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# SOURCES AND FUNCTION OF NEURONAL SIGNALLING MOLECULES IN THE GONADS

**ARTUR MAYERHOFER, MONICA B. FRUNGIERI[1], ANDREAS BULLING, STEPHANIE FRITZ**

*Anatomisches Institut der Technischen Universität München, D-80802 München, Germany*

**Abstract** While the hypothalamic-pituitary-gonadal axis is crucial for the function of the gonads, non-endocrine regulatory influences are exerted by other factors within the gonads. Among these factors are neurotransmitters, such as catecholamines. Several types of receptors for catecholamines exist in the gonads on vascular or endocrine cells. Their activation can alter blood flow, steroidogenesis and gene expression, depending on the target cells. Recently a neuronal-like cell type expressing catecholamine-biosynthetic enzymes and neuronal proteins was identified in testis and ovary of human and non-human primates. Together with the well-known sympathetic innervation, this gonadal nervous system may serve as a source of catecholamines. Dopamine is present in the follicular fluid. Ocytes, while not able to perform de novo synthesis of catecholamines, were shown to utilize dopamine to produce norepinephrine. This catecholamine then acts on beta-adrenoreceptors of follicular cells to increase cAMP. Oocytes may thus indirectly via dopamine and cAMP be able to control their own meiotic arrest. In addition, neurotransmitters may also be synthesized in other, non-neuronal ovarian cells. Thus, cultured human granulosa-luteal cells possess the acetylcholine synthesizing enzyme and the acetylcholine-specific vesicular transporter protein. These cells also express muscarinic-receptors (M1), which are linked to the mobilization of intracellular calcium and cell proliferation. This suggests involvement of the acetylcholine system in follicular growth and in the periovulatory events. In neurons, neurotransmitters alter the properties of the neuronal cell membrane. If this is the case in endocrine cells of the gonads is not yet clear, but the recent identification of voltage-activated potassium and sodium channels in human luteinized granulosa-luteal cells raises this question and opens a door to a new area of investigation.

**Keywords:** testis, ovary, granulosa cells, oocyte, neurotransmitter

## Background

The mammalian ovary and testis depend on gonadotrophic stimulation. However, in addition, the gonads are subjected to multiple influences by locally produced growth factors and neurotransmitters[1-5]. In particular, catecholamines may be an important part in this "neuroendocrinotrophic stimulatory complex" (see summary in 2), a term coined to describe the concert of numerous players interacting to regulate gonadal functions.

Catecholamines are present in sufficiently high concentrations in the gonads (e.g. in the ovarian follicular fluid and testicular interstitial fluid; see 5-6) to be able to activate their receptors on several gonadal cells. Catecholamines, as hormones of the adrenal, can reach the gonads via the blood flow. Moreover, the dense sympathetic innervation of the gonads, observed in many, although not all species, is another important anatomical base for the interest that catecholamines have received in this respect[7-10]. Compared to the blood stream, gonadal innervation may allow a more precise way of delivering neurotransmitters to target cells. This is supported by the fact that testicular nerve fibers form "synapses en passant" with Leydig cells and peritubular cells in the human testis[9-10], implicating release of neurotransmitters into the interstitial space in the close neighborhood of the target cells, bearing receptors. Nerve fibers are also often found in close anatomical proximity of growing follicles in the ovary (Mayerhofer, unpublished). Moreover, gonadal innervation, appears also to be important for a functional direct link between testis/ovary and brain (see 11). For the female gonad, this multisynaptic pathway has recently been mapped using a viral transneuronal labeling method[12].

In general, evidence for functional roles of catecholamines, including norepinephrine (NE), is derived from several experimental approaches. For example, gonadal alpha- and beta-adrenergic receptors exist on steroidogenic cells in many species including the human

[1] **Present address:** Instituto de Biologia y Medicina Experimental, Vuelta de Obligado 2490, 1428 Buenos Aires (Argentina)

**Postal Address:** Dr. Artur Mayerhofer, Anatomisches Institut, Technische Universität München, Biedersteiner Str. 29, D-80802 München, Germany

Fax: +49-89-397035; E-mail: Mayerhofer@lrz.tum.de

(see 13, 1), and activation of these receptors by NE can affect steroid production in both testis and ovary. Dopamine (D1) receptors have been found on human luteinized granulosa cells, which can increase cAMP and phosphorylate a down-stream third messenger, DARPP-32 (dopamine and cAMP-regulated phosphoprotein of Mr 32,000; 5). Interfering with the normal development of ovarian innervation by neonatal denervation led to abnormal ovarian development and function[14], and induction of polycystic ovaries in rats was associated with hyperactivation of the ovarian catecholaminergic system[15]. More recently, we showed that neurotransmitters acting via beta-adrenergic ovarian receptors and cAMP are also responsible for the initiation of FSH-receptor gene expression and subsequent follicular growth in the neonatal rat ovary[16]. Thus neurotransmitters, like in other cellular systems[17], appear to be able to act as growth regulatory factors in the gonads.

Rather than providing a detailed description of these and other well-documented and rather numerous effects of catecholamines and other neurotransmitters, the focus of this short review is on recent developments and results from our group concerning additional sources of neurotransmitters and their novel functions in the gonads.

## Gonadal neuron-like cells

In addition to adrenal catecholamines, reaching the gonads via the blood stream and intragonadal release from the sympathetic innervation, there is evidence for an additional intraovarian and intratesticular source of catecholamines, neuron-like cells[18-21]. These were identified in human and non-human primate species, (Rhesus monkey). They were found to be immunoreactive for neuronal markers, including neurofilaments and the low affinity receptor for nerve growth factor, as well as for tyrosine hydroxylase (TH), the rate limiting enzyme of the catecholamine biosynthetic pathway. Moreover, the TH and the dopamine beta-hydroxylase (DBH) gene are expressed in the Rhesus monkey (Mayerhofer et al., 1998). In situ-hybridization studies, as well as catecholamine histofluorescence allowed to identify elongated bi- or multipolar cells as the sites for catecholamine synthesis. These neuron-like cells, in conjunction with the extrinsic innervation and catecholamines derived from the adrenal could therefore be additional sources of ovarian NE dopamine (DA).

In the human ovary these cells also appear to be present (Mayerhofer, unpublished) and in the human testis, some of these cells were found to express the neuropeptide Y[22]. A fundamental difference between the ovary and the testis became evident during these studies: Cell bodies of neuron-like cells are found in the adult ovary and immature testis, but are largely (human; 21) or completely (monkey; 22) absent from the adult testis. However, even after puberty there is an increase in the density of cross-sectioned, neurofilament-immunoreactive processes in the monkey testis, indicating an overall enrichment of neuronal elements during adulthood. The reasons for these striking differences between the female and male gonad are currently not known and the mechanisms underlying these changes remain to be examined. However, based on the numerous results showing that catecholamines regulate blood flow, steroidogenesis and other aspects of granulosa or Sertoli cell function, these results let us suspect fine tuning influences in the gonads.

Pathologies of the gonads appear to be associated with changes of the catecholaminergic system, as shown in polycystic ovaries[15]. An unexpected plasticity and increased numbers of both nerve fibers and cell bodies were also documented in the testes of infertile men suffering from various degrees of germ cells loss and Sertoli-cell only syndrome[21]. Therefore increases and possibly functional changes of the intratesticular nervous system are associated with idiopathic male infertility in the human and may be among the factors initiating or sustaining these condition.

## Oocytes as a site of neurotransmitter synthesis

Another site of ovarian DBH-gene expression, but not of TH-gene expression, became evident: Besides neuron-like cells oocytes of the monkey ovary contain both DBH mRNA and protein. In addition, they also possess the membrane-associated transporter for DA. DA is a catecholamine found in high concentrations in the follicular fluid. DA, when added to isolated oocytes is rapidly taken up and converted to NE, as measured with a highly sensitive HPLC system[20]. NE released from oocytes then is able to interact with beta-adrenoreceptors present in granulosa cell in the follicle, leading to increased cAMP production. Given that these experimental data reflect the *in vivo* situation, we speculate that via gap junctions, cAMP may serve as inhibitor of meiotic arrest of the oocyte. It could also be involved in the regulation of various granulosa cell functions, an issue that remains to be clarified. These results indicate unexpected and largely unexplored interactions between the gonadal nervous system and germ cells.

## Local production of Neurotransmitters: Acetylcholine

Electron microscopy indicated the presence of catecholamines and neuropeptides in dense-core granules of cross-sectioned nerve processes. In addition, elec-

tron-translucent, "clear" vesicles were observed (Mayerhofer unpublished; 10) which suggested, for example, the presence of acetylcholine or amino acid neurotransmitters. Previous studies showed that acetylcholine and analogues can induce strong increases in intracellular free calcium levels in human luteinized granulosa cells[23]. These cells, as subsequently shown express muscarinic-receptors of the M1 type. Activation of these receptors *in vitro* induces cell proliferation within 24 hours of treatment with a cholinergic agent[24]. We examined the physiological source of ovarian acetylcholine. Using a monoclonal antiserum against the biosynthetic enzyme choline-acetyl-transferase (CHAT) and an antiserum against the specific vesicular transporter of acetylcholine (VACHT), we found that these cells themselves have the prerequisites to produce and store acetylcholine. Moreover, immunostaining of ovarian sections did not show CHAT-immunoreactivity in nerve fibers or cells, but rather in granulosa cells of large follicles. Thus, cultured human granulosa-luteal cells and their counterparts in vivo possess the acetylcholine synthesizing enzyme and its vesicular transporter protein, raising the possibility that the acetylcholine system of the ovary is involved in follicular growth and in the periovulatory events.

## Voltage-activated ion channels - novel bases for neurotransmitter actions in the gonads

In neurons, neurotransmitters exert many of their functions by activation of metabotropic and ionotropic receptors. Catecholamines are among those neurotransmitters that posses only metabotropic receptors, including alpha-, beta- and dopamine receptors (see 5), leading to changes in second messengers (calcium and cAMP) and phosphorylation of third messenger complexes (e.g. DARPP-32; 5). Acetylcholine can interact with both metabotropic (muscarinic) and ionotropic (nicotinic) receptors. Metabotropic or ionotropic actions can result in slow or rapid alteration of electrical membrane properties of target cells. If this is the case also in endocrine cells of the gonads, is not examined and the electrophysiological properties of these cells are not characterized. We have started to address this point and performed whole-cell patch-clamp studies in human luteinized granulosa-cells. These cells were found to possess voltage-activated potassium channels and surprisingly also voltage-activated sodium channels[25]. RT-PCR cloning identified the type of the sodium-channel alpha subunit as one previously found in neuroendocrine cells. The expression of this gene in steroidogenic granulosa-luteal cell and the gonads (unpublished) prompted us to suggest the term "endocrine" voltage-activated sodium channel. Currently, studies are under way that address the question if neurotransmitter can alter the function of the newly described ion channels and would thus provide a new perspective on the mechanisms used by neurotransmitters to act in the gonads.

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