protein kinase I (GK I). The occurrence of the receptors in testicular vasculature and in the peritubular lamina propria suggests relaxation of vessels and tubules in order to modulate testicular blood flow and sperm transport, respectively. Autocrine activities of NO and CNP in Leydig cells may affect cellular functions other than the production of testosterone.

3. MATERIALS AND METHODS

3.1. Isolation of Leydig Cells, Seminiferous Tubules and Testicular Blood Vessels

Testes were obtained from 11 patients aged 30 - 86 years who were undergoing orchietomy as the primary treatment of prostatic carcinoma. One to 2 h after surgery chilled human testes were cut into 4 pieces and transferred to dishes containing Ham's F12/DMEM culture medium (Gibco, Eggenstein, Germany) supplemented with 15 mM