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WE DANCE ROUND *IN A RING AND SUPPOSE, BUT THE SECRET SITS IN THE MIDDLE AND KNOWS.*
ROBERT FROST

THE C-TYPE NATRIURETIC PEPTIDE SYSTEM IN THE TESTIS: A PHYSIOLOGICAL ROLE OF NEUROACTIVE FACTORS IN LEYDIG CELLS

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• Androgen production is the primary function of testicular Leydig cells (1). Recently, Leydig cells of the human testis have been shown to possess, along with well developed smooth endoplasmic reticulum, cytoplasmic vesicles and storage granules similar to those found in neuroendocrine and nerve cells (2). Moreover, a series of nerve cell-specific substances has been detected in Leydig cells (reviewed in 3, 4). However, little is known about the physiological role of these molecules in testicular function.

One of the neuroactive substances recently demonstrated in Leydig cells is C-type natriuretic peptide (CNP) (5). CNP (6) 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 (reviewed in 7), CNP is produced and is of particular physiological relevance in the brain (8-10). This peptide, which mediates its intracellular effects by the second messenger cyclic guanosine monophosphate (cGMP), is an excellent molecule to investigate functional effects of neuroactive substances in the testis, because cGMP is activated only by a small number of messenger molecules, namely the natriuretic peptides, nitric oxide (NO) and carbon monoxide (CO) (see 7). Therefore, the demonstration of components of the cGMP pathway permits to exclude effects of all other substances.

The CNP receptor, designated as GC-B (11), represents a transmembrane protein containing an extracellular ligand-binding domain, and an intracellular guanylate cyclase domain. Whereas CNP specifically binds to GC-B, the related receptor GC-A interacts with ANP and BNP (7). Ligand binding to these receptors results in activation of guanylate cyclase, leading to an intracellular accumulation of cGMP, which in turn regulates a variety of complex, not completely understood cellular functions (12).

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AUTOCRINE C-TYPE NATRIURETIC PEPTIDE EFFECTS IN LEYDIG CELLS AND FUNCTIONALLY RELATED NEUROENDOCRINE CELLS

• Autocrine effects are characterized by production of a substance and a receptor-mediated effect of this substance on the same cell (13). These criteria are fulfilled by Leydig cell CNP as it is produced and leads to a cGMP-mediated elevation of testosterone secretion in these cells (5, 14). Synthesis of CNP could not be detected in other testicular structures (5), suggesting that autocrine CNP effects on Leydig cells are characteristic for these particular cells.

CNP is known to be associated with key neuroendocrine

structures (8, 10, 15). The highest density of CNP receptors was detected in the pineal and pituitary glands (16), while the highest CNP concentrations were found in the pituitary and hypothalamus (8). It is of special interest that in the pituitary CNP is exclusively produced by gonadotropes (17, 18), and that it exerts autocrine effects on these cells (17, 18) as well as on luteinizing hormone-releasing hormone (LHRH) neurons (19, 20). This points to an importance of local CNP effects, especially on the neuroendocrine cells which centrally regulate Leydig cell function. Recently, in granulosa cells of the ovary, which had also been addressed a neuro endocrine organ (21), a local CNP system has been described (22). This suggests that CNP plays a crucial local role in the hormonal control of male and female reproduction. Taken together, the functionally related cells of the hypothalamic-pituitary-gonadal axis use, at least in part, the same autocrine mechanisms to modulate their function (Fig. 1), although the hormones produced by these cells are completely different, i.e. LHRH is a peptide, LH is a glycoprotein, and testosterone is a steroid.

Apart from CNP, NO is the only molecule also known to exert autocrine effects on LHRH neurons (19, 23), gonadotropes (24, 25), and Leydig cells (26, 27). We were able to demonstrate the presence of NO synthase (NOS) and NO receptor (soluble guanylate cyclase) by both immunohistochemistry and Western blotting as well as an NO-induced accumulation

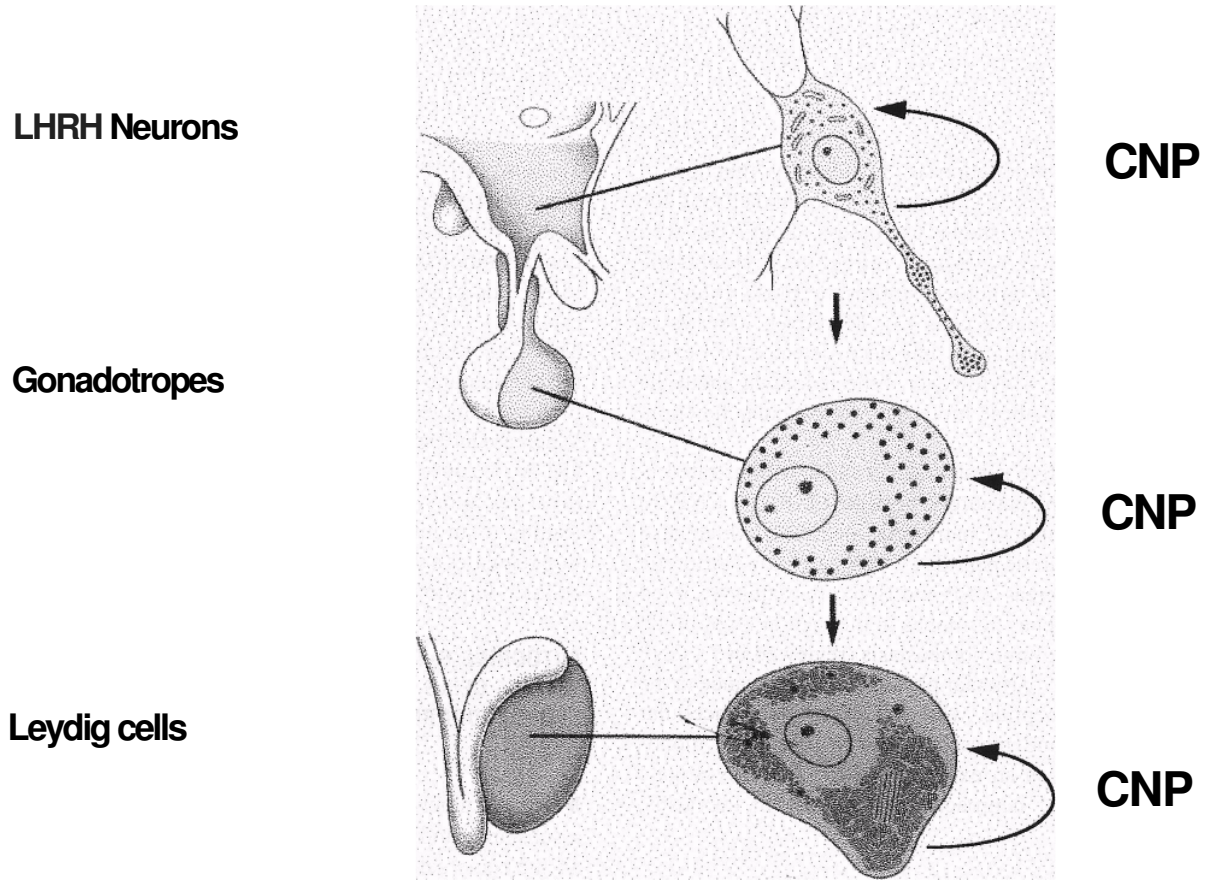

Fig. 1. Autocrine effects of CNP in Leydig cells, gonadotropes, and LHRH neurons. In these cell types CNP is produced and leads to an accumulation of the second messenger cGMP mediated by guanylate cyclase B, the receptor for CNP (5,14,17-20). Moreover, CNP induces an increase of testosterone release by Leydig cells (14), and an increase of LHRH secretion by LHRH neurons (20).

of cGMP in these cell types.

Although ANP and BNP effects on Leydig cells, gonadotropes, and LHRH neurons have been described (14, 19, 24, 28), a local production of these natriuretic peptides remained undetectable in these cells (9, 29), suggesting the absence of autocrine actions. Probably, ANP and BNP effects on target cells are elicited by blood-derived ANP and BNP (30), while autocrine mechanisms in the gonadal axis cells are characteristic of CNP (and NO), but not of cGMP-accumulating agents in general. Autocrine CNP effects have also been described in pinealocytes (31) and in adrenal gland zona glo-merulosa (32), indicating that the (neuro)endocrine CNP actions are not restricted to the gonadal axis, but are also present in other neuroendocrine and steroid-producing cells. Differences in the expression pattern of CNP among Leydig cells, gonadotropes, LHRH neurons, and pinealocytes, respectively (5,17,31), may indicate that only a subpopulation of these cell types synthesizes CNP. It is possible that CNP of a CNP-positive cell also may influence the neighbouring, perhaps CNP-negative, cells in a paracrine manner.

PARACRINE C-TYPE NATRIURETIC PEPTIDE EFFECTS IN LEYDIG CELLS AND FUNCTIONALLY RELATED NEUROENDOCRINE CELLS

• Leydig cells are close to the peritubular lamina propria (1, 33), and to testicular blood vessels (1, 34). Thus, Leydig cell-produced CNP may have direct influence on these structures. Since CNP has been found to act as a vasodilator (35, 36), mediating its effect *via* GC-B, cGMP, and apparently cGMP-dependent protein kinases (GK) (12), our finding of GC-B, cGMP, and GK I in testicular blood vessels as well as the observed CNP-mediated cGMP accumulation in these structures (37) strongly suggests a local vasorelaxant function (3 5). In both seminiferous tubules and tunica albuginea, CNP-induced cGMP (37) may mediate relaxation of myofibroblasts. Whereas endothelin, for example, has been shown to be involved in peritubular cell contraction in the rat (38), the agents responsible for relaxation have not yet been defined. Testicular myofibroblasts express filaments characteristic of fibroblasts and smooth muscle cells (33, 39). An influence of CNP on myofibroblasts may be postulated in the context of the well known effects of this factor on smooth muscle cells in other organs (36). In the testis, CNP may participate in the regulation of the peristaltic activity of the tubules by a direct influence on peritubular myofibroblasts or indirectly, by ef fects on myofibroblasts of tunica albuginea (Fig. 2). Tubular contractions in turn are necessary for sperm transport (40, 41). Furthermore, CNP may influence the permeability of lamina propria, and thus the transport of nutrients into the tubular lumen (39). We were able to detect the components of cGMP pathways in pituitary, hypothalamic, and pineal blood vessels as well as to demonstrate a CNP-induced cGMP ac cumulation in isolated blood vessels of the bovine pineal gland.

POTENTIAL FUNCTIONS OF C-TYPE NATRIURETIC PEPTIDE-IHDUCED cGMP IN HEUROEMDOCRIHE CELLS

• As shown above, CNP has a dual function, exerting *(i)* autocrine effects on Leydig cells similar to other neuro endocrine cells, and *(ii)* paracrine effects on contractile cells (Fig. 2). Whereas paracrine cGMP-mediated effects on contractile cells result in relaxation, the primary function of CNP-induced cGMP accumulation in neuroendocrine cells remains obscure. In Leydig cells, a possible physiological involvement of CNP in androgen regulation has to be considered, since testosterone production constitutes the primary function of these cells. In fact, CNP has been shown to elevate testosterone release (14), but this effect was weaker compared to that of ANP or BNP (14). Thus, the endo-crinely acting ANP and BNP may be responsible for the modulation of testosterone release, whereas the locally acting CNP may affect other cellular functions. Accordingly, an influence of CNP on hormone secretion is absent, especially in the organs with the highest CNP concentration (pituitary), and the highest density of CNP receptors (pineal and pituitary) (17, 24, 31).

Established actions of cGMP involve binding to either cyclic nucleotide-gated (CNG) ion channels, GK or cGMP-dependent phosphodiesterases (reviewed in 12). Whereas direct effects of phosphodiesterases on testosterone production have been excluded (42), and CNG channels (43) as well as GK II (44) are not expressed in Leydig cells, the cytosolic GK I was detected in these cells by both immunoblotting and immunohistochemistry (37). However, stimulation of testosterone production by natriuretic peptides has been shown to result from a promiscuous activation of cAMP-dependent protein kinase by cGMP (42,45). The presence of GK I in this cell type strongly suggests that cGMP, accumulated in response to CNP, exerts further testosterone-independent actions. It is of particular interest that the expression levels of the components of the two cGMP-generating systems remain widely unchanged during each period of Leydig cell development (37), whereas the ability of these cells to produce testosterone dramatically changes (46).

CNP, and therefore cGMP, has only a weak, if any, influence on hormone secretion by neuroendocrine cells. One possible CNP activity is to modulate the effects of the hormones or nerve fibers which primarily control cell function. In gonadotropes, for example, CNP decreases the LHRH effects on these cells (24). In the retina, it has been observed that the

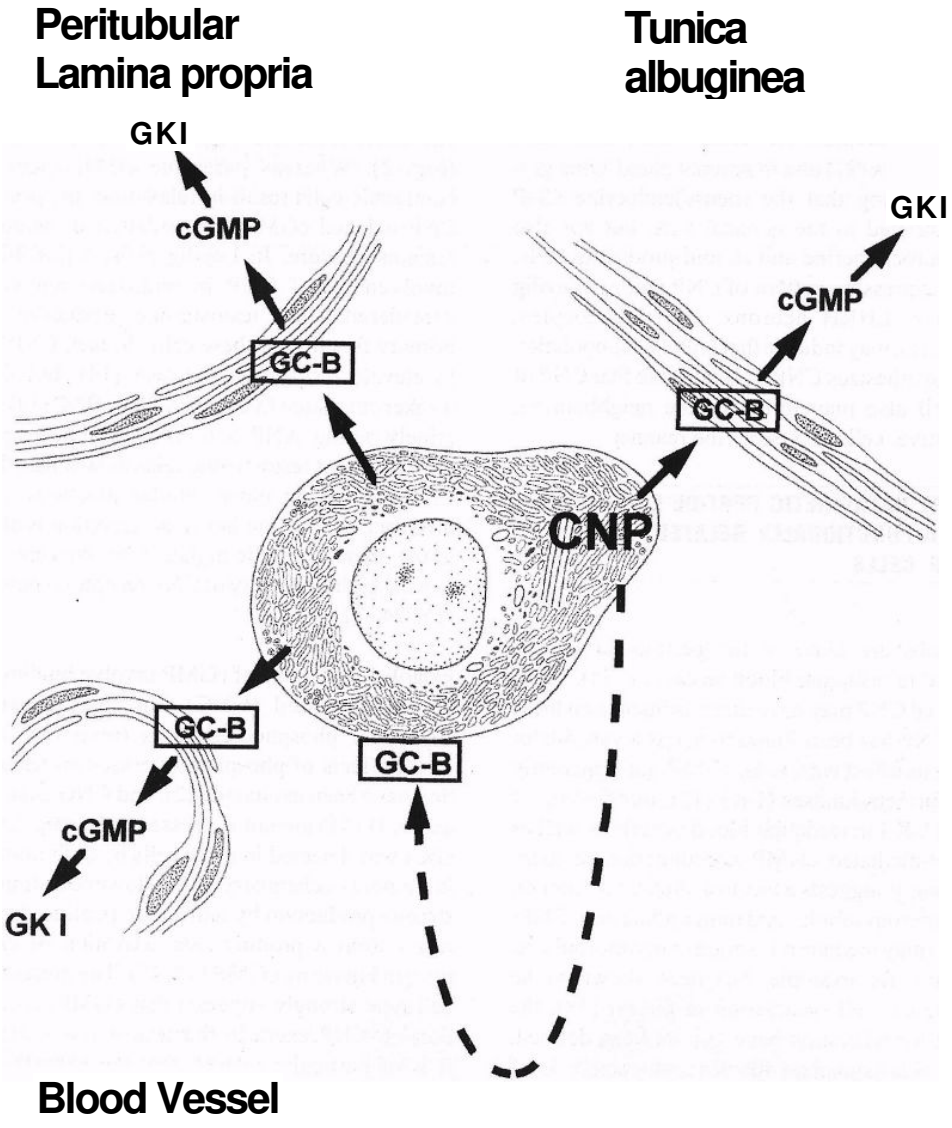

Fig. 2. Schematic presentation of presumed testicular actions of CNP. CNP is produced in human Leydig cells. Based on the presence of specific receptors for CNP, Leydig cells, myofibroblasts of the tunica albuginea, myofibroblasts of the peritubular lamina propria and, vascular smooth muscle cells represent potential sites of CNP activity. This may result in relaxation of contractile cells, presumably mediated by cGMP and GKI (37). In Leydig cells, autocrine actions of CNP may influence testosterone production (14) via a promiscuous activation of cAMP-dependent protein kinase by cGMP (42, 45). Additional hypothetical actions of CNP, possibly involved in the maintenance of normal Leydig cell phenotype by an interaction of cGMP with GK I (37), have to be considered.

function of gap junctions is regulated by cGMP. It is an attractive idea that a similar mechanism may also exist in neuroendocrine cells. Gap junctions between LHRH neurons or Leydig cells, which have recently been shown to be influenced by LH (47) might therefore synchronize hormone secretion of the cells in a cGMP-mediated manner.

Our findings of a reduced expression of functionally active CNP receptors in both mouse (MA10) and human Leydig tumor cells (3 7) may focus on natriuretic peptides as antigrowth factors for a variety of cells, including mesangial, vascular endothelial, smooth muscle cells, and astrocytes (48). ANP receptors for example, are still present in Leydig tumor cells (17). Therefore, the decrease of CNP receptors can not be explained in the context of a general dedifferentiation. Since CNP production still persists in Leydig tumor cells, the interruption of autocrine CNP actions may be interpreted in the context of tumor development. Therefore, down -regulation of CNP receptors might prevent autocrine CNP action, possibly necessary for the maintenance of normal Leydig cell phenotype; a CNP-induced cGMP accumulation was also absent in Leydig tumor cells (37). Key factor of the postulated antigrowth effect of the CNP system in Leydig cells and other neuroendocrine cells might be the loss of fine modulation by CNP-induced cGMP. This raises the question whether cGMP-binding proteins may act as transcription factors. In fact, GK I, which is present in Leydig and other neuroendocrine cells, has recently been shown to affect directly gene expression (49, 50). Nevertheless, little is known about the series of events that underlie growth regulation by natriuretic peptides or cGMP. Signalling into the nucleus, characterization of nuclear targets, and influence on the cell cycle are the main aspects which have to be elucidated.

CONCLUSION

• With respect to the neuroendocrine properties of Leydig cells, the demonstration of CNP represents a further example for the production of neuroactive substances in these cells. Unlike the majority of substances previously detected in the testis, CNP has been shown to be functionally active, and to act *via* an autocrine mechanism in Leydig cells, like in well recognized neuroendocrine cells. Therefore, Leydig cells do not only resemble neuroendocrine cells by the substances produced, but also by their mechanism of activity. With respect to the presumed neuroectodermal origin of Leydig cells, it may be speculated that nerve cell-specific substances found in these cells may be ontogenetic relicts reflecting the origin of the cells, possibly without any functional sig nificance. The functionally active testicular CNP system, however, shows that neuroactive factors found in Leydig cells might play an essential physiological role. Also, the specific autocrine effects of CNP on the cells of the hypothalamic-pituitary-gonadal axis allow us to speculate that CNP agonists and antagonists could gain clinical interest to influence therapeutically reproductive function. Nevertheless, some questions remains to be resolved. For example, the mechanisms, by which CNP is produced and secreted in neuroendocrine cells and the essential function of CNP in neuroendocrine cells have not been elucidated. Investigations on the roles of CNP and NO systems have opened the door to an exciting, but hitherto neglected signal transduction pathway, the cGMP pathway.

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