# The role of monoaminergic and galaninergic systems on the regulation of oxytocin secretion in rat neurohypophyseal cell cultures

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**I.**

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**II.**

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**CONTENTS**

**ABBREVIATIONS**

5-HT: serotonin
AH: adenohypophysis
ADR: adrenalin/epinephrine
Arg: arginine
Asn: asparagine
ATL: atenolol
AVP: arginine-vasopressin
CAT: corynanthine
CIM: cimetidine
CNS: central nervous system
Cys: cysteine
DBB: diagonal brand of Broca
ex: exon
GAL: galanin
GKR: tripeptide processing signal
Gln: glutamine
Gly: glycine
GPCR: G protein-coupled receptor
GTP: guanosine-5'-triphosphate
HA: histamine
HNS: hypothalamo-neurohypohpyseal system
HT: hypothalamus
Ile: isoleucine
KET: ketanserin
LC: locus coeruleus
LDCVs: large dense core vesicles
Leu: leucine
MCNs: magnocellular neurons
MEP: mepyramine
MET: metergoline
MnPO: median preoptic nucleus
M15: galantid
mRNA: messenger ribonucleic acid
NADR: noradrenalin/norepinephrine
NH: neurohypophysis
NTS: nucleus of tractus solitarius
OB: olfactory bulbs
OT: oxytocin
PBS: phosphated buffered saline
Phe: phenylalanine
PNL: propranolol
PNZ: perinuclear zones adjacent to the supraoptic and tuberomammillary nuclei
Pro: proline
PVN: paraventicular nuclei
RER: rough endoplasmic reticulum
RIA: radioimmunoassay
SCN: chiasma opticum
S.E.M.: standard error of mean
Ser: serine
SON: supraoptic nuclei
TGN: trans-Golgi network
TMN: tuberomammillary neurons
TPE: thioperamide
Tyr: tyrosine
Val: valine
VLM: ventrolateral medulla
VP: vasopressin
YOB: yohimbine

## INTRODUCTION

Homeostasis in animals is regulated by the nervous, the endocrine and the immune systems. Glial cells play significant role in the relationship between the endocrine and the nervous systems. Hormonal effects on glia cell may have an essential role in neuronal development, metabolism and activity {1}. Glial cells are also involved in the modulation of hormone release. Pituicytes and microglial cells in the neurohypophysis (NH) may influence hormonal secretion by regulating neurovascular connections. Additionally, astroglia cells in the hypothalamus (HT) regulate the number of synaptic inputs to a neuronal population that is involved in pituitary hormone release {2,3}.

HT and pituitary gland produce both immuno-suppressive and immuno- enhancing effects. Several neurotransmitters, neuropeptides and neurohormones affect immune functions both *in vivo* and *in vitro* {4}.

### Hypothalamus

Pituitary hormone secretion is controlled by the HT. The HT is one of the most evolutionarily conserved and essential region of the mammalian brain. The hypothalamo-neurohypophyseal system (HNS) is defined as consisting of large neurons, 20-40 μm cell body diameter, of the supraoptic nuclei (SON) and paraventricular nuclei (PVN). The hypothalamic nuclei have axons terminating on the blood capillaries of the posterior pituitary. {5}. PVN are divided into two regions: lateral and medial subdivision. Parvocellular oxytocin (OT) and arginine-vasopressin (AVP) producing neurons have smaller cell bodies and project to the median eminence, brain stem, spinal cord, limbic and olfactory areas {6,7,8}. There are some topographical segregation in magnocellular neurons (MCNs) {9,10,11,12}. Within the SON OT neurons are located mainly rostrally and dorsally. Within the PVN OT neurons are found predominantly in the rostral region.

Not all centrally projecting AVP and OT neurons are located within the SON and the PVN. Immunoreactive neurons occupy the bed nucleus of stria terminalis, the preoptic area, the septal region, and the locus coeruleus (LC) {13,14}.

An intact HT is essential for normal endocrine function. There are three types of hypothalamic neurosecretory cells. One of them is the MCNs, secretes AVP and OT {10,15}. The cell body is located in the SON and PVN and its projections to the neural lobe release neurohormone via nerve endings.

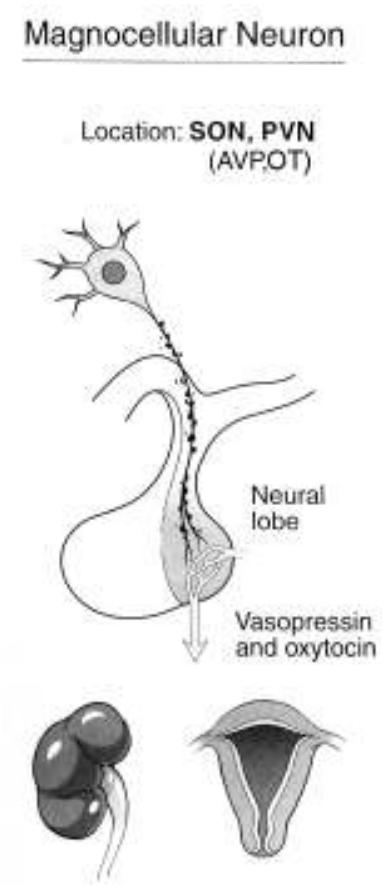


**Figure 1.** MCN which secretes the AVP and OT. The cell body is located in the SON and the PVN. (From Larsen, Kronberg, Melmed, Polonsky: Textbook of endocrinology, Philadelphia, Saunders; 2002 p.83.)

The other two types of hypothalamic neurosecretory cells are the parvicellular hypophyseotropic neurons and the hypothalamic projection neurons {16,17}. These collections of neurons coordinate endocrine, autonomic, and behavioral responses.

**Pituitary gland**

The HT receives sensory inputs from the external and the internal environment. It integrates sensory and hormonal inputs and provides coordinated responses through motor outputs to key regulatory sites. These regulatory sites include: the anterior pituitary gland, the posterior pituitary gland, the cerebral cortex, the premotor and motor neurons in the brain stem and spinal cord, and the autonomic preganglionic neurons {5,18}.

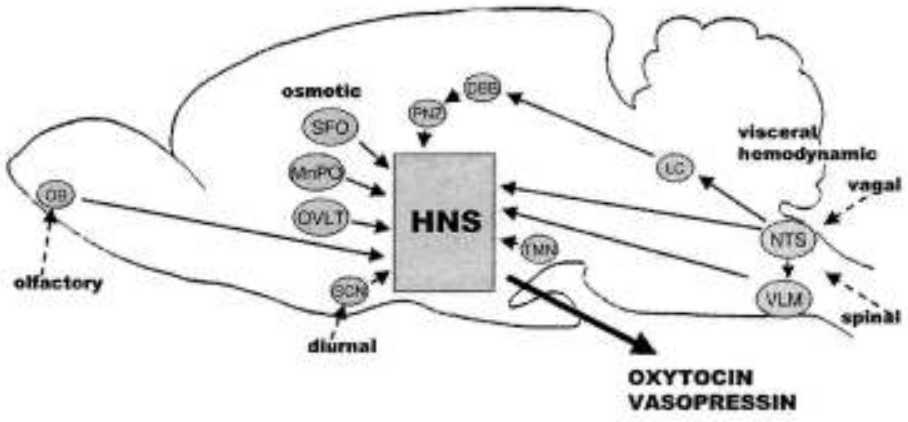


**Figure 2.** Afferent inputs to the HNS (From Burbach, Luckman, Murphy, Gaine: Gene regulation into magnocellular hypothalamo-neurohpypophyseal system, Physiological Rewiev, 2001 Vol(81) p.1201.)

The pituitary gland is regulated by 3 interacting elements: hypothalamic inputs, feedback effects of circulating hormones, paracrine and autocrine secretions of pituitary {18}.

The pituitary gland can be divided into two major parts: the adenohypophysis (AH) and the NH. AH is subdivided into three lobes: the pars distalis; the pars intermedia and the pars tuberalis. NH is composed of pars nervosa, and the infundibular stalk. The infundibular stalk is surrounded by pars tuberalis and together they constitute the hypophyseal stalk {19}.

In human the pituitary gland is situated within the sella turcica and is overlain by the diaphragma sella; through which the stalk connects to the median eminence of the HT. The sella turcica is located at the base of the skull, and forms the thin roof of the sphenoid sinus. The lateral walls, comprising either bone or dural tissue about the cavernous sinuses, are traversed by the third, fourth, and sixth cranial nerves and the internal carotid arteries. The dural roofing protects the gland from compression by the cerebrospinal fluid pressure. The posterior pituitary gland is directly innervated by supraopticohypophyseal and tuberohypophyseal nerve tracts of the posterior stalk {19,20}.

The HT contains nerve cell bodies that synthesize hypophysiotropic releasing and inhibiting hormones as well as the neurohypophyseal hormones of the posterior pituitary {15}.

The anterior and intermediate lobes of pituitary are derived from the Rathke's pouch, an outgrowth of pharyngeal cavity, and migrate during development to surround the neural lobe {21}.

The NH arises from neural ectoderm and is associated with third-ventricle development. During embryogenesis, neuroepithelial cells of the lining of the third ventricle mature into MCNs while migrating laterally to and the optic chiasm to form the SON. Migration also occurs to the walls of the third ventricle to form the PVN. The major components of the neural lobe are a collection of axon terminals arising from magnocellullar secretory neurons, from the PVN, and from SON of the HT {22}.

These axon terminals are in close association with a capillary plexus and they secrete substances, including AVP and OT into the hypophyseal veins, and into the general circulations {23}.

The median eminence lies in the center of the tuber cinereum. It is composed of an extensive array of blood vessels and nerve endings. The median eminence can be considered to be the functional link between the HT and the pituitary gland {24,25}.

**Neuropeptides**

The axon terminals of the MCNs contain neurosecretory granules and membrane bound packets of hormones stored for subsequent release. The blood supply of the posterior pituitary comes directly from the inferior hypophyseal arteries {24}. The MCNs are clustered into a subdivision of the SON and PVN {26,27}. All of the OT-erg and VP-erg neurons in the SON project their axons to the NH {28}. The organization of the PVN is much more complex. The MCNs are divided into at least three portions such as OT-erg, VP-erg and parvicellular (smaller cells, synthesize other peptides and opioids and project to the median eminence, the brain stem and the spinal cord) {29,30}.

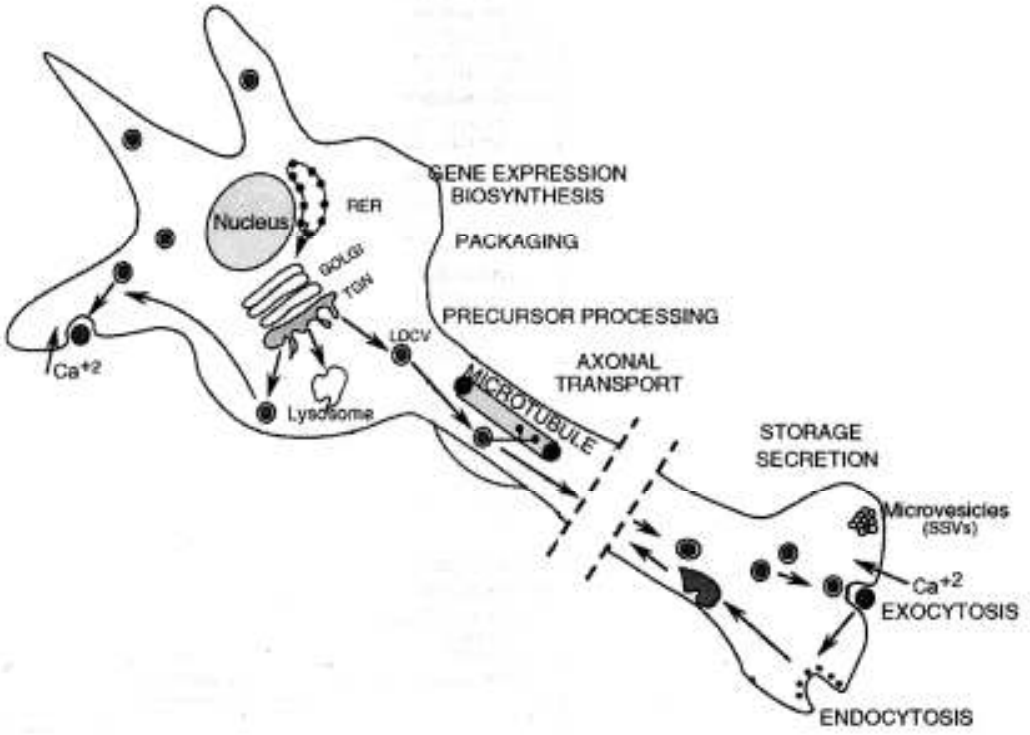


**Figure 3.** Peptidergic neuron (From Burbach, Luckman, Murphy, Gaine: Gene regulation into magnocellular hypothalamo-neurohpypophyseal system, Physiological Rewiev, 2001 Vol(81) p. 1203)

The MCNs of the hypothalamic nervous system, which synthesize and secrete the nonapeptide, OT, represent a special class of peptidergic neurons called neurosecretory cells. The biosynthesis and secretion of neuropeptides, OT, in the HNS requires continual transcription and translation of peptide precursor proteins. The process of the biosynthesis of OT is as follows: gene expression, protein biosynthesis, and packing of the protein into large dense core vesicles (LDCV) in the cell body, where the nucleus, rough endoplasmic reticulum (RER), and Golgi apparatus are located. Enzymatic processing of the precursor proteins into the biologically active peptides occurs primarily in the LDCVs. Processing often occurs during the process of anterograde axonal transport of the LDCVs to the nerve terminals on the microtubule tracks in the axon. The LDCVs are usually stored in preparation for secretion in the nerve terminals. Action potential causes an influx of calcium ion through calcium channels. The increased calcium ion concentration causes a cascade of molecular events that leads to neurosecretion. Recovery of the excess LDCV membrane is performed by endocytosis and it is retrogradedly transported to the cell body for reuse or degradation in lysosomes.

The control of hormone synthesis resides at the level of transcription. Stimuli for secretion of OT also stimulate transcription and increase the mRNA content in the MCNs {31}. The transport of neurosecretory vesicles from the site of synthesis to the posterior lobe along microtubule is also regulated by demand for neuropeptides. When synthesis is turned off, the transport stops, and when the synthesis is increased, transport is upregulated {5,32}. The MCNs, specific for OT, have intrinsic individual characteristic electrical firing patterns. These patterns are modulated by the paracrine and autocrine action of hormone released by the dendrites into the extracellular space surrounding the MCNs in the SON and PVN {33,34}.

**Oxytocin and oxytocin receptor**

OT was the first peptide hormone to have its structure determined and the first to be chemically synthesized in biologically active form {35,36}.

OT is a nonapeptide with a disulfide bridge between Cys residues 1 and 6 which result in a cyclic part and a 3 residues tail in COOH-terminal. OT contains isoleucine (Ile) and leucine (Leu) at positions 3 and 8 {37}.

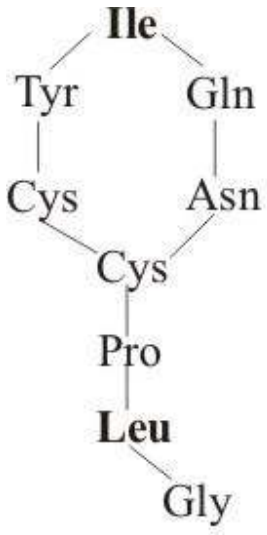


**Figure 4.** Structure of OT

This figure shows OT and related peptides in invertebrates and vertebrates {38}.

**OT(-related) peptides**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Cys | Tyr | Phe | Arg | Asn | Cys | Pro | Ile | Gly-NH2 | Cephalotocin | Octopus vulgaris |
| Cys | Tyr | Ile | Asn | Asn | Cys | Pro | Leu | Gly-NH2 | Aspargtocin | cartilaginous fishes (sharks) |
| Cys | Tyr | Ile | Gln | Asn | Cys | Pro | Val | Gly-NH2 | Valitocin | cartilaginous fishes (sharks) |
| Cys | Tyr | Ile | Ser | Asn | Cys | Pro | Gln | Gly-NH2 | Glumitocin | cartilaginous fishes (rays) |
| Cys | Tyr | Ile | Ser | Asn | Cys | Pro | Ile | Gly-NH2 | Isotocin | bony fishes |
| Cys | Tyr | Ile | Gln | Asn | Cys | Pro | Ile | Gly-NH2 | Mesotocin | mammals, birds, reptiles, amphibians, lungfishes |
| Cys | Tyr | Ile | Gln | Asn | Cys | Pro | Leu | Gly-NH2 | Oxytocin | mammals |
| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | | |

**Figure 5.** The primary sequences of various peptides of the OT superfamily. At positions 1, 5, 6, and 7 the amino acids are the same. Peptide names and the species or group names (in which it has been identified) can be seen after the sequences.

Two evolutionary molecule lineages have been proposed {39,40}:

Isotocin-mesotocin-OT

Vasotocin-VP

It is thought that these genes evolved from an ancestral gene about 400 million years ago. The high identity rate in amino acid sequence of vertebrate and invertebrate peptides led the previous establishment {38,41}.

The OT and AVP genes are on the same chromosome in all species (for example: chromosome 2 in the mouse; chromosome 3 in the rat; chromosome 20 in the human) but are transcribed in opposite directions. The intergenic distance between these genes is 3.5 kb in the mouse; 11 kb in the rat and 12 kb in the human {42,43,44}.

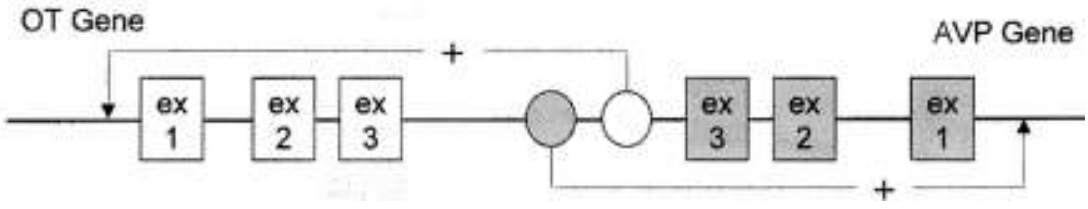


**Figure 6.** OT and AVP gene

OT is synthesized as parts of large precursor proteins from which OT is cleaved. There is separate precursor for OT and its associated neurophysin {45}. The human gene for OT-neurophysin encodes the oxytocin prepropeptide and consists of 3 exons {41,44,46}:

Exon 1: -translocator signal

-nonapeptide hormone

- tripeptide processing signal (GKR)

-neurophysin first 9 residues

Exon 2: -neurophysin central part

Exon 3: -neurophysin COOH terminal

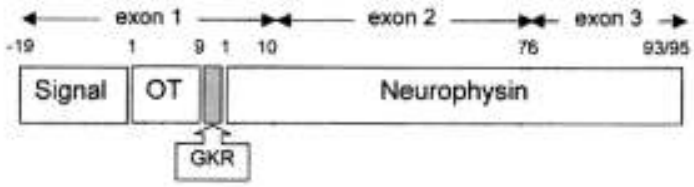


**Figure 7.** Organization of OT propeptide

The OT propeptide, after the cleavage and modifications, is transported down the axons to terminals in the posterior pituitary. OT and its carrier molecule, neurophysin, are stored in axon terminals. Neurophysin functions are: proper targeting, packaging and storage of OT within granula before release into the bloodstream. The binding strength between OT and its neurophysin is much higher in an acid compartment, such as the neurosecretory granula. The dissociation of the complex is facilitated as the complex enters the alkaline plasma {47,48}.

The human OT receptor consists of 389 amino acid polypeptides with 7 transmembrane domains and belongs to the class 1 G protein coupled receptor family. The OT receptor encoding sequences from the rat and the mouse have also been identified {49,50}. The OT receptor gene contains 3 introns and 4 exons. Exons 1 and 2 correspond to the 5'-prime noncoding region. Exons 3 and 4 encode the amino acids of the OT receptor {51}.

The OT receptor is a typical member of the rhodopsin–type (class 1) G protein-coupled receptor (GPCR) family. The seven transmembrane α-helices, are highly conserved, and they may be involved in a common mechanism for activation and signal transduction to the G protein. In the class 1 GPCR family, an Asp in transmembrane domain 2, the tripeptide at the interface of transmembrane 2, and the first.

OT receptors are functionally coupled with $G_{q/11}$ α class GTP binding proteins, which stimulate (together with $G_{\beta\gamma}$) the activity of the phospholipase C-β isoform. This leads to the generation of inositol-trisphosphate and 1,2-diacylglycerol. Inositol-trisphosphate triggers $Ca^{2+}$ release from intracellular stores; whereas, diacylglycerol stimulates protein kinase C which phosphorylates unidentified target proteins. Finally, in response to an increase of intracellular [$Ca^{2+}$], a variety of cellular events are initiated {53,54}.

**Peripheral oxytocin system**

Female reproduction system: The pregnant uterus is one of the traditional targets of OT. In rats, OT gene expression was shown to be present in the placenta and the amnion {55} and in humans in the amnion, the chorion and the deciduas {56}. Around the onset of labor uterine sensitivity to OT markedly increases. This is associated with both an upregulation of OT receptor mRNA levels and a significant increase in the density of myometrial OT receptors {57}. (During late gestation OT gene expression is upregulated in intrauterine tissues, although the OT peptide itself was not found at concentrations significantly higher than in the circulating blood {58}.) Both OT and OT receptor genes are expressed in human cumulus cells surrounding the oocytes. Thus local OT may participate in fertilization and early embryonic development in humans {59}.

Male reproduction system: In humans the complete OT system appears to be present is the testis, the epididymis, and the prostate {60}. Within the rat testis OT is present in the interstitial Leydig cells, which are the main store of testosterone in the male. The two main functions of testicular OT are the regulation of seminiferous tubule contractility and the modulation of steroidogenesis {61}. OT was shown to increase the activity of 5α-reductase in both the testis and the epididymis. Thus, OT may have an autocrine/paracrine role in modulating steroid metabolism in these tissues {62}. OT can stimulate growth of the prostate in the rat, particularly the mitotic activity in the glandular epithelium {63}.

Mammary tissues: Central OT-erg neurons are essential components for the initiation and maintenance of successful lactation {64}. OT production has been suggested as a preventative factor in the development of breast cancer both pre- and postmenopausally {65}.

Kidney: OT is a nonhypertensive natriuretic agent and is involved in normal osmoregulation. The natriuretic effect of OT is mainly due to a reduction in tubular $Na^{+}$ reabsorption {66,67}. In adult rats, OT binding sites were localized in the cortex, on the loops of Henle of the juxtamedullary nephrons {68}.

Heart and Cardiosvascular System: Peripherally injected OT decreases mean arterial pressure in rats. Blood volume expansion, via baroreceptor input to the brain, causes the release of OT which then circulates to the heart {69}. The OT concentrations in the aorta and vena cava were higher than those in the right atrium of the heart. OT might play a direct role in volume and pressure regulation in a paracrine/autocrine manner {70}.

Other locations: The thymus is responsible for the selection of the peripheral T-cells. Neuropeptides and their receptors have been described in the thymus, supporting the concept of interaction between the neuroendocrine and immune system at T-cell differentiation {71}. OT has been identified in the human thymus by immunoreactivity {72}.

In adipocytes OT has a so-called insulin-like activity: it stimulates glucose oxidation, lipogenesis, and increases pyruvate dehydrogenase activity {73}.

OT and AVP have been identified in human and rat pancreas extracts at higher concentration than those found in the peripheral plasma {74}. Neurohypophyseal hormones induce the release of glucagons and insulin from the pancreas {75}.

OT and AVP have been identified by immunoreactivity in human and rat adrenal glands {76}. OT was found to be localized in both the adrenal cortex and the medulla {77}.

**Aminergic and peptidergic neurotransmitters**

The principal neurohormones are known to be released not only via physiological stimulation, but also via coexisting peptides by increasing or inhibiting hormone secretion. The secretion of OT is regulated by different aminergic and peptidergic neurotransmitters, including adrenalin (ADR), noradrenalin (NADR) {78}, dopamine {79,80}, serotonin (5-HT) {81,82,83}, histamine (HA) {84,85,86} and galanin (GAL) {84,88,89}.

**Catecholamines**

The catecholamines: dopamine, ADR, and NADR are neurotransmitters and/or hormones in the periphery and the central nervous system (CNS). Dopamine is a precursor of noradrenalin; it has a biological activity in the periphery, particularly in the kidney, and serves as a neurotransmitter in several important pathways in the CNS. NADR is a neurotransmitter in the brain as well as in postganglionic sympathetic neurons. ADR (formed by N-methylation of noradrenalin) is a hormone released from the adrenal gland, and stimulates catecholamine receptors in different organs. Small amounts of adrenalin are also found in the CNS, particularly in the brainstem {90}.

There are 16 dopamine- and NADR-containing cell groups and three ADR-containing cell groups in the CNS {91,92}.The axons from these cells project to a widespread area in the CNS. Some of the cell groups have a more focused projection and more specific functional effects. The LC is one of the best-studied cell groups of NADR-ergic neurons and it has many ascending and descending projection from the pons.

Some of the most-studied of dopaminergic neurons include the tuberoinfundibular neurons involved in endocrine regulation, and the nigrosriatal neurons, involved in body movement and Parkinson's disease {91}.

The brain contains many different catecholamine receptors. There are five different dopamine receptors: $D_1$-like ($D_1$, $D_5$) and $D_2$-like ($D_2$, $D_3$, $D_4$) {93}. The effect of NADR and ADR are mediated through nine distinct receptors grouped into three families ($\alpha_1$, $\alpha_2$, $\beta$), each containing three subtypes encoded by separate genes {94}.

**Serotonin**

5-HT was localized to three key systems in the body: platelets, the gastrointestinal tract, and the brain.

5-HT -containing neuronal cell bodies are restricted to discrete clusters or groups of cells located along the midline of the brainstem. Their axons innervate nearly every area of the CNS. The majority of 5-HT-ergic soma was found in the raphe nuclei, however, not all the cell bodies in the raphe nuclei are 5-HT-ergic {95}.

Ascending projection from the raphe nuclei to forebrain structures is organized in a topographical manner. The dorsal and median raphe nuclei give rise to distinct projections to forebrain regions. The median raphe projects heavily to the hippocampus, the septum, and the HT, whereas, the striatum is innervated by the dorsal raphe. The dorsal and median raphe send overlapping neuronal projections to the neocortex. Within the dorsal and median raphe, cells are organized in particular zones or groups that send axons to specific areas of the brain {96}. 5-HT is one of the many neurotransmitters which participate in the hypothalamic control of pituitary secretion {97}.

Three families of 5-HT GPCR are: the 5-$HT_1$ and 5-$HT_5$ families, 5-$HT_2$ family and the family that includes: 5-$HT_4$, 5-$HT_6$, and 5-$HT_7$. The 5-$HT_3$ receptor is a ligand-gated ion channel and is separate family. Each 5-HT receptor can be activated by 5-HT, but differences exist in: signal transduction mechanisms, neuroanatomical distribution and affinities for synthetic chemicals. These differences create opportunities for drug discovery and make 5-HT receptor subtypes a potential therapeutic target {81,98,99}.

**Histamine**

HA is a mediator of several physiological and pathological processes within and outside the nervous system. HA is formed and released from CNS neuron and is an important regulator of several brain functions. As a physiological mediator, HA is best known as an endogenous stimulant of gastric secretion. HA is also released from mast cells and basophils by: antigens, certain peptides, and small basic drugs. HA participates in inflammation and in the regulation of immune response {100,101,110}.

The brain stores and releases HA from more than one type of cell. Mast cells are a family of bone marrow derived secretory cells that store and release high concentrations of histamine. They are found throughout many connective tissues of the body and are also present within and surrounding the brain of many species. They are prevalent in the thalamus and HT, as well as in the dura mater, the leptomeninges, and the choroid plexus {101,103}.

In all vertebrates studied HA-ergic neurons are found in the tuberomammillary nucleus of the posterior basal HT. HA-ergic fibers project widely to most regions of the central nervous system. Although nearly all CNS areas contain some HA-ergic fibers, the density of innervation is heterogeneous. The highest densities are found in several hypothalamic nuclei, the medial septum, the nucleus of diagonal band of Broca (DBB) and the ventral tegmental area. Moderate densities are found in the cerebral cortex, the amygdale, the striatum, and the substantia nigra {104,105,106,107}.

A number of substances are colocalized with HA and its biosynthetic enzyme in hypothalamic tuberomammillary neurons. GAL may be a presynaptic inhibitor of neuronal HA release; similar to its proposed actions on cholinerg and 5-HT-ergic fibers {108}.

Tuberomammillary cells receive monoaminergic input from ADR-ergic (C1-C3), NADR-ergic (A1-A2), and 5-HT-ergic (B5-B9) cells {100,107}.

Newly synthesized neuronal HA is transported into tuberomammillary neuronal vesicles. The depolarization of nerve terminals activates the exocytotic release of HA by voltage- and Ca-dependent mechanism {103,109}.

The activity of HA-ergic neurons is regulated by $H_3$ autoreceptors and by other transmitter receptors. HA acts on four GPCR. Within the brain, the $H_1$, $H_2$, and $H_3$ receptors all have unique regional distribution but none is localized exclusively to neurons. $H_4$ is detected predominantly in the periphery, for example bone marrow and leukocytes {110,111,112,113}.

HA in the brain may act as both a neuromodulator and the classic transmitter. A number of other neurotransmitter systems can interact with HA-ergic neurons. Activation of brain $H_3$ receptor decreases the release of acetylcholine, dopamine, NADR, 5-HT, and certain peptides. However, HA may also increase the activity of some of these systems through $H_1$ and/or $H_2$ receptors {114,115,116,117}.

**Galanin**

GAL is a 29/30 amino acid neuropeptide which belongs to the gut and brain neuropeptide family {118,119}. GAL is present in both the nervous system and the peripheral tissues. GAL immunopositive neurons were localized in the following areas: raphe nuclei; various parts of the reticular formation; vagal nuclei; and the gelationous part of the spinal trigeminal nucleus {120}.
The ability of GAL to impair cognitive performance and to stimulate feeding behaviour, affects the circulating levels of hormones. GAL inhibits the secretion of insulin, acetylcholine, 5-HT, and NADR. GAL also stimulates release of prolactin and growth hormone {121,122}.
In many rat and human CNS structures, GAL is co-expressed with other neurohormones or neuromediators. GAL is active in the neuroendocrine system and has been suggested to have paracrine as well as endocrine effects in the HT-pituitary axis. Neurons in the HT containing both GAL and AVP were very common in the SON and also occurred in the PVN and suprachiasmatic nuclei. The SON and PVN also contained some neurons with immunoreactivity for both GAL and OT {123,124}.
The GAL is mediated via GPCR and ion channels. GAL receptors show a wide spread distribution in the nervous system and are also found in neurons innervating the gastrointestinal tract and the pancreas {121}. GAL-R1 receptors are present in several hypothalamic nuclei (partly in neurons synthesizing GAL), rat stomach and small intestine. The Gal-R2 type is differentially distributed, e.g. in the peripheral tissues and in different parts of the brain {122}. Gal-R3 relatively abundant is in peripheral tissues {125}.

**AIM**

The changes in the intracellular level of OT is described under monoaminergic (ADR, NADR, 5-HT, HA) and peptidergic (GAL) regulation in the magnocellular cells (PVN, SON) of the HT. However, the relevant literature contains no data which satisfactorily demonstrate that independently of the HT the OT secretion from the NH can be directly influenced by the monoaminergic and GAL-ergic systems. Our aim throughout the present study was the investigation of this topic. The following issues had to be dealt with prior to the work:

**1.a** The creation of an *in vitro* model system (NH cell culture) for the study of regulation of OT secretion at the cellular level.
**1.b** The setting up of a modified radioimmunoassay (RIA) method for the determination of the released OT content from the functionally active NH culture.

**2.a** The investigation of changes in OT release NH cell culture following of ADR, NADR, 5-HT, HA administration.
**2.b** The study of the functional presence of OT release-connected special receptor types of the monoaminergic compounds in the presence of agonists and/or antagonists of specific receptor subtypes.

**3.** The study of GAL receptor regulated OT release in connection with peptidergic regulation.

**4.** The determination of the interaction between monoaminergic and peptidergic receptor functions of OT release as a probable component of regulation.

## MATERIALS AND METHODS

### *Cell culture technique*

Since pituicytes are the equivalent of modified glial cells, we made use of experience with glial cell cultures, mainly relating to the enzymatic dissocation technique {126,127}. Details of the cell culture procedure were described earlier {128,129,130,131}. Under sterile conditions, pituitary of male Wistar rats weighing 180-230 g was removed under pentobarbital anesthesia (4.5 mg/kg Nembutal, Abbott, USA), immediately after decapitation. The posterior lobe was carefully separated from the intermediate lobe under a preparative microscope. The tissue was digested enzymatically with 0.2% trypsin (Sigma, Germany) in phosphate-buffered saline (PBS) for 60 min., and with 0.05% collagenase (Sigma) for an additional 60 min at 37 ˚C. The enzymatic hydrolysis was stopped by the addition of 100 µg/ml trypsin inhibitor (Sigma). Mechanical disintegration of the tissue was performed on nylon blutex sieves (pore sizes 100 µm, 80 µm and 48 µm in series). The viability was 99-100%, and the cell count was determined to be $2x10^6$/ml. The dispersed cells were placed into 24-well plastic plates (Costar, USA) coated with 5% rat-tail collagen (Sigma). The starting cell density was $10^5$ cells/ml of medium (Dulbecco's modified Eagle's Medium [DMEM]), (Sigma) supplemented with 20% Fetal Calf Serum (Gibco, USA), 100 µg/ml penicillin and 100 µg/ml streptomycin. The cell cultures were maintained at 37 °C in a humidified atmosphere of 5% $CO_2$ in air. The culture medium was changed daily.

### *Incubation procedure*

The OT levels of the cultures were examined on days 13 and 14. The OT content of the medium had become constant by this time, and this period therefore seemed appropriate for the measurement of OT levels {128,129}. From the supernatant media, 500 µl samples were removed and stored at -80 ˚C until peptide RIA was performed {79,129}.

Function of NH cell culture was checked by $K^+$ ($[K^+]$= 30 mM). $[K^+]$ is an aspecific stimulus and it triggers OT release which was measured from the supernatant after 20 min.

| | **pg OT/mg protein** (n:8; ±S.E.M.) |
|---|---|
| **control** | 153,51±1,06 |
| **30 mM $[K^+]$** | 313,3±3,59 |

Table 1.

For the determination of dose effect curves, in the various series, different monoamines (5-HT, HA, ADR, NADR) or GAL were added in different concentrations to each medium and the OT

concentration of condensed media was measured by RIA after incubation for 1 h. In order to study the kinetic curves of OT secretion, the supernatant media were changed after incubation with different drugs for 1h. During the following 90 min, 100 µl samples were removed from the media every 10 min during the first 30 min, and every 30 min thereafter, and the OT levels were determined.

For the study of the interactions of 5-HT and 5-HT antagonists, WAY-100635, ketanserin (KET) and metergoline (MET), $10^{-6}$ M 5-HT was added to each sample of medium following a 20-min pre-incubation with 5-HT. $10^{-6}$ M WAY-100635, $10^{-6}$ M KET and $10^{-6}$ M MET were then administered to the media, and the OT content of the supernatants was measured 1h later. In the other experimental series, the pre-incubation was carried out following the addition of $10^{-6}$ M WAY-100635, $10^{-6}$ M KET and $10^{-6}$ M MET; 20 min later, $10^{-6}$ M 5-HT was administered to each sample of medium, and the hormone concentrations of the supernatant were measured 1 h later.

For the study of interaction of HA and the HA antagonists mepyramine (MEP), cimetidine (CIM) and thioperamide (TPE), $10^{-6}$ M HA was added to each sample of medium following a 20-min preincubation with HA. $10^{-6}$ M MEP, $10^{-6}$ M CIM and $10^{-6}$ M TPE was added to each sample of medium, and the OT contents of the supernatants were measured 1 h later. In the other experimental series, the preincubation was carried out following the addition of $10^{-6}$ M MEP, $10^{-6}$ M CIM and $10^{-6}$ M TPE; 20 min later, $10^{-6}$ M HA was administered to each sample of medium, and the OT concentrations of the supernatant were measured 1 h later.

For the study of the interaction of ADR and the α-receptor antagonists corynanthine (CAT) and yohimbine (YOB) and of NADR and β-receptor antagonist propranolol (PNL) and atenolol (ATL), the antagonist was added in a dose of $10^{-6}$ M to each sample of medium following a 20 min pre-incubation with ADR or NADR, and the OT content of the supernatants was measured 1 h later. In the other experimental series, the pre-incubation was carried out with $10^{-6}$ M CAT or YOB, and 20 min later $10^{-6}$ M ADR was administered; alternatively, $10^{-6}$ M PNL or ATL were administered, and 20 min later, $10^{-6}$ M NADR was added to each sample of medium. In all cases the OT concentration of the supernatant was measured 1 h after administering ADR/NADR.

For the study of interaction of GAL and GAL receptor antagonist, galantid (M15), the pre-incubation was carried out with $10^{-6}$ M M15 and 20 min later, $10^{-6}$ M GAL was administered. The OT concentration of the supernatant was measured 1 h after administering GAL.

For study of interaction GAL and monoamines (ADR, NADR, HA, 5-HT), GAL was added in a dose of $10^{-6}$ M to each sample of medium following a 20 min pre-incubation with $10^{-6}$ M

ADR/NADR/HA/5-HT, and OT content of the supernatants was measured 1 h later. In the other experimental series, the pre-incubation was carried out with $10^{-6}$ M ADR, NADR, HA or 5-HT, and 20 min later, $10^{-6}$ M GAL was administered. In all cases the OT concentration of supernatant was measured 1 h later administering GAL. In the 3rd experimental series, the pre-incubation was carried out with $10^{-6}$ M GAL antagonist M15, 20 min later, $10^{-6}$ M GAL was administered and finally another 20 min later $10^{-6}$ M ADR/NADR/HA/5-HT was added. The OT concentration of the supernatant was measured 1 h later.

All agonists, antagonists and NH hormone used were from Sigma, Steinheim, Germany except: WAY-100635 Tocris Cookson, Bristol, United Kingdom; Galanin (rat, 1-29) and galantid which were synthesized by Dr. L. Balazspiri (Department of Medical Chemistry, University of Szeged, Szeged, Hungary).

***Oxytocin radioimmunoassay***

Hormone contents of cell culture supernatants were determined 2 h after the change of the medium. The OT levels were measured by RIA based on the technique described by Vecsernyés et al {132}. Synthetic OT was used and radiolabeling. OT antibody was generated against the OT-(ε-aminocaproic acid)-thyroglobulin conjugate in rabbits of the New Zealand strain. The cross-reaction was 92.7 % with oxypressin. $^{125}$I-labeling of OT was performed by the chloramines T method {133}. Reverse phase high performance liquid chromatography was used for the purification of the labeled hormone {134}. The standard curve covered the range 1.0-128 pg per assay tube. The sensitivity of the assay for OT was 1 pg/tube. OT levels are given in pg/mg protein.

***Protein measurement***

A modified Lowry method was used for the determination of total protein volume {81,84,135}

***Statistical analysis***

Statistical analysis of OT concentration was performed with the Kruskal–Wallis test. The Kruskal-Wallis one-way analysis of variance by ranks is a non-parametric method for testing equality of population medians among groups. It is identical to a one-way analysis of variance with the data replaced by their ranks {136}.

A probability level of less than 0.05 was accepted as a significant difference vs. control values. Data are reported as standard error of means ±S.E.M.

**RESULTS**

The changes in OT secretion as a function of the time elapsed from the beginning of the culture procedure are demonstrated in Figure 8. X-axis shows the days and the hormone secretion (pg hormone/mg protein) is shown on Y-axis. On days 1 through 4 OT level was undetectable in the supernatant. The OT contents of the medium appeared on day 5 or 6, and gradually increased up to day 13 or 14. The hormone secretion had become constant by this time.

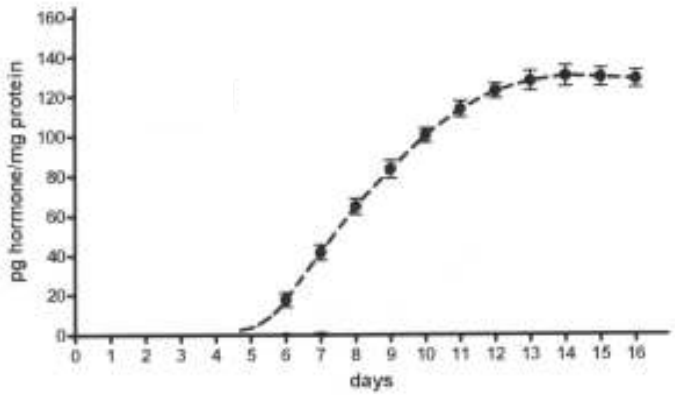


**Figure 8**. OT secretion following the beginning of the culture procedure. The OT contents gradually increased from day 5 or 6, and had become constant by day 13 or 14. (n=10, means ±S.E.M.)

The dose-effect relationships between the HA and OT secretion and 5–HT and OT secretion are demonstrated in Figure 9.

(A) Following the addition of a $10^{-9}$ M dose of HA, a significant increase was observed in the OT concentration in the cell culture medium. After the administration of the increasing doses ($10^{-8}$-$10^{-4}$ M) of HA, a linear enhancement was detected in the OT content of the supernatant medium. The dose-effect correlations between administration of the HA antagonist MEP, CIM or TPE and NH hormone secretion were also studied. The OT concentration was measured following the administration of $10^{-10}$-$10^{-5}$ M doses of MEP, CIM or TPE, but the OT levels did not display any significant changes (n=8, control:145.9±3.8; $10^{-6}$ M MEP: 142.9±2.8; $10^{-6}$ M CIM: 141.6±6.7; $10^{-6}$ M TPE: 144.4±2.9 pg OT/mg protein).

(B) The addition of a $10^{-11}$ M dose of 5-HT, a significant increase was observed in the OT concentration of the cell culture medium. After the administration of increasing doses ($10^{-10}$-$10^{-7}$) of 5-HT, also a linear enhancement was detected in the hormone concentration of the supernatant medium. There was no longer an elevation following the addition of higher doses ($10^{-7}$-$10^{-4}$ M). The dose-effect correlations between the 5-HT antagonist WAY-100635, KET or MET and OT secretion were also studied. (The OT concentrations were measured following administration of $10^{-10}$-$10^{-5}$ M doses of WAY-100635, KET or MET, but the OT hormone levels did not display any significant changes (n=8, control: 149.5±9.7; $10^{-6}$ M WAY-100635: 150.4±13.2; $10^{-6}$ M KET: 147.4±10.8; $10^{-6}$ M MET: 143.1±10.1 pg OT/mg protein).

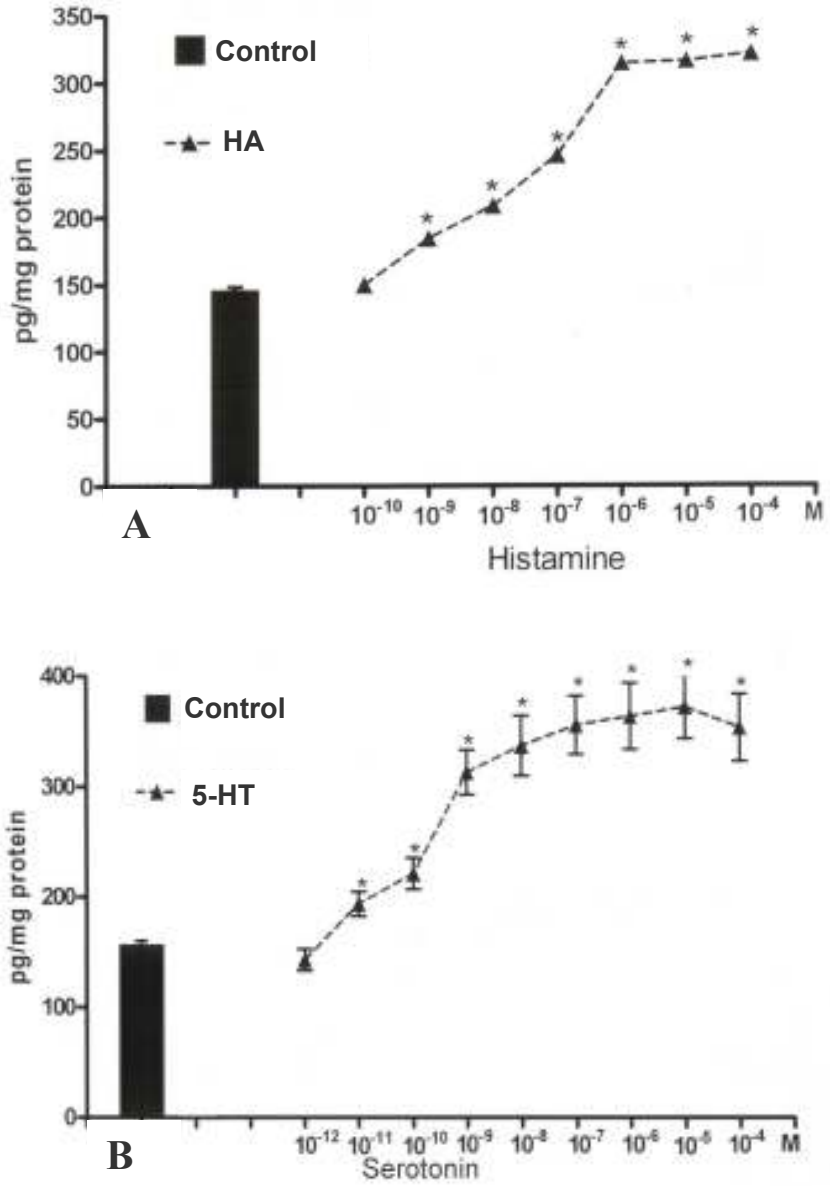


**Figure 9**. Dose-effect relationship between HA and OT releasing as well as 5-HT and OT releasing. (A) Following the administration of increasing doses ($10^{-10}$-$10^{-4}$ M) of HA, a linear enhancement was detected in the OT contents of the supernatant medium. (B) In the case of 5–HT protocol following the addition of a $10^{-11}$ M dose of 5-HT significant increase was observed in the OT concentration of the cell culture media. (n=8, means ±S.E.M., * a significant difference vs. the control)

The dose-effect relationships between the ADR and OT secretion and NADR and OT secretion are demonstrated in Figure 10.

(A) With the addition of a $10^{-9}$ M dose of ADR, a significant increase was observed in the OT concentration of the cell culture medium. After the administration of the increasing doses ($10^{-8}$-$10^{-5}$ M) of ADR, a linear enhancement was detected in the OT content of the supernatant medium. The dose-effect correlations between administration of the ADR antagonist CAT and YOB, and OT secretion were also studied. The OT concentration was measured following the administration of $10^{-8}$-$10^{-5}$ M doses of CAT and YOB, but the OT levels did not display any significant changes (n=10, control: 145.9±3.8; $10^{-6}$ M CAT: 155.18±2.31; $10^{-6}$ M YOB: 155.12±3.02 pg OT/mg protein).

(B) Following the addition of a $10^{-8}$ M dose of NADR, a significant increase was observed in the OT concentration of the cell culture medium. After the administration of increasing doses ($10^{-7}$-$10^{-5}$) of NADR, also a linear enhancement was detected in the hormone concentration of the supernatant medium. The dose-effect correlations between administration of the NADR antagonist ATL and PNL, and OT secretion were also studied. The OT concentration was measured following the administration of $10^{-8}$-$10^{-5}$ M doses of ATL and PNL, but the OT levels did not display any significant changes (n=6, control: 145.9±3.8; $10^{-6}$ M ATL: 152.76±2.13; $10^{-6}$ M PNL: 153.13±3.08 pg OT/mg protein).

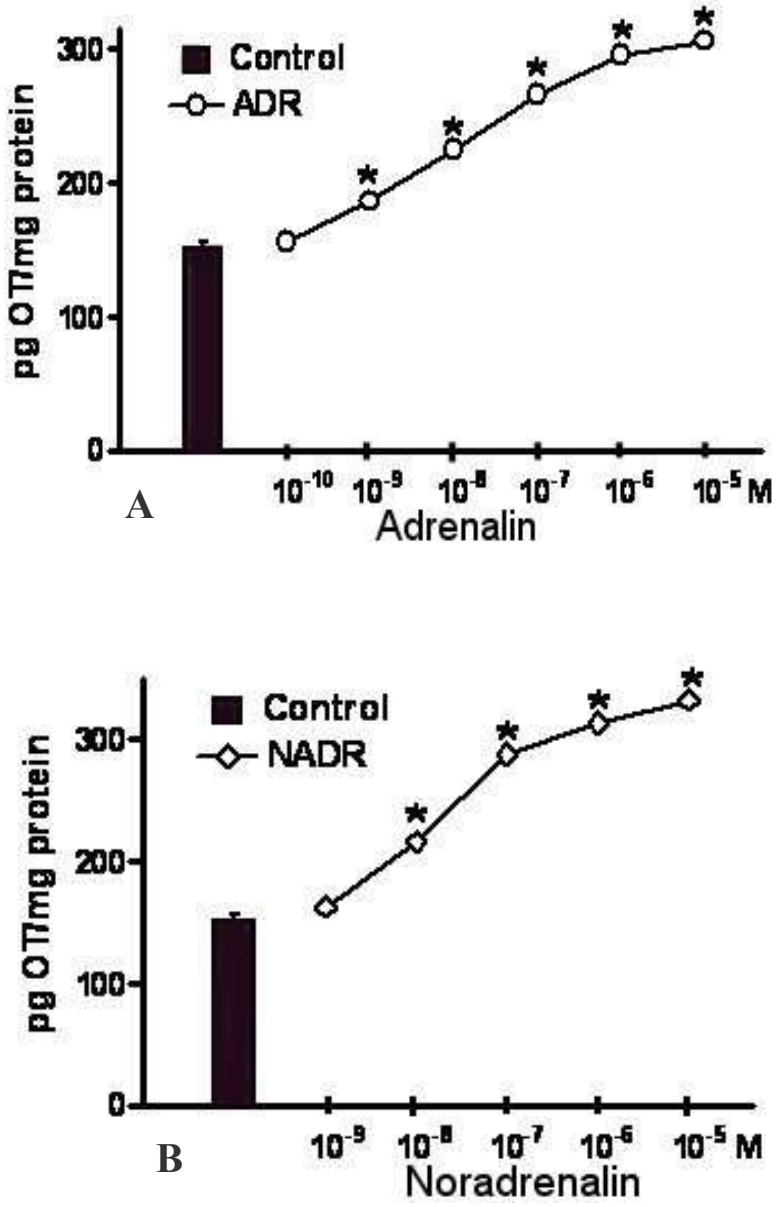


**Figure 10**. Dose-effect relationship between ADR and OT secretion as well as NADR and OT secretion. (A) Following the administration of increasing doses ($10^{-9}$-$10^{-5}$ M) of ADR, a linear enhancement was detected in the OT contents of the supernatant medium. (B) In the case of NADR protocol following the addition of a $10^{-8}$ M dose of NADR significant increase was observed in the OT concentration of the cell culture media. (n=10 or 6, means ±S.E.M., * a significant difference vs. the control)

The dose-effect relationship between the GAL and OT secretion is shown in Figure 11.

In the presence of $10^{-9}$-$10^{-6}$ M doses of GAL, significant decrease was observed in the OT secretion of the cell culture medium. The dose-effect correlation between administration of the GAL antagonist galantid (M15) and NH hormone secretion was also studied. The OT concentration was measured following the administration of $10^{-8}$-$10^{-5}$ M doses of M15, but the OT levels did not display any significant changes (n=5, control: 145.9±3.8; $10^{-6}$ M M15: 153.14±2.68; pg OT/mg protein).

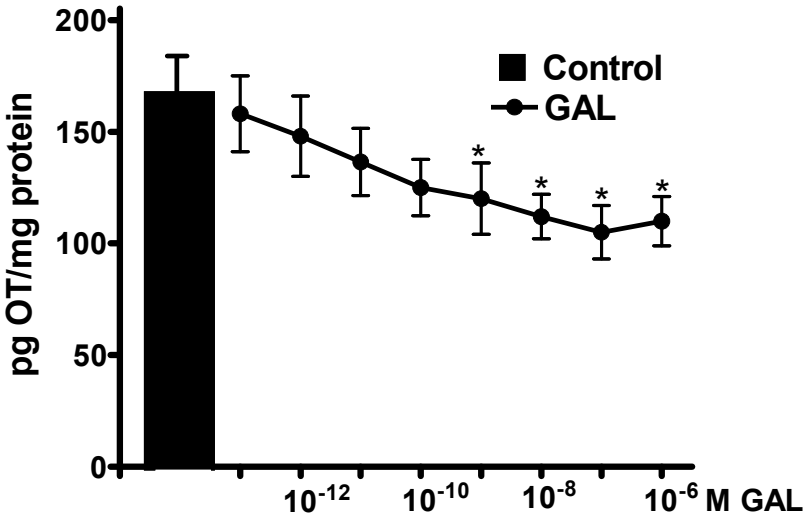


**Figure 11**. Dose-effect relationship between GAL and OT releasing. Following the administration of $10^{-9}$-$10^{-6}$ M doses of GAL, a decrease was detected in the OT contents of the supernatant medium. (n=5, means ±S.E.M., * a significant difference vs. the control)

The kinetic curves of the OT levels in the supernatant media are depicted in Figure12.

Following HA/5-HT administration, a significant increase was detected in the OT concentrations of the cell culture media. During the 20 min (in the case of 5–HT protocol, 30min) observation period, the OT levels rose abruptly in the control medium and remained constantly high after 20 min (in the case of 5–HT protocol, 30min).

(A) HA administration significantly increased OT production. A partial blocking of OT secretion was observed following HA antagonist+HA administration. The OT level increased significantly 10 min after the addition of MEP, CIM or TPE+HA to the supernatant media. However, the enhancement of the OT curves lessened in comparison with that measured following HA administration without MEP or CIM pre-incubation. TPE did not influence the OT release enhancement induced by HA.

(B) 5-HT administration significantly increased OT production. A partial blocking effect of OT secretion was observed following 5-HT antagonist and 5-HT administration. The OT levels were increased significantly 10 min after WAY-100635, KET or MET+5-HT addition to the supernatant media. However, the enhancement of the OT curves was reduced in comparison with the OT contents measured following 5-HT administration without 5-HT antagonist pre-incubation.

The kinetic curves of the OT levels in the supernatant media are depicted in Figure 13.

Following ADR/NADR administration, a significant increase was detected in the OT concentrations of the cell culture media. During the 30 min observation period, the OT levels rose abruptly in the control medium and remained constantly high after 30 min.

(A) ADR administration significantly increased OT production. A partial blocking of OT secretion was observed following ADR antagonist+ADR administration. The enhancement of the OT curves lessened in comparison with that measured following ADR administration without CAT pre-incubation. YOB did not influence the OT release enhancement induced by ADR.

(B) NADR administration significantly increased OT production. A partial blocking effect of hormone secretion was observed following NADR antagonist and NADR administration. The enhancement of the OT curves decreased in comparison with the OT contents measured following NADR administration without PNL pre-incubation. ATL did not influence the OT release enhancement induced by NADR.

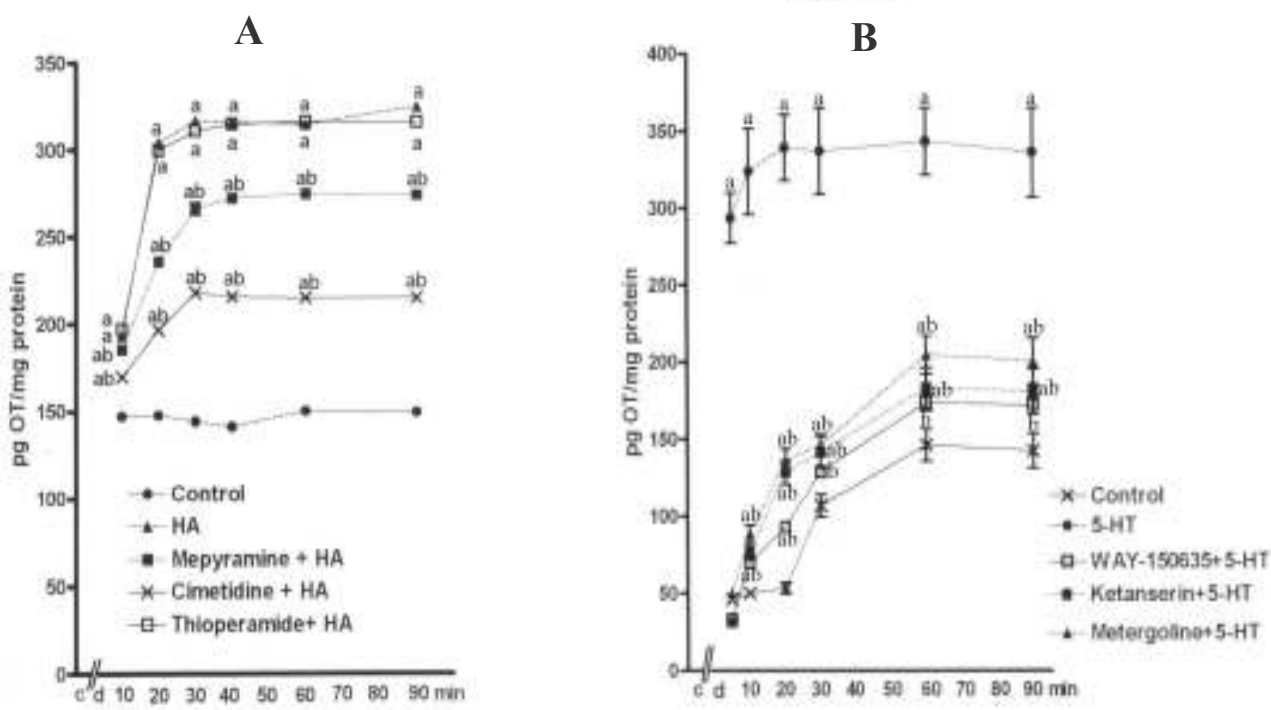


**Figure 12.** Kinetic curves of OT secretion as functions of the incubation time (min). (A) HA or 5-HT administration significantly increased the hormone concentration. A partial blocking effect of OT secretion was observed following the addition of the HA antagonist MEP or CIM before HA administration. TPE did not influence the HA induced OT concentration enhancement. (B) A partial blocking effect of elevation of OT secretion was observed following each 5-HT antagonist before 5-HT administration.

(n=6 or 8, means±S.E.M., a: significant difference vs. the control, b: significant difference vs. HA/5-HT administration, c: pre-incubation time 60 min., d: washing 3x)

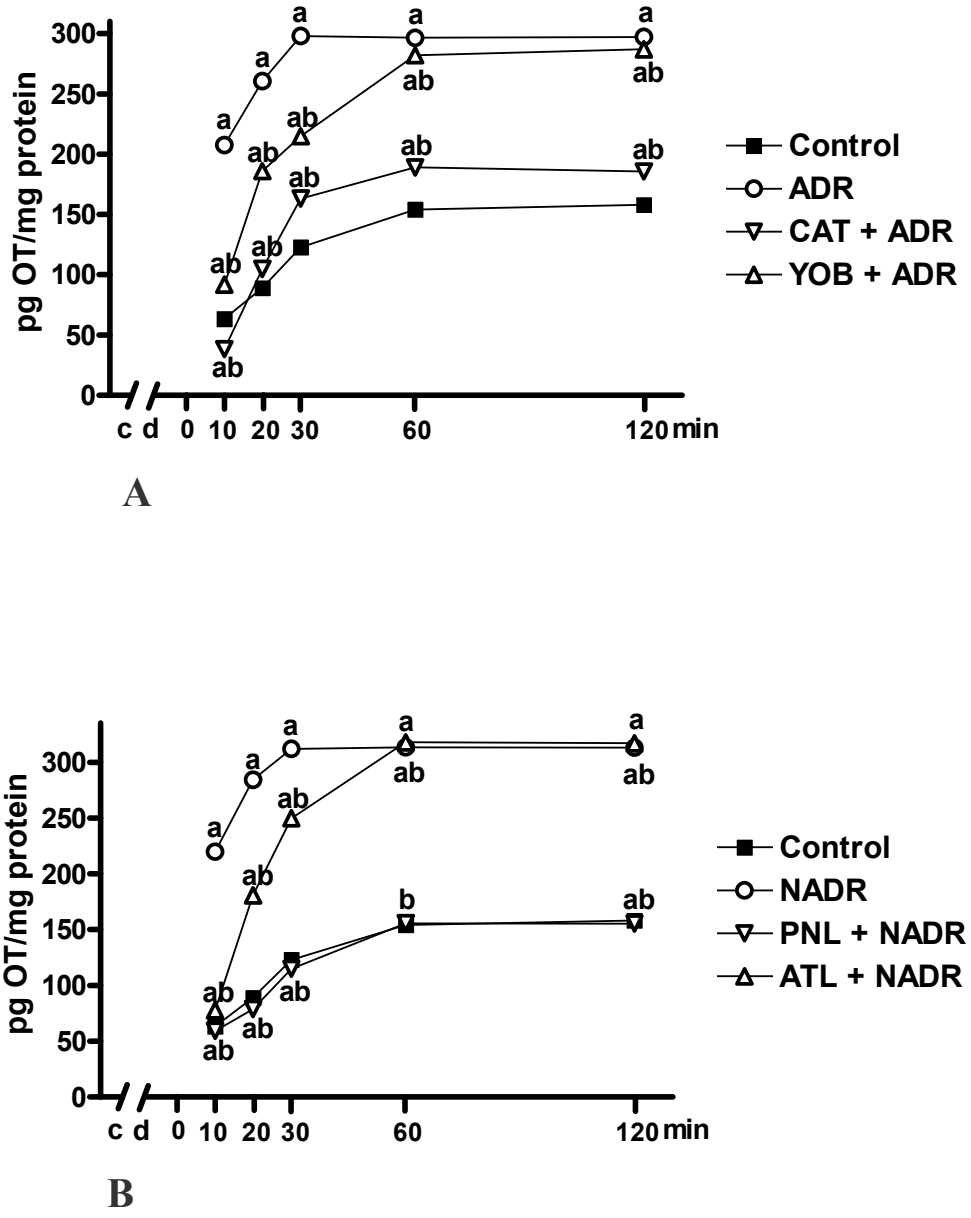


**Figure 13.** Kinetic curves of OT secretion as functions of the incubation time (min). ADR and NADR administration significantly increased the hormone concentration. (A) A partial blocking effect of OT secretion was observed following the addition of the ADR antagonist CAT before ADR administration. YOB did not influence the ADR induced OT concentration enhancement. (B) A partial blocking effect of the elevation of OT secretion was observed following NADR antagonist PNL before NADR administration. ATL did not influence the NADR induced OT concentration enhancement. (n=6 or 8, means±S.E.M., a: significant difference vs. the control, b: significant difference vs. ADR/NADR administration, c: pre-incubation time 60 min., d: washing 3x)

The kinetic curves of the OT levels in the supernatant media are depicted in Figure 14.
Following GAL administration, a significant decrease was detected in the OT concentrations of the cell culture media compared to control. A partial blocking of OT decreased secretion was observed following GAL antagonist (M15) +GAL administration. The reduction of the OT curves was enhanced in comparison with that measured following GAL administration without M15 pre-incubation.

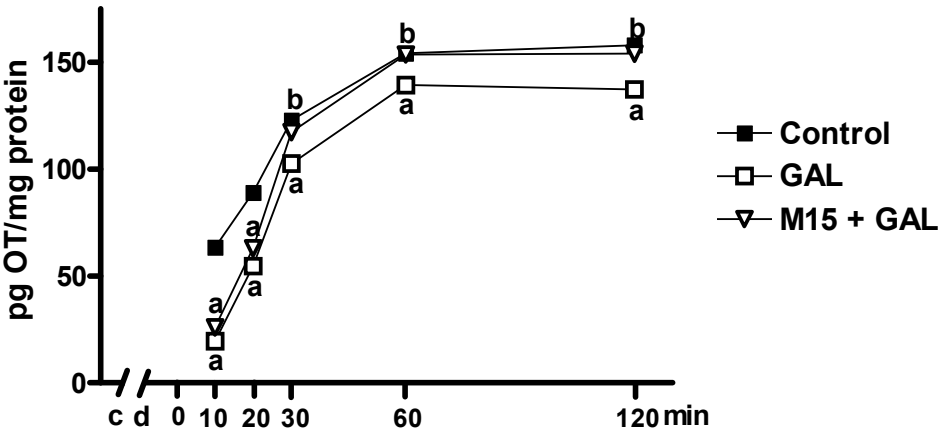


**Figure 14.** Kinetic curves of OT releasing as functions of the incubation time (min). GAL administration significantly decreased the hormone concentration. M15 administration before GAL treatment blocked the decreasing effect of GAL on the OT secretion. (n=6, means±S.E.M., a: significant difference vs. the control, b: significant difference vs. GAL administration, c: pre-incubation time 60 min., d: washing 3x)

Elevated hormone secretion were observed after HA/5-HT administration in Figure 15.

The OT concentrations did not change after the administration of the HA antagonists to the control medium (A). When the MEP and CIM treatments were applied before HA administration, the HA-induced elevations of the OT levels significantly decreased, but the levels remained above the control concentrations. TPE did not significantly inhibit the hormone secretion increase when administered before HA. The HA antagonists did not significantly reduce the OT levels when HA was administered before MEP, CIM or TPE treatment.

After administration of the 5-HT antagonists to the control medium, the OT concentration did not change (B). When the WAY-100635, KET and MET treatments were applied before 5-HT administration, the 5-HT-induced elevation of the hormone levels significantly decreased, but they remained above the control concentrations. The 5-HT antagonists did not significantly reduce the hormone levels when 5-HT was administered before WAY-100635, KET or MET treatment.

Figure 16. shows the effect of ADR, NADR and their antagonist.

Following incubation for 1 h, elevated OT production was observed after ADR administration (A). The OT concentration did not change after the addition of the α-receptor antagonists CAT or YOB to the control medium. When CAT treatment was applied before ADR administration, the ADR-induced increase of OT release was blocked. YOB did not inhibit the OT secretion enhancement when administrated before ADR. The α-receptor antagonists did not significantly reduce the OT levels when ADR was administered before CAT or YOB treatment.

In the second series (B), the effect of NADR on the OT secretion was studied in the NH cell culture. After incubation with NADR for 1 h, an increased OT content was observed. The OT concentrations did not change following the administration of the β-receptor antagonist PNL and ATL. When the PNL was added before the NADR treatment, β-receptor antagonist PNL prevented the NADR-induced increase of OT release. When the NADR treatment preceded PNL administration, the OT concentration did not increase. ATL did not inhibit the OT secretion enhancement when administrated before NADR.

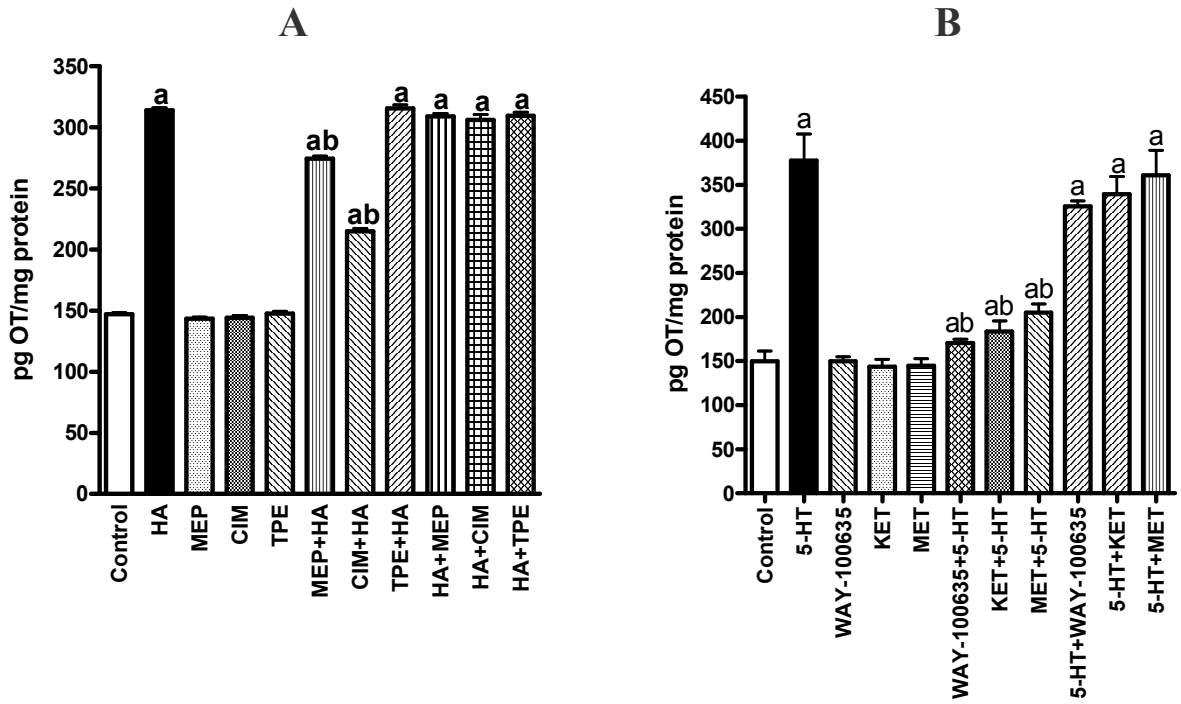


**Figure 15.** Effect of HA and HA antagonist (MEP, CIM, TPE) compounds as well as 5-HT and 5-HT antagonist (WAY-100635, KET, MET) compounds on OT secretion. Following incubation for 1 h elevated hormone levels were observed after HA/5-HT administration. (A) HA antagonist and 5-HT antagonist treatments did not induce any changes themselves in the OT secretion. When MEP or CIM treatment was applied before HA administration the HA-induced OT decreased. TPE did not block the HA-induced hormone enhancements. When HA was administered before the HA antagonists, there was no significant reduction in OT concentration increase. (B) Each 5-HT antagonist application before 5-HT administration reduced the 5-HT induced OT elevation. When 5-HT was administered before the 5-HT antagonists, there was no significant reduction in the increased OT concentration. (n=8, means±S.E.M., a: significant difference vs. control, b: significant difference HA/5-HT administration)

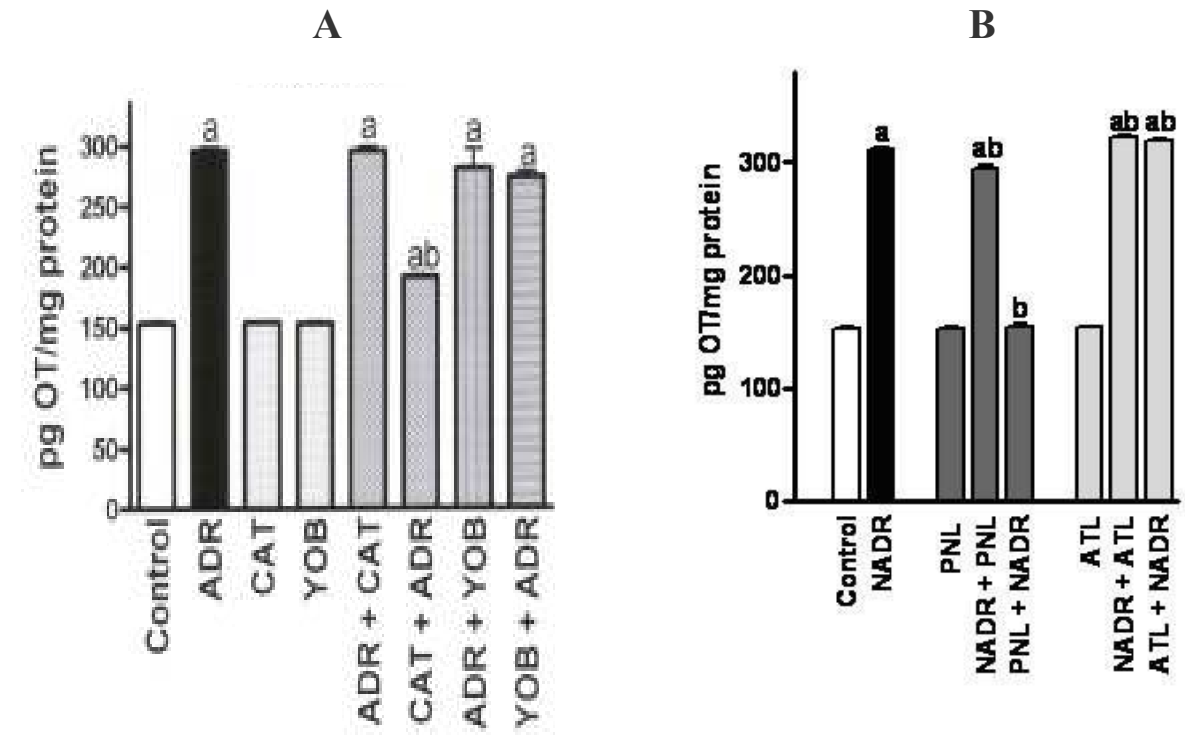


**Figure 16.** (A) Effects of ADR and adrenalin antagonists (CAT, YOB) as well as (B) NADR and NADR antagonist (PNL, ATL) on OT release. Following incubation for 1 h elevated hormone levels were observed after ADR/NADR administration. (A) ADR and NADR antagonist treatments themselves did not induce any changes in hormone secretion. When ADR or NADR was administered before the antagonists there was no significant reduction in OT concentration increase. When CAT and PNL were applied before catecholamine administration the induced OT levels decreased. YOB and ATL did not block ADR and NADR induced hormone enhancement. (n=6, means±S.E.M., a: significant difference vs. control, b: significant difference ADR/NADR administration)

Figure 17. represents interaction of GAL and monoamine-system in connection with the OT secretion.

Following pre-incubation for 1 h, reduced OT release was observed after GAL administration. The OT concentration did not change after the addition of the GAL antagonist M15 to the control medium.

When GAL treatment was applied before monoamine (ADR, NADR, HA and 5–HT) administration, the monoamine-induced increase of OT release was blocked. When monoamine treatments were applied before GAL administration, OT secretion was enhanced. When M15 treatment precedes GAL pre-incubation and this was followed by administration of the monoamines, monoamine-induced OT release was observed.

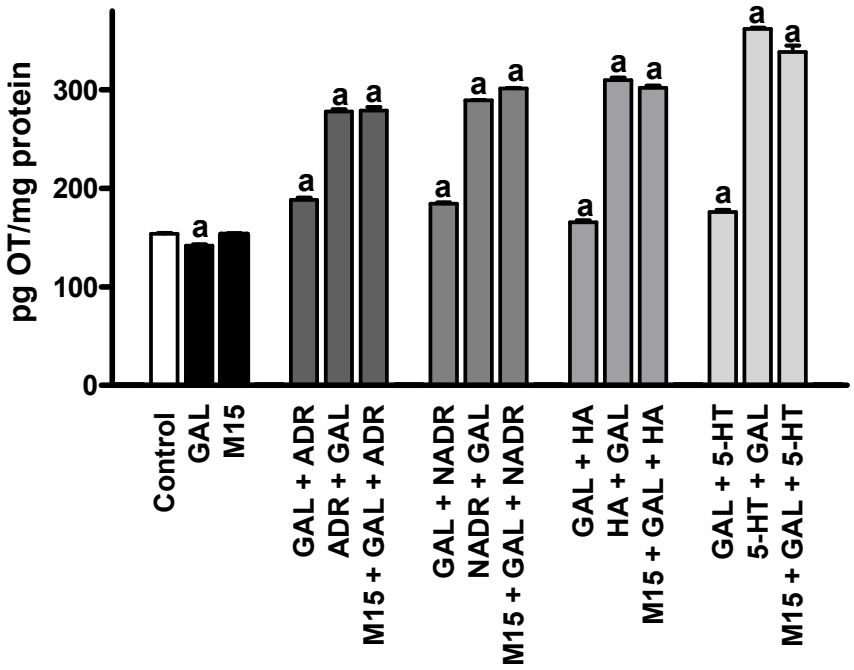


**Figure 17.** Effects of GAL monoamine-induced OT release. GAL blocked the enhancing effect of monoamines on OT secretion. GAL antagonist, M15, inhibited GAL-reduced action on ADR, NADR, HA and 5–HT induced OT release. (n=6, means±S.E.M., a: significant difference vs. control)

## DISCUSSION

Many data have been reported regarding the question of whether the pituicytes can synthesize neuropeptides. OT present in the NH is synthesized in the hypothalamic neurons, located in the SON and the PVN and the other accessory cell groups. Synthesized OT is packed in neurosecretory granules and transported by axons to the NH, where it is secreted into the circulation {5,18}. In accordance with this generally accepted hypothesis, the NH plays a role only as a storage site for OT {142} and the morphological and functional experiments have generally focused interest on the hypothalamic regulation.

Release of the hormones from the posterior pituitary reflects the convergence of extensive afferent pathways on the MCNs. These afferent pathways transmit information about physiological parameters (such as blood pressure and volume, reproductive functions, fluid and electrolyte balance, stress). These afferents utilize a broad spectrum of chemicals including all recognized fast-acting and classical neurotransmitters as well as a large number of neuropeptides {138}.

In contrast with the storage of NH, FA. Laszlo and his colleagues earlier found that both VP and OT are produced by the pituicytes in isolated NH cell cultures {127}. This finding is supported by different experimental series {79,80,87}.

Accordingly, the role of NH cells (pituicytes) in the monoamine-induced changes in OT secretion is not a well-studied problem.

There is sufficient evidence for innervations of OT in the SON, the PVN and anterior commissural nuclei by NADR-ergic, dopaminergic and 5–HTergic fibers.

The ***ADR-***ergic system is an essential regulator of neuronal, endocrine, cardiovascular, vegetative, and metabolic functions. There adrenoceptors can be divided into three different groups: $\alpha_1$-receptors ($\alpha_{1A}$, $\alpha_{1B}$, $\alpha_{1D}$), $\alpha_2$-receptors ($\alpha_{2A}$, $\alpha_{2B}$, $\alpha_{2C}$), and β-receptors ($\beta_1$, $\beta_2$, $\beta_3$). The $\alpha_1$-receptor type usually mediates responses in the effector organ. $\alpha_2$-receptors are essential feedback regulators of neurotransmitter release. $\alpha_2$-adrenoceptors can also regulate a number of other neurotransmitters in the central and peripheral nervous systems. $\alpha_2$-receptors are involved in the regulation of pain perception and a number of behavioral functions. Both alpha-adrenoceptors are important for the control of vascular tone. β-receptors are mostly known for

their role in the regulation of cardiovascular, uterine and peripheral metabolic functions {143,144}.

We found that CAT {145} totally prevented the ADR induced elevation of OT when the $\alpha_1$-receptor antagonist treatment was applied before ADR administration, while the $\alpha_2$-receptor antagonist, YOB {146} proved ineffective in blocking the OT elevation induced by ADR. Accordingly, we conclude that $\alpha_1$-receptors are involved in the ADR induced increases of OT secretion in isolated NH cell culture. The $\beta_{1+2}$-receptor antagonist, PNL {147} has a preventive effect when administered before NADR addition, however, $\beta_1$ receptor antagonist, ATL did not block NADR induced hormone enhancement. Consequently the $\beta_2$ receptor has a role in the NADR mediated OT secretion in primer NH cell cultures. These data support earlier result described by authors, NADR, acting via α-1 subtype, is a critical neuromessenger for the transduction of the suckling signal to the OT secretory response {86,137}. Stimulation of beta-ADR-ergic receptor inhibits OT secretion; this may be due, at least in part, to an action of an adrenal catecholamine, which may act centrally and/or directly on the NH {140}. Our results lead us to conclude that there is no significant difference between ADR and NADR-induced increases of OT release.

***5–HT*** -immunoreactive fiber areas are concentrated in specific parts of the parvocellular division of the PVN, whereas in the magnocellular division of the nucleus, and in the SON, they are found mostly in regions where OT-erg cells predominate {97}.

15 receptor subtypes in the groups have been recognized {148}: the 5-$HT_1$ subfamily of receptors contains subtypes 5-$HT_{1A-F}$, the 5-$HT_2$ contains subtypes 5-$HT_{2A-C}$, and finally, the subfamily of miscellaneous 5-HT receptors contains subtypes 5-$HT_{3-7}$. 5-HT receptors participate in the physiological functions. The restraint stress-induced OT response seems to be mediated via the 5-$HT_{1A}$, the 5-$HT_{2A}$ and the 5-$HT_{2C}$ receptors. The dehydration and hemorrhage-induced OT responses are mediated by at least 5-$HT_{2A}$ and 5-$HT_{2C}$ {82}.

In our present study, the selective 5-$HT_1$ receptor antagonist WAY-100635, the 5-$HT_2$ receptor antagonist KET {149,150} and the 5-$HT_{1,2}$ receptor antagonist MET {151} were used. We found that every antagonist (WAY-100635, KET, MET) significantly reduced the 5-HT-induced elevation of OT when the 5-HT antagonist treatment was applied before 5-HT administration. Accordingly, we conclude that the 5-$HT_1$ and 5-$HT_2$ receptors are involved in the 5-HT induced increase of OT secretion in isolated NH cell cultures. This observation is in accordance with

Jørgensen's finding, 5-HT induced OT secretion involves 5-$HT_{1A}$, 5-$HT_{2C}$ and 5-$HT_4$ receptors {83}.

The neurotransmitter ***HA*** participates in the neuroendocrine regulation of pituitary hormone secretion by an indirect action at the hypothalamic level where HA-ergic neurons are abundant. OT is stimulated by HA, probably by an effect of the SON and PVN {85}.

To date, four HA receptors have been recognized {152,153}: the $H_1$ receptor gene was cloned by Yamashita et al. in 1991 {154} and the $H_2$ receptor by Gantz et al. 1991 {155}. The identification of the $H_3$ receptor came nearly a decade later {156}, and recently the $H_4$ was discovered {110}. Concerning the functions of the HA receptors, many findings have been published, but not all their activities have been fully defined. The $H_1$ receptor is involved in allergic manifestations. The $H_2$ receptor controls gastric acid secretion in the gut. The $H_3$ receptor plays an important role in neurotransmitter release in the CNS. The $H_4$ receptor might modulate immune cell functions. We found that both MEP and CIM {157} significantly reduced the HA-induced elevation of OT when the HA antagonist treatment was applied before HA administration. Accordingly, we conclude that mainly the $H_1$ and $H_2$ receptors are involved in the HA-induced increases of OT secretion in isolated NH cell cultures, while the $H_3$+$H_4$ receptor antagonist, thioperamide proved ineffective in blocking the OT elevation induced by HA. This observation is in accordance with the findings of other authors, HA stimulates OT secretion via activation of postsynaptic $H_1$ and $H_2$ receptors {86,139}. HA acts centrally, via $H_1$-receptors, during partition and may have an excitatory effect on OT release. $H_2$ receptors may have a dual effect, increasing the synthesis of OT while inhibiting its premature release {141}.

ADR-ergic antagonists can moderate the OT release-increasing effect of ADR or NADR only if the antagonists are administered before ADR or NADR treatment; if ADR or NADR precedes the application of the antagonists, the adrenergic receptor antagonists prove ineffective: the increase in hormone release is not changed at all. We observed the same phenomenon in connection with 5-HT or HA regulation. The 5-HT- or HA–induced elevation of NH hormone secretion in NH cell cultures could be blocked by prior administration of 5-HT or HA antagonists; after this 5-HT or HA treatment proved ineffective. This phenomenon can be explained in that a 20-min period is probably long enough for the OT-increasing action of ADR or NADR, and thus the ADR-ergic antagonists are ineffective.

The role of ***GAL*** in the regulation of OT secretion is not a totally solved problem. In this regard, earlier studies have indicated an apparent absence of specific GAL-binding sites in the rat

pituitary gland {158}. GAL also failed to affect OT secretion from neurosecretosomes (isolated neurosecretory terminals) {159}, suggesting the absence of receptors for the full-length peptide in the NH {160}. It was recently described that GAL acts via at least three receptor subtypes, which differ in amino acid sequence, distribution, pharmacology and signal transduction {161}. MCNs in the PVN and SON express GALR1 receptors {162,163}, whereas they do not express GALR2 receptors {162,164}. GALR2 mRNA is reported to be present in the anterior but not the posterior pituitary, and GALR1 transcripts are not found in this tissue {165}. The low levels of the GALR3 receptor were expressed in the pituicytes {166}. Study of the role of the NH resulted in the observation of the expressions of a number of peptides, including GAL, in the rat NH, and some of these peptides have been shown to modulate the secretion of OT *in vitro* {142,167}. The present findings and our earlier observation {80} indicate that NH hormone can be directly influenced by GAL-ergic system, and the GAL-ergic control of NH hormone secretion in rat occurs independently of the HT, at the level of the NH. Our results permit the supposition that GAL receptors exist on the membranes of pituicytes cultured *in vitro*, but the potentially involved GAL receptor subtypes are still unknown.

It is interesting that GAL can moderate the NH hormone release-increasing effect of monoaminergic compounds only if GAL is administered before monoaminergic treatment; if monoaminergic compound administration precedes the application of GAL, galanin proves ineffective: the increase in OT releasing is not changed at all. We observed the same phenomenon when M15 administration precedes the application of GAL which followed by monoaminergic compounds administration: OT secretion enhanced in all cases. We demonstrate GAL has a moderate decreasing effect.

## SUMMARY

1. A biologically active standard NH cell culture was performed. Pituicyte cultures are good models for the examination of OT release. The released OT was measured by modified RIA.
The secretion of OT was influenced by monoaminergic compounds (ADR, NADR, 5-HT, HA) and GAL in the NH cell cultures. These changes are presented by dose- and time-kinetic curves.

2. ADR and NADR dose-dependently increase the OT-level in the supernatant. There was no significant difference between ADR- and NADR-induced OT releases. Using the specific α and β receptor antagonists we conclude that $\alpha_1$-receptors are involved in the ADR-induced increase of OT secretion, while the $\beta_2$-receptor has a role in NADR-mediated OT secretion.

3. A linear enhancement was detected in the OT content of the supernatant following the administration of increasing doses of 5-HT. Based on our observations; we conclude that the 5-$HT_1$ and 5-$HT_2$ receptors are involved in the 5-HT induced increase of OT secretion in NH cell cultures.

4. A dose-effect relationship was detected between HA and increasing OT in the supernatant. Our results show that $H_1$ and $H_2$ receptors are involved in the HA-induced increase of OT release in NH cell cultures.

5. Following the administration of $10^{-6}$ M and $10^{-5}$ M of GAL, a decrease could be seen in the OT content of the supernatant. Using the GAL antagonist galantid (M15) before the GAL administration, the decrease was prevented.

6. When GAL was administered before monoamine (ADR, NADR, 5-HT, HA) treatment, the monoamine-induced increase of OT secretion was blocked. Our results permit the supposition that GAL receptors exist on membranes of cultured pituicytes *in vitro*, but the potentially involved GAL receptor subtypes are still unknown.

7. Our results indicate that OT release is influenced directly by the monoaminergic system. The monoaminergic control of OT secretion from the NH tissue and the interaction between the monoaminergic and GAL-ergic systems from the aspect of OT secretion occur independently from the HT at the level of the posterior pituitary.

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