The Oxytocin Receptor System:
Structure, Function, and Regulation

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Gimpl, Gerald, and Falk Fahrenholz. The Oxytocin Receptor System: Structure, Function, and Regulation. *Physiol Rev* 81: 629–683, 2001.—The neurohypophysial peptide oxytocin (OT) and OT-like hormones facilitate reproduction in all vertebrates at several levels. The major site of OT gene expression is the magnocellular neurons of the hypothalamic paraventricular and supraoptic nuclei. In response to a variety of stimuli such as suckling, parturition, or certain kinds of stress, the processed OT peptide is released from the posterior pituitary into the systemic circulation. Such stimuli also lead to an intranuclear release of OT. Moreover, oxytocinergic neurons display widespread projections throughout the central nervous system. However, OT is also synthesized in peripheral tissues, e.g., uterus, placenta, amnion, corpus luteum, testis, and heart. The OT receptor is a typical class I G protein-coupled receptor that is primarily coupled via G_q proteins to phospholipase C-β. The high-affinity receptor state requires both Mg^{2+} and cholesterol, which probably function as allosteric modulators. The agonist-binding region of the receptor has been characterized by mutagenesis and molecular modeling and is different from the antagonist binding site. The function and physiological regulation of the OT system is strongly steroid dependent.

However, this is, unexpectedly, only partially reflected by the promoter sequences in the OT receptor gene. The classical actions of OT are stimulation of uterine smooth muscle contraction during labor and milk ejection during lactation. While the essential role of OT for the milk let-down reflex has been confirmed in OT-deficient mice, OT's role in parturition is obviously more complex. Before the onset of labor, uterine sensitivity to OT markedly increases concomitant with a strong upregulation of OT receptors in the myometrium and, to a lesser extent, in the decidua where OT stimulates the release of $PGF_{2\alpha}$. Experiments with transgenic mice suggest that OT acts as a luteotrophic hormone opposing the luteolytic action of $PGF_{2\alpha}$. Thus, to initiate labor, it might be essential to generate sufficient $PGF_{2\alpha}$ to overcome the luteotrophic action of OT in late gestation. OT also plays an important role in many other reproduction-related functions, such as control of the estrous cycle length, follicle luteinization in the ovary, and ovarian steroidogenesis. In the male, OT is a potent stimulator of spontaneous erections in rats and is involved in ejaculation. OT receptors have also been identified in other tissues, including the kidney, heart, thymus, pancreas, and adipocytes. For example, in the rat, OT is a cardiovascular hormone acting in concert with atrial natriuretic peptide to induce natriuresis and kaliuresis. The central actions of OT range from the modulation of the neuroendocrine reflexes to the establishment of complex social and bonding behaviors related to the reproduction and care of the offspring. OT exerts potent antistress effects that may facilitate pair bonds. Overall, the regulation by gonadal and adrenal steroids is one of the most remarkable features of the OT system and is, unfortunately, the least understood. One has to conclude that the physiological regulation of the OT system will remain puzzling as long as the molecular mechanisms of genomic and nongenomic actions of steroids have not been clarified.

I. INTRODUCTION

The neurohypophysial hormone oxytocin (OT) was the first peptide hormone to have its structure determined and the first to be chemically synthesized in biologically active form (143). It is named after the "quick birth" ($\omega\kappa\upsilon\xi$ = quick; $\tau o\kappa o\xi$ = birth) which it causes due to its uterotonic activity (124). OT was also found to be responsible for the milk-ejecting activity of the posterior pituitary gland (428). The structure of the OT gene was elucidated in 1984 (270), and the sequence of the OT receptor was reported in 1992 (299).

OT is a very abundant neuropeptide. This became obvious in a study where the most prevalent hypothalamic-specific mRNAs were analyzed. OT was found to be the most abundant of 43 transcripts identified (202). Today, we recognize that OT exerts a wide spectrum of central and peripheral effects. The actions of OT range from the modulation of neuroendocrine reflexes to the establishment of complex social and bonding behaviors related to the reproduction and care of the offspring. Overall, the cyclic nonapeptide OT and its structurally related peptides facilitate the reproduction in all vertebrates at several levels.

In this review, we summarize the present knowledge of the OT receptor system gained in the different fields of research, thereby focusing mainly on the work over the past decade. For more details to the different topics, the reader is referred to many excellent reviews that have been recently published (1, 42, 43, 155, 160, 161, 261, 265, 266, 295, 298, 335, 369, 389, 413, 443, 572, 573, 621). In the following two sections, we describe the structural features of the OT receptor system on the molecular level. OT has long been considered to be restricted to stimulation of uterine contractions during labor and milk ejection

during lactation. These classical functions of the OT system are treated in the first parts of section IV. The fact that OT is found in equivalent concentrations in the neurohypophysis and plasma of both sexes suggests that OT has further physiological functions. The expression of OT and its receptor has now been identified in a variety of peripheral tissues. For example, evidence was provided that OT acts in concert with atrial natriuretic peptide (ANP) in the control of body fluid and in cardiovascular homeostasis in the rat. It is important to note that most of our knowledge about functions of OT derives from studies with rats. However, localization and expression patterns of OT receptors show marked species differences, suggesting that some of the described OT activities may be species specific. Over the past decade, particularly the central actions of OT have been intensively studied revealing a profound regulation by steroids. The regulation by steroids is in fact a common theme throughout the review and is probably the most remarkable feature of the OT receptor system. Finally, in the last section, we summarize the contributions that have been reported on behavioral effects mediated or modulated by OT. Ironically, the classical "oxytocic" function of OT is again open for discussion due to the results with OT-deficient mice.

II. OXYTOCIN AND OXYTOCIN-LIKE PEPTIDES

A. Evolutionary Aspects

All neurohypophysial hormones are nonapeptides with a disulfide bridge between Cys residues 1 and 6. This results in a peptide constituted of a six-amino acid cyclic part and a COOH-terminal α-amidated three-residue tail. Based on the amino acid at position 8, these peptides are

TABLE 1. *Oxytocin and related peptides*

	1	2	3	4	5	6	7	8	9	
Oxytocin	Cys	Tyr	Ile	Gln	Asn	Cys	Pro	Leu	Gly(NH$_2$)	Placentals, some marsupials, ratfish (*Hydrolagus colliei*)
Mesotocin	*	*	*	*	*	*	*	Ile	*	Marsupials, nonmammalian tetrapods, lungfishes
Isotocin	*	*	*	Ser	*	*	*	Ile	*	Osteichthyes
Glumitocin	*	*	*	Ser	*	*	*	Gln	*	Skates (Chondrichthyes)
Valitocin	*	*	*	*	*	*	*	Val	*	Sharks (Chondrichthyes)
Aspargtocin	*	*	*	Asn	*	*	*	*	*	Sharks (Chondrichthyes)
Asvatocin	*	*	*	Asn	*	*	*	Val	*	Sharks (Chondrichthyes)
Phasvatocin	*	*	Phe	Asn	*	*	*	Val	*	Sharks (Chondrichthyes)
Cephalotocin	*	*	Phe	Arg	*	*	*	Ile	*	*Octopus vulgaris* (Molluscs)
Annetocin	*	Phe	Val	Arg	*	*	*	Thr	*	*Eisenia foetida* (Annelids)
Vasotocin	*	*	*	*	*	*	*	Arg	*	Nonmammalian vertebrates, cyclostomes
Vasopressin	*	*	Phe	*	*	*	*	Arg	*	Mammals
Lysipressin	*	*	Phe	*	*	*	*	Lys	*	Pig, some marsupials
Phenypressin	*	Phe	Phe	*	*	*	*	Arg	*	Macropodids (Marsupials)
Locupressin	*	Leu	*	Thr	*	*	*	Arg	*	*Locusta migratoria* (Insects)
Arg-conopressin	*	Ile	*	Arg	*	*	*	Arg	*	*Conus geographicus* (Molluscs)
Lys-conopressin	*	Phe	*	Arg	*	*	*	Lys	*	*Lymnaea stagnalis* (Molluscs)

Asterisks indicate amino acid residues that are identical to the corresponding residues in the oxytocin sequence. [Modified from Acher et al. (1).]

classified into vasopressin and OT families: the vasopressin family contains a basic amino acid (Lys, Arg), and the OT family contains a neutral amino acid at this position (Table 1). Isoleucine in position 3 is essential for stimulating OT receptors and Arg or Lys in position 8 for acting on vasopressin receptors. The difference in the polarity of these amino acid residues is believed to enable the vasopressin and OT peptides to interact with the respective receptors (42).

Virtually all vertebrate species possess an OT-like and a vasopressin-like peptide. Bony fishes (Osteichthyes), predecessors of the land vertebrates, possess isotocin and vasotocin. Thus two evolutionary molecular lineages have been proposed: an isotocin-mesotocin-OT line, associated with reproductive functions, and a vasotocin-vasopressin line involved in water homeostasis. Because vasotocin has been found in the most primitive cyclostomes, the OT and vasopressin genes may have arose by duplication of a common ancestral gene after the radiation of cyclostomes. Based on calculations from the nucleotide level of OT and vasopressin gene, the ancestral gene encoding the precursor protein should be more than 500 million years old. The exceptional structural stability of the nonapeptides during evolution suggests a strong selective pressure, e.g., by coevolution with the corresponding receptors and/or with specific processing enzymes. A conspicuous diversity of OT-like peptides is found in cartilaginous fishes (Chondrichthyes). These marine fishes use urea rather than salts for osmoregulation. It was hypothesized that the OT-like hormones gained their high diversity in Chondrichthyes as they have been relieved from the control of ionic homeostasis (1). Notably, OT, the typical hormone of placental mammals, has been identified in the Pacific ratfish, a Chondrichthyes species.

Mesotocin is the OT-like hormone found in most terrestrial vertebrates from lungfishes to marsupials, which includes all nonmammalian tetrapods (amphibians, reptiles, and birds). Only two South American marsupials express OT exclusively, whereas all other marsupials have mesotocin. In the Northern brown bandicoot (*Isoodon macrourus*) (488) and the North American opossum (*Didelphis virginiana*) (102), OT is present together with mesotocin. Overall, mesotocin has the largest distribution in vertebrates after vasotocin found in all nonmammalian vertebrates and isotocin identified in bony fishes. Despite this invariability, no clear physiological role has been ascribed to this peptide so far. It is unknown whether the marsupial species that are endowed with both OT and mesotocin have two distinct receptors. The earthworm *Eisenia foetida* is the most primitive species from which an OT-related peptide (annetocin) has been isolated (429). Injection of annetocin in the earthworm or in leechs results in induction of egg-laying behavior (430).

B. Gene Structure

In all species, OT and vasopressin genes are on the same chromosomal locus but are transcribed in opposite
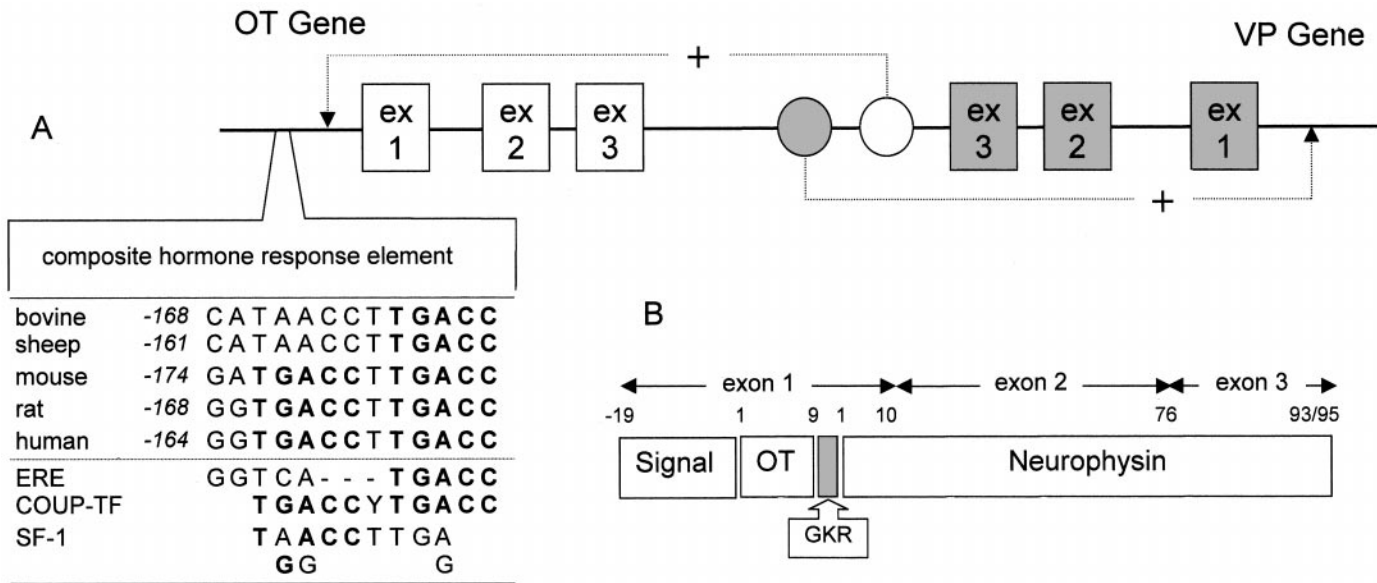
FIG. 1. Organization of the oxytocin (OT) and vasopressin (VP) gene structure including schematic depiction of the putative cell-specific enhancers (open circle, enhancer of OT gene; shaded circle, enhancer of VP gene). [Modified from Gainer et al. (200).] *A*: details of the approximately −160-bp region (composite hormone response element) of the upstream OT gene promoter conserved across five species including the sequences of the response elements estrogen response element (ERE), chicken ovalbumin upstream promoter transcription factor I (COUP-TF), and steroidogenic factor-1 (SF-1) are indicated. [Modified from Ivell et al. (273a).] *B*: domain organization of preprooxytocin including the processing sites. The precursor is split into the indicated fragments by enzymatic cleavages, one involving a glycyl-lysyl-arginine (GKR) sequence and leaving a carboxamide group at the COOH-terminal end of OT. Signal, signal peptide.

directions (Fig. 1). The intergenic distance between these genes range from 3 to 12 kb in mouse (224), human (500), and rat (391). This type of genomic arrangement could result from the duplication of a common ancestral gene, which was followed by inversion of one of the genes. The human gene for OT-neurophysin I encoding the OT pre-propeptide is mapped to chromosome 20p13 (472) and consists of three exons: the first exon encodes a translocator signal, the nonapeptide hormone, the tripeptide processing signal (GKR), and the first nine residues of neurophysin; the second exon encodes the central part of neurophysin (residues 10–76); and the third exon encodes the COOH-terminal region of neurophysin (residues 77–93/95).

The high homology of the OT-like precursor polypeptides is well documented in the sequence of pre-proannetocin from *Eisenia foetida*, a primitive invertebrate. It consists of a signal peptide, annetocin (flanked by a Gly COOH-terminal amidation signal and a Lys-Arg dibasic endoproteolytic sequence), and a neurophysin domain. Notably, 14 cysteine residues that play a crucial role in constructing the correct tertiary structure of a neurophysin are completely conserved in the *Eisenia* neurophysin domain (499).

The OT prepropeptide is subject to cleavage and other modifications as it is transported down the axon to terminals located in the posterior pituitary (74). The mature peptide products, OT and its carrier molecule neuro-

physin, are stored in the axon terminals until neural inputs elicit their release (475). The main function of neurophysin, a small (93–95 residues) disulfide-rich protein, appears to be related to the proper targeting, packaging, and storage of OT within the granula before release into the bloodstream. OT is found in high concentrations (>0.1 M) in the neurosecretory granules of the posterior pituitary complexed in a 1:1 ratio with neurophysin. In such complexes, OT-neurophysin dimers are the basic functional units as suggested by the crystal structure of the neurophysin-OT complex (486). Cys-1 and Tyr-2 in the OT molecule are the principal neurophysin binding residues. In particular, the protonated α-amino group (Cys-1) in OT forms an essential contact site to neurophysin via electrostatic and multiple hydrogen bonding interactions. Due to its dependence on amino group protonation (pK_a ~6.4), the binding strength between OT and neurophysin is much higher in an acidic compartment like the neurosecretory granules (pH ~5.5). Conversely, the dissociation of the complex is facilitated as the complex is released from the neurosecretory granules and enters the plasma (pH 7.4).

C. Gene Regulation

Due to the lack of an appropriate cell culture system, the regulation of OT gene expression was studied in het-

erologous systems and in transgenic mice. The expression patterns found with bovine OT transgenes in mice suggested very complex mechanisms for the cell type-specific expression of OT genes. A bovine OT transgene consisting of the OT structural gene flanked by 600 bp of upstream and 1,900 bp of downstream sequences contained sufficient information to direct expression to murine oxytocinergic magnocellular neurons, within which it was subject to physiological regulation (240). However, enlargement of OT transgene constructs by addition of 700 bp of contiguous downstream sequences repressed the hypothalamic expression. Analysis of various gene constructs in transgenic mice led to the proposal that cell-specific enhancers for OT and vasopressin gene expression are not located on the 5′-upstream regions of these genes, but are present in the intergenic region 0.5–3 kb downstream of the vasopressin gene (Fig. 1). So, constructs containing genomic DNA from 0.5 to 9 kb 5′-upstream of the OT and vasopressin genes but with no endogeneous 3′-downstream sequences did not show significant expression in the hypothalamic magnocellular neurons (200).

The OT mRNA in the rat shows an increase in poly(A) tail length in response to the activation of the hypothalamoneurohypophysial system, e.g., during pregnancy, lactation, and dehydration. This could augment mRNA stability and may be an additional level of OT gene control (95, 623). Hexanucleotide AGGTCA motifs and variations thereof are present in the proximal 5′-flanking region of cloned OT genes. This motif is part of binding sites for all members of the nuclear receptor superfamily, except the glucocorticoid, mineralocorticoid, progesterone, and androgen receptor. Various combinations of this motif exist, ranging from single hexanucleotides, direct or inverted repeats with spacing varying from one to at least six nucleotides (436). Thus potentially several members of the nuclear receptor family including many orphan receptors could interact with the OT gene and regulate its expression. The human and rat OT promoters could be stimulated by the ligand-activated estrogen receptors ERα and ERβ, the thyroid hormone receptor THRα, and the retinoic acid receptors RARα and RARβ in a variety of cells (3, 477, 478). However, it is important to note that these results were obtained from cotransfection experiments in cell lines, i.e., under nonphysiological circumstances.

A highly conserved DNA element exists at ~160 nucleotides upstream from the transcriptional initiation site (Fig. 1). Deleting the region between −172 and −148 resulted in complete loss of thyroid hormone responsiveness and most of the responsiveness to estrogen and retinoic acid (4). This special "composite" hormone response element is composed of three TGACC motifs. Two of them form an inverted repeat with a spacing of three nucleotides that differs in one nucleotide from the palin-

dromic, canonical estrogen response element (ERE) (79). The rat and human OT promoter shows a good homology with the classic palindromic ERE. Accordingly, in a heterologous transfection system, the rat and human but not the bovine OT gene promoter could be stimulated by estradiol (477). In the appropriate cellular context, the strongest activators were ERα and ERβ. The composite hormone response element was suggested to synergize with proximal elements for the estrogen responsiveness and was found to be essential for the positive regulation by retinoic acid (341, 478). However, estrogen receptor expression was not detected in oxytocinergic cells of the rat hypothalamus (37). Thus direct estrogen-dependent activation may not regulate the OT gene expression in magnocellular neurons in vivo. An estrogen responsiveness was reported for a parvocellular OT-expressing cell group that contains ERβ (112). Although THRs have been localized in supraoptic nucleus (SON) and paraventricular nucleus (PVN), only a small influence of thyroid hormones on OT gene expression was found in rats in vivo (4).

The rat uterus displays a marked upregulation of OT gene expression before delivery. The main site of steroid-induced uterine OT gene expression was the endometrial epithelium. A strong increase in OT mRNA (~150-fold) preceded the increase in uterine OT binding sites that occurs very shortly before the onset of labor (326, 526). The estrogen-induced rise in uterine OT mRNA was probably mediated via the common hormone response element in the OT gene promoter. The palindromic structure at the composite hormone response element at approximately −160 bp was identified as necessary and sufficient for estrogen induction of the OT gene promoter. However, the high level of uterine OT mRNA at term was not achieved by any of the steroid treatment regimens tested so far (624).

Several investigations have focused on the role of nuclear orphan receptors in the regulation of the OT gene. Unlike in the hypothalamus, the bovine OT gene could be tissue-specifically expressed in the gonads by a minimal functional promoter contained within 600 bp of the transcription start site (15, 586). As mentioned above, the bovine OT gene is unresponsive to estradiol. Nuclear orphan receptors of corpus luteum granule cells have been identified to interact with the common hormone response element (~−160 bp, Fig. 1): chicken ovalbumin upstream promoter transcription factor I (COUP-TFI) and steroidogenic factor-1 (SF-1). The levels of these factors could be responsible for the regulation of the endogeneous OT gene in this tissue. SF-1 is a factor with constitutive activating properties on the OT gene (586). The orphan COUP-TFI repressed the activation of the rat OT gene induced by retinoic acid, thyroid hormone, and estrogens through competitive binding to the composite hormone response element (79). Another orphan receptor

identified in the hypothalamus, testis receptor 4 (TR4), interacts, unlike all other nuclear receptors, at a region further downstream (−112/−77 bp) of the common response element in the OT gene (80).

The 5′-flanked region of the rat OT gene also contains binding sites for class III POU homeodomain proteins. Brn-2, a member of this family, is involved in the regulation of OT genes in magnocellular neurons. Brn-2 null mice lack magnocellular vasopressin- and OT-expressing neurons in SON and PVN (225). Brn-2 is required for a specific step in the developmental fate of magnocellular neurons. However, POU class III proteins did not display a significant regulatory activity on the OT gene in heterologous expression systems (80).

Taken together, OT gene regulation in vivo appears to be governed by multiple enhancers and repressors interacting in a complex yet ill-defined fashion.

III. OXYTOCIN RECEPTORS

A. Gene Structure and Regulation

Kimura et al. (299) first isolated and identified a cDNA encoding the human OT receptor using an expression cloning strategy. The encoded receptor is a 389-amino acid polypeptide with 7 transmembrane domains and belongs to the class I G protein-coupled receptor (GPCR) family. To date, the OT receptor encoding sequences from pig (215), rat (489), sheep (480), bovine (44), mouse (315), and rhesus monkey (492) have also been identified.

The human OT receptor mRNAs were found to be of two sizes, 3.6 kb in breast and 4.4 kb in ovary, endometrium, and myometrium. The OT receptor gene is present in single copy in the human genome and was mapped to the gene locus 3p25–3p26.2 (254, 386, 519). The gene spans 17 kb and 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. Intron 3, which is the largest at 12 kb, separates the coding region immediately after the putative transmembrane domain 6. Exon 4 contains the sequence encoding the seventh transmembrane domain, the COOH terminus, and the entire 3′-noncoding region, inluding the polyadenylation signals (Fig. 2). Although many GPCRs have an intronless gene structure, the genes for some other members of the GPCR family including the human vasopressin V2 receptor (511) contain an intron at the same location after transmembrane domain 6. The transcription start sites lie 618 and 621 bp upstream of the initiation codon as demonstrated by primer extension analysis. Nearby, a TATA-like motif and a potential SP-1 binding site is found in the human OT receptor gene. The 5′-flanking region also contains invert GATA-1 motifs, one c-Myb binding site, one AP-2 site, two AP-1 sites, but no complete ERE.
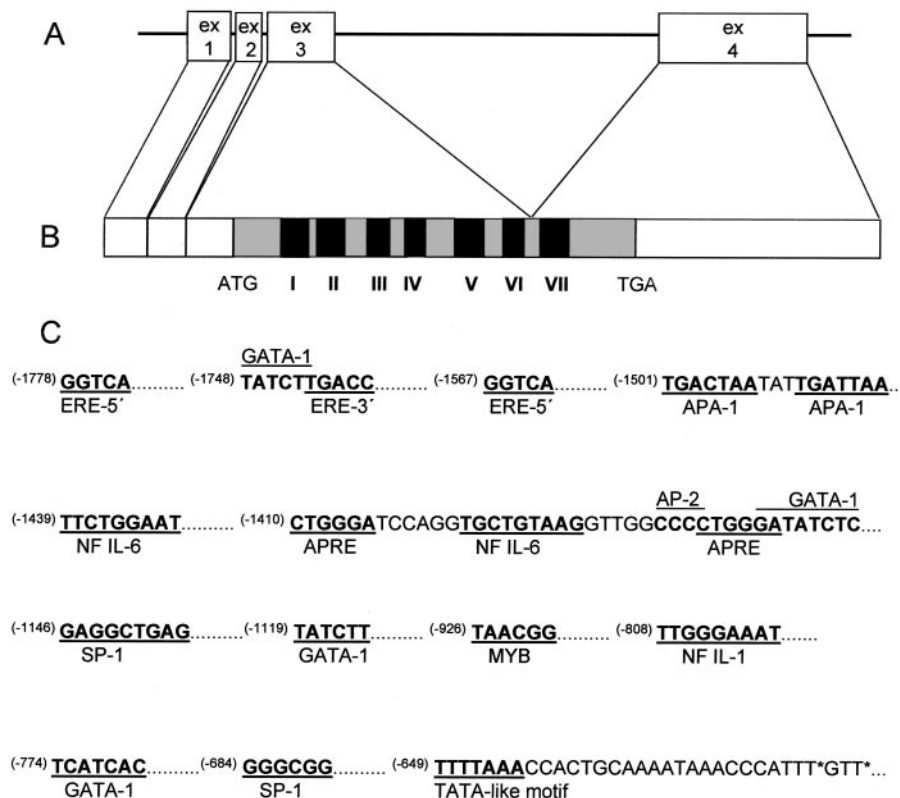
FIG. 2. Organization of the human OT receptor gene including the localization of consensus sequences for transcription factors. The human OT receptor gene consists of four exons. Exons 3 and 4 encode the amino acid sequence for the OT receptor. The start (ATG) and stop (TGA) codons of the receptor cDNA are indicated. The DNA sequences encoding for transmembrane regions I–VII are indicated by black areas. [Modified from Inoue et al. (254).]

Instead, there were two half-palindromic 5′-GGTCA-3′ motifs and one half-palindromic 5′-TGACC-3′ motif of ERE. Moreover, there were two nucleofactor interleukin-6 (NF IL-6) binding consensus sequences and two binding site sequences for an acute phase reactant-responsive element at the 5′-flanking region (254).

In the OT receptor gene of the mouse, the promoter region lacks an apparent TATA box but contains multiple putative interleukin-response elements, several half-palindromic motifs, and a classical ERE (315).

In case of the rat OT receptor gene expression at parturition, three transcripts (2.9, 4.8, and 6.7 kb) were identified that differ in the length of their 3′-untranslated regions (489). The promoter region of the rat OT receptor gene also contains multiple putative interleukin-response elements, NF IL-6, and acute-phase response elements (APRE) (489). Further sequence analysis of 4 kb of the 5′-flanking DNA of the rat OT receptor gene revealed the presence of a cAMP response element (CRE) as well as several other potential regulatory elements, including AP-1, AP-2, AP-3, AP-4 sites, an ERE, and a half-steroid response element. A palindromic ERE was identified ~4 kb 5′ of the translational start site. An OT receptor reporter construct of this promoter in the human cancer breast cell line MCF-7 demonstrated pronounced induction by both forskolin and phorbol ester, but contrary to in vivo findings, only a weak transcriptional response to estradiol (39). Constructs of the CRE and half-steroid response elements from the promoter of the rat OT receptor gene function as active enhancers. This suggests a potential role for protein kinase A and C pathways in OT receptor gene regulation. The protein kinase A pathway, for example, may be activated when forskolin treatment promotes upregulation of OT receptors in cultured rabbit amnion cells (235, 236). Protein kinase C may act to increase fos/jun activity at AP-1 sites in response to phorbol ester treatment (41). In a mammary tumor cell line (Hs578T) that expresses inducible, endogenous OT receptors, a DNA region containing an ets family target sequence (5′-GGA-3′), and a CRE/AP-1-like motif was required for both basal and serum-induced OT receptor gene expression. The ets factor $GABP\alpha/\beta$ slightly induced OT receptor gene expression in this human breast cell line. The gene expression was markedly potentiated following cotransfection with c-*fos*/c-*jun* (242).

APREs are typically found in genes for acute-phase proteins such as α_2-macroglobulin or T kininogen, which are induced by infection or inflammation. The presence of these elements in the promoter region of the human and rat OT receptor gene suggests that the acute induction of OT receptor expression could be a phenomenon similar to the induction of acute-phase reponse genes. The decidua has "macrophage-like" properties and functions. Possibly, inflammatory cytokines are able to induce labor and, thereby, take usage of the transcriptional activation of the OT receptor gene.

However, the lack of classical EREs in a promoter does not exclude a potential direct effect of estrogens on gene expression, since the present half-palindromic ERE motifs can also act synergistically to mediate estrogen activation as shown in the ovalbumin gene (284). Gonadal steroids have an important influence on the uterine OT receptor mRNA accumulation in vivo. Estrogens administered to ovariectomized rats increased OT receptor binding sites and increased OT receptor mRNA accumulation severalfold. Although progesterone leads to a marked decline of OT receptor binding sites, the mRNA levels of OT receptor were nearly unchanged (622). This and several other findings (528) imply the involvement of nongenomic effects of progesterone (see sect. III*F*).

The OT receptor gene is differentially expressed in various tissues. In uterus or hypothalamus, the OT receptor regulation correlates with the pattern of sex steroids, in particular estradiol. As shown with knock-out mice, $ER\alpha$ is not necessary for basal OT receptor synthesis but is absolutely necessary for the induction of OT receptor binding in the brain by estrogen (612). However, it is unclear whether OT receptor gene transcription is predominantly regulated by estrogen. The continuous presence of receptors in certain brain regions after gonadectomy suggests the existence of alternate mechanisms of regulation. In this context, a study of the tammar wallaby, an Australian marsupial, is interesting. This species has a twin uterus attached to a double cervix so that each uterus forms an independent environment. During pregnancy, only one uterus becomes gravid, the other remains empty and can thus be regarded as a natural control for uterine changes occurring during pregnancy. It was shown that mesotocin receptor concentrations and the responsiveness to mesotocin differed between the gravid and nongravid myometrium during pregnancy. This indicates that the stimulating agent for the mesotocin receptor is unlikely a circulating factor but rather a local factor, possibly of fetal or placental origin (438). Another well-studied system is the OT receptor gene expression in bovine endometrial cells. In vivo, bovine endometrial OT receptors are upregulated in a cycle-dependent fashion. This regulation appears to be completely at the transcriptional level. Even if the receptors are downregulated in vivo, they show upregulation when explanted and cultured in vitro (516). This indicates that the OT receptor regulation is partly due to gene suppression in vivo. Despite the presence of steroid receptors in bovine endometrial cells, the level of OT receptor mRNA could neither be affected by progesterone or estradiol nor by a progesterone withdrawal protocol. The only factor that affected the OT receptor mRNA level was interferon-γ. As in vivo, this cytokine suppressed the OT receptor mRNA production (267).

Nuclear protein binding and transfection experiments suggested that constitutive upregulation is a feature of the OT receptor promoter (267). So, specific gene suppression is likely to play an important role for physiological control of the OT receptor expression. Of interest, a genomic element within the third intron of the human OT receptor gene was found to be associated with transcriptional gene suppression. The intronic region was hypermethylated in nonexpressing tissues, but relatively hypomethylated in the myometrium of the cycle and at term, when the OT receptor gene is upregulated (390). Taken together, it was concluded that sex steroids have an indirect effect on both the OT and OT receptor genes, possibly involving intermediate transcription factors or cofactors (273).

The transcriptional regulation of OT receptor shows species-specific differences. The brain OT receptor varies across species in its distribution as well as in its regional regulation by gonadal steroids (261, 262). For the OT receptor as for many other genes, the DNA sequences located in the 5′-flanking region upstream from the coding region are primarily responsible for conferring tissue-specific expression. Transgenic mice carrying 5 kb of the 5′-flanking region of the prairie vole OT receptor gene showed the typical expression pattern of prairie vole OT receptors in mice (616). However, the regulatory elements that confer the specific expression patterns for the OT receptor gene in vivo are yet unknown.

One of the fundamental questions concerns the possible existence of OT receptor subtypes (443, 572). Such subtypes have been suggested to be present, e.g., in the rat uterus (101, 103), kidney (34), or brain (5, 132), to explain differential pharmacological profiles or immunoreactivity patterns. Application of polymerase chain reaction methods and Southern analysis in several tissues known to possess OT binding activity failed to identify a gene encoding a further OT receptor subtype. However, the applied techniques only screen for genes with high homology to the uterine-type OT receptor, and therefore, a putative further OT receptor with low homology to the uterine-type OT receptor would have been kept undetected (43, 572).

B. Receptor Structure

The OT receptor is a typical member of the rhodopsin-type (class I) GPCR family. The seven transmembrane α-helices are most highly conserved among the GPCR family members. Conserved residues among the GPCRs (outlined in black in Figs. 3 and 4) may be involved in a common mechanism for activation and signal transduction to the G protein. On the basis of studies with model GPCRs, it is assumed that the switching from the inactive to the active conformation is associated with a change in the relative orientation of transmembrane domains 3 and 6, which then unmasks G protein binding sites. In the class I GPCR family, an Asp in transmembrane domain 2 (Asp-85 in human OT receptor, see Figs. 3–5) and a tripeptide (E/D RY) at the interface of transmembrane 2 and the first intracellular loop are believed to be important for receptor activation (57). With respect to Asp-85, this was confirmed for the human OT receptor. When Asp-85 is exchanged by the residues Asn, Gln, or Ala, agonist binding and signal transduction of the receptor becomes impaired (166, 587). Mutations at the conserved tripeptide motif DRY (DRC in case of the OT receptor) result in an either inactive or a constitutively active OT receptor (see below) (166) (for an overview of the published mutagenesis studies see Fig. 5 and Table 2).

The cysteine residues in the first and second extracellular loops are highly conserved within the GPCR family and are probably connected by a disulfide bridge. Two other well-conserved Cys residues reside within the COOH-terminal domain. Most likely, they are palmitoylated as demonstrated for the V2 receptor (491) and other GPCRs and anchor the cytoplasmic tail in the lipid bilayer. However, for the V2 receptor as well as for the rat OT receptor, elimination of palmitoylation sites by mutagenesis failed to produce significant alterations in receptor function (505).

The OT receptor has two (mouse, rat) or three (human, pig, sheep, rhesus monkey, bovine) potential N-glycosylation sites (N-X-S/T consensus motif) in its extracellular NH_2-terminal domain. For the "core" OT receptor, a molecular mass of ~40–45 kDa can be calculated on the basis of the amino acid sequence derived from the known cDNA sequences of several species. In photoaffinity labeling experiments using myometrial membranes obtained from guinea pig during late pregnancy, a 68- to 80-kDa protein was specifically labeled by a photoreactive OT antagonist (306) developed by Elands et al. (151). Deglycosylation of the photolabeled receptor with endoglycosidase F gave rise to protein with 38–40 kDa (306). Similarly, in the same tissue, a 78-kDa protein was labeled by a photoreactive vasopressin analog (164). In contrast, in membranes from rat mammary gland and rabbit amnion cells, photoreactive OT analogs specifically incorporated into a 65-kDa binding protein (237, 399). It is possible that the different molecular masses for the myometrial versus the mammary gland and amnion OT receptor are due to differential glycosylation patterns. With the assumption of a mass of ~10 kDa for a typical glycosylation core, all of the potential glycosylation sites could be occupied by glycosylation moieties. Recombinant deglycosylation mutants of the human OT receptor have been created by site-directed mutagenesis by exchanging Asp for Asn in positions 8, 15, and 26. The deglycosylated receptors were highly expressed in HeLa cells and showed unaltered receptor binding characteristics (296). Thus the receptor

```
OTR             MEGALAANWSAEAANASAAPPGAEGNRTAGPPRRNEALARVEVAVLC   47
V2R               MLMASTTSAVPGHPSLPSLPSNSSQERPLDTRDPLLARAELALLS   45
V1aR   MRLSAGPDA GPSGNSSPWWPLATGAGNTSREAEALGEGNGPPRDVRNEELAKLEIAVLA   59
V1bR            MDSGPLWDANPTPRGTLSAPNATTPWLGRDEELAKVEIGVLA   42
                                                        *********

OTR    LILLLALSGNACVLLAL  RTTRQKHSRLFFFMKHLSIADLVVAFQVLPQLLWDITFRFY  106
V2R    IVFVAVALSNGLVLAALARRGRRGHWAPIHVFIGHLCLADLAVALFQVLPQLAWKATDRFR  106
V1aR   VTFAVAVLGNSSVLLALHRTPR  KTSRMHLFIRHLSLADLAVAFFQVLPQMCWDITYRFR  118
V1bR   TVLVLATGGNLAVLLTLGQLGR  KRSRMHLFVLHLALTDLAVALFQVLPQLLWDITYRFQ  101
       ***** TM 1 *********      ************* TM 2 *********

OTR    GPDLLCRLVKYLQVVGMFASTYLLLLMSLDRCLAICQPLRSLR    RRTDRLAVLATWLGC  164
V2R    GPDALCRAVKYLQMVGMYASSYMILAMTLDRHRAICRPMLAYRHGSGAHWNRPVLVAWAFS  167
V1aR   GPDWLCRVVKHLQVFGMFASAYMLVVMTADRYIAVCHP LKTLQQPARRSRLMIAAAWVLS  178
V1bR   GPDLLCRAVKYLQVLSMFASTYMLLAMTLDRYLAVCHP LRSLQQPGQSTYLLIAAPWLLA  161
       ********* TM 3 **************                  **************

OTR    LVASAPQVHIFSLRE    VADGVFDCWAVFIQPWGPKAYITWITLAVYIVPVIVLATCYG  221
V2R    LLLSLPQLFIF  AQRNVEGGSGVTDCWACFAEPWGRRTYVTWIALMVFVAPTLGIAACQV  226
V1aR   FVLSTPQYFVFS  MIEVNNVTKARDCWATFIQPWGSRAYVTWMTGGIFVAPVVILGTCYG  237
V1bR   AIFSLPQVFIFSL  REVIQGSGVLDCWADFGFPWGPRAYLTWTTLAIFVLPVTMLTACYS  220
       ** TM 4 ******            ********* TM 5 **********

OTR    LISFKIWQNLRLKTAA       AAAAEAPEGAAAGDGGRVALARVSSVKLISKAKIRTVK  275
V2R    LIFREIHASLVPGP            SERPGGRRRGRRTGSPGEGAHVSAAVAKTVR  271
V1aR   FICYNIWCNVRGKTASRQSK       GAEQAGVAFQKGFLLAPCVSSVKSISRAKIRTVK  291
V1bR   LICHEICKNLKVKTQAWRVGGGWRTWDRPSPSTLAATTRGLPSRVSSINTISRAKIRTVK  281
       ***                                             *****

OTR    MTFIIVLAFIVCWTPFFFVQMWSVWDAN       APKEASAFIIVMLLASLNSCCNPWIYMLFT  333
V2R    MTLVIVVVYVLCWAPFFLVQLWAAWD       PEAPLEGAPFVLLMLLASLNSCTNPWIYASFS  329
V1aR   MTFVIVTAYIVCWAPFFIIQMWSVWDPMSVWTESENPTITITALLGSLNSCCNPWIYMFFS  352
V1bR   MTFVIVLAYIACWAPFFSVQMWSVWDKNAPDEDSTNVAFTISMLLGNLNSCCNPWIYMGFN  342
       *********** TM 6 ********          *********** TM 7 *********

OTR    GHLFHELVQRFLCCSASYLKGRRLGETSASKKSNSSSFVLSHRSSSQRSCSQPSTA      389
V2R    SSVSSELRSLLCCARGRTPPSLGPQDESCTTASSSLAKDTSS                   371
V1aR   GHLLQDCVQSFPCCQNMKEKFNKEDTDSMSRRQTFYSNNRSPTNSTGMWKDSPKSSKSIKF 413
V1bR   SHLLPRPLRHLACCGGPQPRMRRRLSDGSLSSRHTTLLTRSSCPATLSLSLSLTLSGRPRP  403

V1aR   IPVST                                                        418
V1bR   EESPRDLELADGEGTAETIIF                                        424
```

FIG. 3. Primary sequence alignments of the human OT receptor (OTR), the human vasopressin 2 receptor (V2R), the human vasopressin 1A receptor (V1aR), and the human vasopressin 1b receptor (V1bR). The putative transmembrane helices 1–7 are underlined (asterisks). The residues conservative within the subfamily (~25% of the whole sequence) are outlined in gray, while those conservative for the whole G protein-coupled receptor superfamily are outlined in black.

glycosylation appears not to be necessary for proper expression and has no effect on the functional properties of the receptor. Similar findings have been reported for other GPCRs, e.g., the vasopressin V2 receptor (251).

C. Ligand Binding Characteristics

The high homology of the nonapeptides of the evolutionary line isotocin-mesotocin-OT (see sect. II*A* and
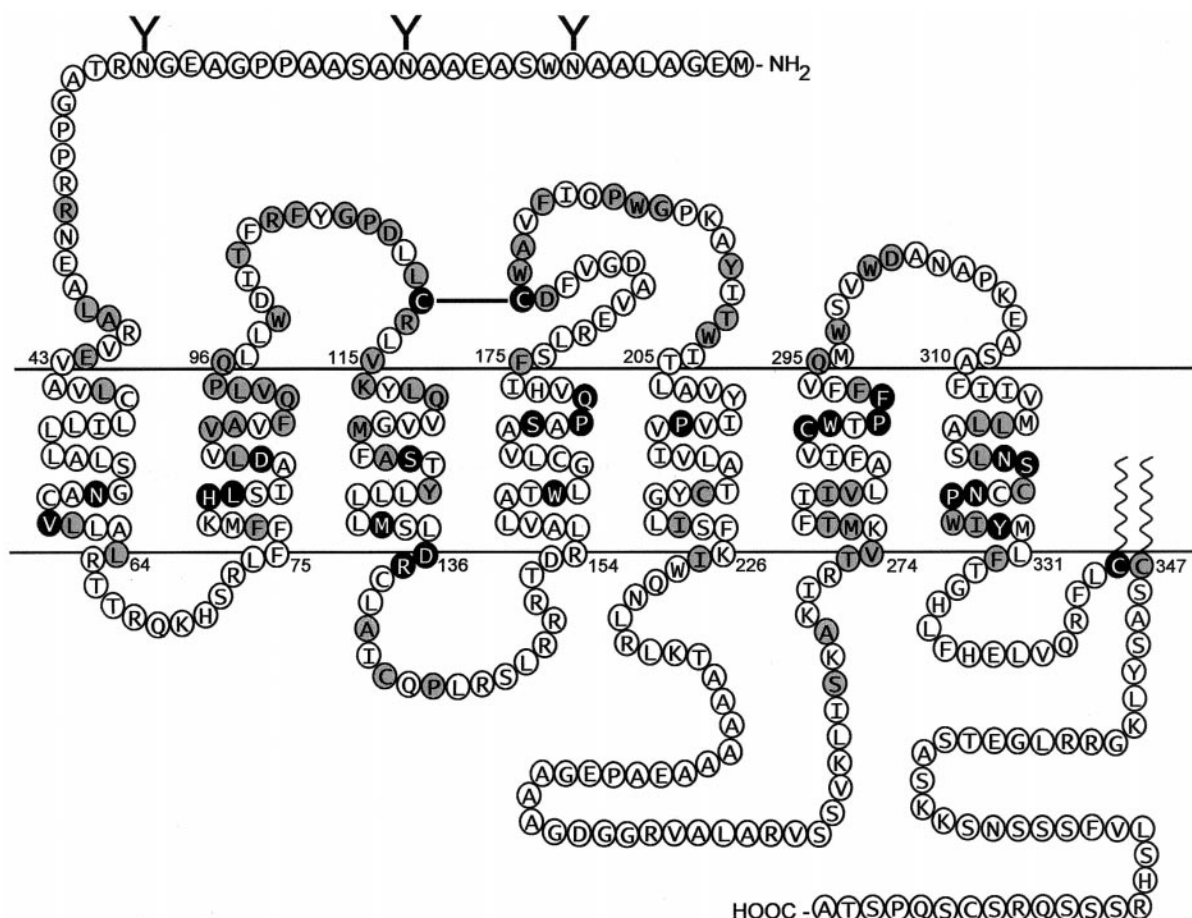
FIG. 4. Schematic structure of the human OT receptor with amino acid residues shown in one-letter code. The residues are marked in the same manner as in Fig. 3, i.e., residues conservative within the OT/vasopressin receptor subfamily are outlined in gray, and residues conservative for the whole G protein-coupled receptor superfamily are outlined in black. The putative *N*-glycosylation ("Y") and palmitoylation (at C346/C347) sites are marked.

Table 1) is also reflected in the high homology of the corresponding receptors. Accordingly, the mammalian OT receptors share the highest degree of sequence similarity with the toad mesotocin receptor (70%) (6) and the isotocin receptor of teleost fish (66%) (229), whereas the sequence homologies with the vasopressin V1 (nearly 50%) and V2 receptors (40%) are significantly lower. About 100 amino acids ($\sim$ 25%) are invariant among the 370–420 amino acids in the human receptors for vasopressin V2, V1a, V1b, and OT (see Figs. 3 and 4). The highest homology between the vasopressin/OT receptor types is found in the extracellular loops and the transmembrane helices. The NH$_2$ terminus and the COOH terminus have lower similarities, and the intracellular loops are the least of all conserved. Structural common features of the OT/vasopressin receptor family could play an important role in ligand/receptor recognition, e.g., the sequences FQVLPQ at the end of transmembrane domain 2, the sequences GPD (APD in mesotocin receptor) in the first extracellular loop, and DCWA (DCRA and DCWG in

mesotocin and isotocin receptor) in the second extracellular loop.

For small molecules like catecholamines, the ligands bind in a cavity between the α-helical segments formed by transmembrane domains 3–6. Peptide ligands, on the other side, bind more superficially and also interact with extracellular loops and/or the NH$_2$-terminal domain. For the binding of the peptides OT and arginine vasopressin (AVP), residues located in the transmembrane domains as well as residues within extracellular domains are involved in ligand binding. Because the OT/vasopressin peptides as well as their receptors are well conserved, the ligand binding interaction should consist of both common and selective contact sites. As derived from molecular modeling in combination with mutagenesis studies, the agonist binding site for the vasopressin/OT peptides was proposed to be located in a narrow cleft delimited by the ringlike arrangement of the transmembrane domains. An equivalent position was described for the binding of cationic neurotransmitters. In the rat V1a receptor, the con-
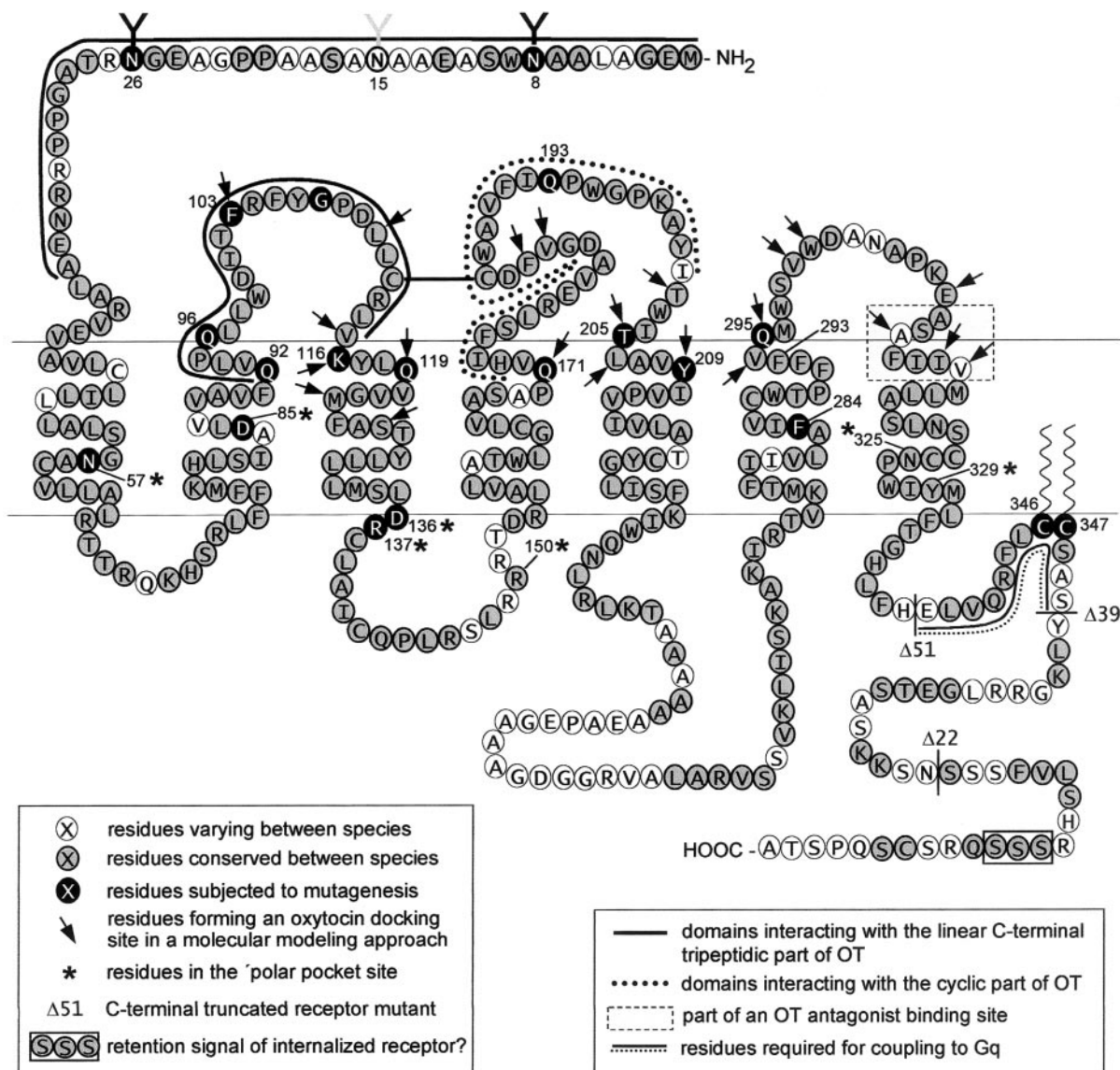
FIG. 5. Schematic model of the human OT receptor indicating amino acid residues that are putatively involved in ligand-binding and associated signal transduction events (described in sect. III, *B–E*). The amino acid residues in gray solid circles are conserved (identical) between the OT receptors from different mammalian species (human, rhesus monkey, pig, bovine, sheep, rat, and mouse). Residues in open circles show interspecies variation. At these positions, an amino acid substitution may be tolerated through mammalian evolution without influencing the functional properties of the receptor. Residues in black solid circles have been subjected to mutagenesis (see Table 2 and sect. III, *B–E*, for more details). The glutamine and lysine residues highly conserved within the vasopressin/OT receptor family may partly define an agonist-binding pocket that is common to all the different subtypes of this receptor family (42, 398). According to a molecular modeling approach (166), an OT docking site has been proposed (corresponding residues are marked by arrows). In the inactive receptor conformation, the highly conserved arginine (R137) may be constrained in a pocket that is formed by polar residues (indicated by asterisks). After agonist binding, this arginine side chain may be shifted out of the "polar pocket," thereby unmasking a G protein binding site. Receptor domains putatively interacting with OT, a peptide OT antagonist and $G_q\alpha$ are marked by lines (for details see sect. III, *C* and *D*).

served Gln residues in the transmembrane domains 2, 3, 4, and 6 and a Lys residue localized in transmembrane domain 3 were replaced by Ala residues. All the receptor mutants had a decreased affinity for the agonists vasopressin, OT, and vasotocin (see Table 2 and Fig. 5). Because the corresponding Gln and Lys residues are highly conserved, it was proposed that the agonist-binding

pocket is common to all the different subtypes of this receptor family (42, 398).

Photoaffinity labeling of the first extracellular loop of the bovine vasopressin V2 receptor using a photoreactive lysine vasopressin analog provided direct evidence for the involvement of the first extracellular loop in agonist binding (305). In the first extracellular loop, the homologous

TABLE 2. *Mutagenesis of vasopressin/oxytocin receptors and its influence on oxytocin binding*

Mutated Receptor/ Cell System	Mutation	Residue in hOTR*	Expression Level	Affinity for OT	Reference Nos.
hOTR/HeLa	N8D	8	>100%	Unaltered	296
hOTR/HeLa	N15D	15	>100%	Unaltered	296
hOTR/HeLa	N15/26D	15/26	Variable	Unaltered	296
hOTR/HeLa	N8/26D	8/26	Variable	Unaltered	296
hOTR/COS-7	N57A	57		No binding, no signaling	166
hOTR/COS-7	D85A	85	<10%	No binding, no signaling (low affinity for AVP)	166
hOTR/COS-7	D85N	85		No binding, no signaling	587
hOTR/COS-7	D85E	85	ND	Decreased 7-fold (for OTA: increased 10-fold)	587
rV1aR/COS-7	D97A	85	100%	Decreased >12-fold	398
rV1aR/COS-7	Q104A	92	100%	Decreased >6-fold	398
rV1aR/COS-7	Q108A	96	100%	Decreased >12-fold	398
pV2R/COS.M6	Y102F	103	200%	Increased 2-fold	463
pV2R/COS.M6	Y102D	103	ND	Decreased >30-fold	562
pV2R/COS.M6	Y102N	103	ND	Decreased 3-fold	562
bV2R/COS.M6	D103Y	103	50%	Unaltered	562
rV1aR/COS-7	Y115F	103	ND	Increased 19-fold	105
hV1aR/COS-7	Y115F	103	ND	Increased 8-fold	105
rV1aR/COS-7	Y115D	103	ND	Unaltered	105
rV1aR/COS-7	Y115L	103	ND	Decreased 2.4-fold	105
pV2R/COS.M6	R105Y	106	50%	Decreased 1.4-fold	†
bV2R/COS.M6	R106L	106	60%	Unaltered	562
hOTR/COS-7	G107A	107	ND	Unaltered	587
rV1aR/COS-7	K128A	116	50%	Decreased >6-fold	398
rV1aR/COS-7	Q131A	119	100%	Decreased >12-fold	398
hOTR/COS-7	D136A	136		No binding, no signaling	166
hOTR/COS-7	D136Q	136		No binding, no signaling	166
hOTR/COS-7	R137A	137	10%	Decreased 1.8-fold (constitutively active)	166
rV1aR/COS-7	Q185A	171	150%	Decreased >12-fold	398
pV2R/COS.M6	E197Q	193	50%	Unaltered	†
rV1aR/COS-7	T223A	205	100%	Increased 2.6-fold	398
hOTR/COS-7	Y209F	209	50%	Increased 2.5-fold (for OTA: decreased 40-fold)	106
hOTR/COS-7	Y209A	209		No binding, no signaling	106
hOTR/COS-7	F284Y	284	85%	Unaltered	106
hOTR/COS-7	F284A	284		No binding, no signaling	106
hOTR/COS-7	F293I	293	ND	Unaltered	587
rV1aR/COS-7	Q317A	295	50%	Decreased >6-fold	398
rOTR/CHO-K1	C351S	346	100%	Unaltered	241
rOTR/CHO-K1	C352S	347	100%	Unaltered	241

    The receptor mutants are characterized by the receptor type (for abbreviations, see legend to Fig. 3; species: r, rat; h, human; p, pig), the cell line in which the receptor has been expressed, and the name of the mutation using single-letter code for amino acids and numbering of residues according to their primary sequence (e.g., in the mutant N15, 26D, the asparagines at positions 15 and 26 in the human OT receptor have been mutated to aspartates). * The indicated residues represent the equivalent positions of the mutagenized amino acid within the human OT receptor (hOTR) (see Figs. 4 and 5, alignment in Fig. 3). The expression levels are given in percent of expression of the corresponding wild-type receptor. ND, not determined. † Postina and Fahrenholz, unpublished data.

residues F103, Y115, and D115 in the human OT, V1a/V1b, and V2 receptor were found to be crucial for the determination of the ligand selectivity (105, 562). For example, the mutation in the equivalent position (Y115F) of the rat V1a receptor led to a 19-fold increase of OT binding compared with the native receptor (105). Molecular modeling of ligand binding interaction to the V1a receptor supported the view that the side chain of Arg-8 in AVP projects outside the transmembrane core of the receptor and could interact with Tyr-115 located in the first extracellular loop. Arg-8 in AVP is known to be necessary for its high-affinity binding to the V1a receptor. When Tyr-115 in the V1a receptor is replaced by an Asp and a Phe, the amino acids naturally occurring in the V2 and in the OT receptor subtypes, the agonist selectivity of the V1a receptor switches accordingly (Table 2) (105). The corresponding residue also determined the agonist specificity of the bovine and pig V2 receptor (562). Thus this residue certainly contributes to agonist selectivity. Additionally, by a peptide mimetic approach it was found that a synthetic dodecapeptide, which is homologous to the first extracellular loop of the human OT receptor, inhibits the binding of tritiated AVP to the human OT receptor (245). The second extracellular loop is also thought to be im-

portant for hormone binding of the human OT receptor, since it is conserved only within the nonapeptide receptor family (Fig. 5).

The OT receptor has a weak ligand selectivity profile: hormones with the same cyclic part and either Arg-8 (in arginine vasotocin) or Leu-8 (in OT) are bound with the same affinity, whereby Ile-3 (in OT) in the cyclic hormone part contributes more to affinity than Phe-3 (in oxypressin). This indicates that the cyclic part of OT is more important in conferring binding selectivity for the OT receptor compared with the linear tripeptidic part of the hormone. Using chimeric "gain in function" V2/OT receptor constructs, Postina et al. (463) demonstrated that the NH$_2$ terminus and the first and second extracellular loops were necessary for agonist binding and selectivity. In particular, the exchange of the NH$_2$ terminus of the V2 receptor for the corresponding first extracellular domain of the OT receptor resulted in a sixfold increase in binding affinity for OT. Presumably, the NH$_2$ terminus of the OT receptor takes part in hormone binding and probably interacts with the hydrophobic leucyl residue in position 8 of the ligands. The NH$_2$-terminal domain and the first extracellular loop of the OT receptor are proposed to interact with the linear COOH-terminal tripeptidic part of OT, whereas the second extracellular loop of the OT receptor could be identified to interact with the cyclic hormone part (463) (Fig. 5).

Concerning the binding for OT and AVP, the OT receptor is relatively unselective with only about 10-fold higher affinity of the receptor for OT (297, 463). AVP acts as a partial agonist on the OT receptor. To elicit the same response as induced by OT, ~100-fold higher concentrations of AVP are necessary (106, 297). However, AVP becomes a full agonist when two aromatic residues of the OT receptor (Y209 and F284) are replaced by the residues F and Y present at equivalent positions in the vasopressin receptor subtypes (Table 2). These two residues are therefore crucial for the response of the OT receptor to the partial agonist AVP (106).

Chimeric constructs encoding parts of the white sucker fish [Arg^8]vasotocin receptor and parts of the isotocin receptor have shown that the NH$_2$ terminus and a region spanning the second extracellular loop and its flanking transmembrane segments contribute to the affinity of the [Arg^8]vasotocin receptor (230). For the isotocin receptor from teleost fish, it has been shown that the sixth transmembrane helix and/or the fourth extracellular domain are involved in ligand binding (230).

Several studies indicate that the binding site of OT antagonists is different from the agonist binding site. Studies with chimeric receptors provided evidence that the binding site for the peptide OT antagonist (151) was formed by the transmembrane helices 1, 2, and 7, with a major contribution to binding affinity by the upper part of helix 7 (see Fig. 5). These regions did not participate in

OT binding (463). Most mutations affecting agonist binding affinities (398) have little effect on antagonist binding affinities (42).

D. Signal Transduction and G Protein Coupling

GPCRs may also be constitutively active, in the absence of any agonist. This was first shown for the β-adrenergic receptor where mutations in the third intracellular loop, or simply overexpression of the receptor, resulted in constitutive receptor activation. The human V2 receptor mutant D136A (396) and the human OT receptor mutant R137A (166) represent such constitutively active receptors. Both positions are located within the conserved DRY motif (DRC in the OT receptor) at the cytoplasmic side of transmembrane domain 3. The invariably conserved Arg has been hypothesized to be constrained in a hydrophilic pocket formed by conserved polar residues in transmembrane domains 1, 2, and 7 (see "polar pocket" site in Fig. 5) (166, 424). Receptor activation was suggested to involve protonation of the Asp in this motif causing Arg to shift out of the polar pocket leading to cytoplasmic exposure of buried sequences in the second and third intracellular loops. In accordance with this hypothesis, mutating Asp in this motif resulted in increased agonist-independent activity of some receptors including the V2 receptor (208, 396). Although for the V2 receptor (487) as for some other receptors, mutations of the Arg residue within this motif result in uncoupled receptor forms, the human OT receptor mutant R137A possesses an increased basal activity (166). Thus, in this mutant, conformational constraints are released that normally stabilize the wild-type OT receptor in its inactive ground state. Activation of the OT receptor might occur similarly as proposed for the α_{1B}-adrenergic receptor, i.e., by the opening of a solvent-exposed site in the cytosolic domains that has been hypothesized to be involved in G protein recognition (166, 503).

OT receptors are functionally coupled to $G_{q/11}\alpha$ class GTP binding proteins that stimulate together with G$\beta\gamma$ the activity of phospholipase C-β isoforms. 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. For example, the forming Ca^{2+}-calmodulin complexes trigger activation of neuronal and endothelial isoforms of nitric oxide (NO) synthase. NO in turn stimulates the soluble guanylate cyclase to produce cGMP. In smooth muscle cells, the Ca^{2+}-calmodulin system triggers the activation of myosin light-chain kinase activity which initiates smooth muscle contraction, e.g., in myometrial or

mammary myoepithelial cells (495). In neurosecretory cells, rising Ca^{2+} levels control cellular excitability, modulate their firing patterns, and lead to transmitter release. Further Ca^{2+}-promoted processes include gene transcription and protein synthesis.

In most cell systems studied so far, OT-induced intracellular Ca^{2+} increase is greater in the presence of extracellular Ca^{2+} than that in its absence. This suggests that OT has also effects on calcium influx through voltage-gated or receptor-coupled channels. The effect was nifedipine insensitive (495). OT was also shown to inhibit Ca^{2+}/Mg^{2+}-ATPase activity in sarcolemmal membranes from the rat uterine myometrium (532). This could sustain transient increases in intracellular Ca^{2+} concentrations and thereby prolong the effects of OT. In rat, guinea pig, and human myometrial cells, the OT-stimulated phosphoinositide hydrolysis was suggested to be mediated by pertussis toxin-sensitive and/or pertussis toxin-resistant G proteins (20, 359, 452). OT-stimulated GTPase and phospholipase C activities were attenuated by incubation with an antibody directed against the COOH termini of $G_q\alpha$ and $G_{11}\alpha$ in rat and human myometrial cells (314). In human myometrium, the coupling of OT receptors to a ~80-kDa G protein with transglutaminase activity, termed G_h, was proposed from experiments with solubilized OT receptor-G protein ternary complexes (38). Phospholipase C-δ1 was suggested as the effector for this kind of signal transduction (435). In Chinese hamster ovary (CHO) cells expressing the rat OT receptor, OT stimulated increases in intracellular $[Ca^{2+}]$, extracellular signal-related kinase-2 (ERK-2) phosphorylation, and PGE_2 synthesis (279, 539). OT also induces PGE_2 synthesis in uterine endometrial and amnion cells. In cultured uterine myometrial cells, OT caused tyrosine phosphorylation of mitogen-activated protein (MAP) kinase through an islet-activating protein-sensitive G protein (421). Solubilization experiments in combination with pertussis toxin sensitivity assays indicated that rat OT receptors can couple to both $G_{q/11}$ and G_i proteins in transfected CHO cells as well as in pregnant rat myometrium (539, 540).

Which are the receptor domains conferring G protein specificity? Functional analysis of V1a/V2 hybrid receptors demonstrated that the second intracellular loop of the V1a receptor and the third intracellular loop of the V2 receptor each are required and sufficient for efficient coupling to $G_{q/11}$ and G_s, respectively (344). Cytoplasmic loops 2 and 3 are also proposed to be implicated in receptor G protein coupling for many other GPCRs. In case of the OT receptor, this appears to be more complex. Several intracellular domains of the receptor could be involved in the specificity and/or efficacy of coupling to $G_{q/11}$. This was concluded from the finding that various coexpressed intracellular receptor domains interfered with the OT-stimulated inositol phosphate production (470, 495). Hoare et al. (241) provided evidence that prox-

imal parts of the COOH terminus of the rat OT receptor are required for coupling to G_q (Fig. 5). Whereas OT receptors with COOH-terminal truncations of 22 and 39 residues showed no effect on receptor function, the OT receptor lacking 51 COOH-terminal residues revealed an interesting phenotype: OT-induced intracellular $[Ca^{2+}]$ transients could be produced, although the phosphoinositide pathway was apparently not activated. However, it remained unclear which signals could mediate this Ca^{2+} release from intracellular stores. The Δ51 mutant receptor had a reduced affinity for OT and was uncoupled from G_q-mediated pathways. A coupling of this receptor to G_i was concluded, since the OT-induced Ca^{2+} transients were sensitive to pertussis toxin and to a $G\beta\gamma$ sequestrant. Because the Δ39 mutant was still able to couple to both $G_{q/11}$ and G_i, the sequence comprising the residues 339–350 of the rat OT receptor is required for interaction with $G_{q/11}$, but not G_i (241) (Fig. 5). Because of the high conservation of the COOH terminus of the OT receptor between various species, similar signal transduction mechanisms may also occur for OT receptors from species other than rats. It is possible that the fidelity of receptor G protein interaction is decreased when OT receptors are strongly upregulated, e.g., in myometrium near term. Moreover, it is known that phosphorylation of GPCRs not only induce their desensitization but may also modify their coupling specificity (123).

E. Receptor Internalization and Downregulation

When receptors are persistently stimulated with agonists, they desensitize. This process can occur by numerous mechanisms operating at the transcriptional, translational, and protein levels. Rapid, i.e., within seconds to minutes, homologous desensitization of GPCRs consists of two steps, phosphorylation and subsequent arrestin binding. The receptor uncouples from G proteins and undergoes endocytosis, internalization, or sequestration. Receptor sequestration is viewed as an early step in the downregulation of receptors that occurs after prolonged (hours to days) agonist stimulation and that may either end in degradation within lysosomes or in recycling back to the plasma membrane. These processes have been best studied for the adrenergic receptors (330). Birnbaumer and co-workers (52, 252, 253) have analyzed the internalization process for the vasopressin V1a and V2 receptors in some detail.

Like most other GPCRs, OT receptors may undergo rapid homologous desensitization following persistent agonist stimulation (162). Within 5–10 min after agonist stimulation, >60% of the human OT receptors expressed in HEK 293 fibroblasts were internalized (Gimpl and Fahrenholz, unpublished data), similar to as found for the human V2 receptor expressed in the same system (450)

and in LLC-PK$_1$ cells (276). Internalization of OT receptors occurs mainly by a clathrin-dependent pathway. But when stably expressed in HEK 293 cells, a fraction of OT receptors (10–15% of total) is localized in caveolae-like membrane microdomains (210). The internalization mechanism for this receptor population has not been examined. The internalized OT receptor is not recycled back to the cell surface (Gimpl and Fahrenholz, unpublished data). This indicates that the OT receptor behaves more like the V2 receptor and unlike the V1a receptor that rapidly recycles back to the cell surface (252). Using mutagenesis experiments and chimeric receptor constructs, Innamorati et al. (253) identified a serine cluster (Ser-362 to Ser-364) in the COOH-terminal tail of the V2 receptor acting as a retention signal for the internalized V2 receptor. The human OT receptor contains 17 potential phosphorylation sites including two serine clusters in its COOH terminus. One may speculate that these clusters also contribute to prevent the recycling of the internalized OT receptor (Fig. 5).

Exposure of human myometrial cells to OT for up to 20 h resulted in an almost 10-fold reduction in OT binding capacity (451). Although the total amount of OT receptor protein appeared not to be affected by OT treatment for up to 48 h, the OT receptor mRNA was reduced, which may be due to transcriptional suppression and/or destabilization of mRNA (451). When HEK 293 cells expressing the human OT receptor were treated for 18 h with high (μM) concentrations of OT, ~50% of the initial binding capacity remained at the cell surface (278). In WRK1 cells, OT was able to induce a desensitization of the vasopressin (VP) receptors when present for 18 h (89). Up- or downregulation of receptors could also be affected by yet ill-defined cross-talk mechanisms. Evidence for a cross-talk between the corticotropin-releasing hormone (CRH) and OT signal transduction pathways was provided in human myometrial cells at term (217). Furthermore, stimulation of β_2-adrenergic receptors causes heterologous upregulation of OT receptors in the nonpregnant estrogen-primed rat myometrium. In this system, a threefold increase in OT receptor mRNA, an ~100% rise in receptor binding, and an augmented contractile response of isolated uterine strips to OT were observed (156).

F. Effects of Steroids

1. Cholesterol

Both solubilized and membrane-associated OT receptors require at least two essential components for high-affinity OT binding: divalent cations such as Mn^{2+} or Mg^{2+} and cholesterol. Compared with many other GPCRs, the GTP sensitivity of the agonist binding to the OT receptor is rather modest. All attempts to purify functional OT receptors have been unsuccessful to date. With the use of 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO) as detergent, it is possible to solubilize functional OT receptors from different sources (174, 234, 301, 527). However, a common observation is that following solubilization, OT receptors lose characteristic binding properties, the affinity for OT becomes lower, and/or additional low-affinity state receptors appear in the extract. Unfortunately, low-affinity [e.g., dissociation constant (K_d) >10–50 nM] receptor populations cannot be characterized adequately by conventional radioligand binding assays. The necessity to separate free from bound ligand concomitantly leads to a dissociation of low-affinity ligand-receptor interactions. Solubilization with CHAPSO is known to lead to a substantial cholesterol depletion of the soluble extract, and we could demonstrate that substitution with cholesterol markedly enhanced the OT binding of soluble OT receptors (165, 301, 302). This became first evident in reconstitution of soluble OT receptors using liposomes of defined composition. A saturable high-affinity OT binding was obtained only with liposomes that contained a critical amount of cholesterol (301). Moreover, when OT receptors were expressed in insect cells, which naturally have plasma membranes with low cholesterol content, the receptors are mainly in a low-affinity state (K_d >100 nM). After addition of cholesterol to the culture medium, a fraction of OT receptors is converted from a low- to a high-affinity state (K_d ~1 nM) (212). The low-affinity state was identified as a physiological active receptor state, and the conversion of the affinity states to each other is, at least to a certain degree, reversible. The interaction of cholesterol with OT receptors is of high specificity and is not due to mere changes of membrane fluidity (209). Furthermore, cholesterol stabilizes both membrane-associated and solubilized OT receptors against thermal denaturation (210). Taken together, our data suggest a direct and cooperative molecular interaction of cholesterol with OT receptors. Cholesterol acts as an allosteric modulator and stabilizes the receptor in a high-affinity state for agonists and antagonists. In many but not in all cell systems, populations of high- and low-affinity OT receptors have been observed (119, 135, 209, 456). This could reflect uneven cholesterol distributions within the plasma membrane of these cells. We would expect that high-affinity state OT receptors are preferentially localized in cholesterol-rich subdomains of the plasma membrane. Likewise, receptor heterogeneity with respect to affinity states should be highest in cell systems with abundant cholesterol-rich domains such as rafts or caveolae structures, e.g., in myometrial cells at term (70). In fact, we recently provided evidence for a partial enrichment of high-affinity OT receptors in cholesterol-rich plasma membrane domains in HEK 293 fibroblasts stably expressing the human OT receptor (210, 211).

Divalent metal ions like Mg^{2+} are long known to

increase the response of target cells to OT and to shift the dose-response curve to the left. Thus addition of Mg^{2+} was found to increase both the OT binding capacity and the affinity state of the OT receptor (525). This is surprisingly similar to what cholesterol does. In addition, Mg^{2+} has been proposed to display its effect on the OT receptor interaction by influencing positive cooperativity (457). Mg^{2+} increases the potency of OT analogs in stimulating uterine contractions whereby the effects of Mg^{2+} were observed to be inversely related to the potency of the peptide. Relatively inactive peptides like 7-glycine OT became significantly more potent when the Mg^{2+} concentration bathing the uterine smooth muscle in vivo was increased from 0 to 0.5 mM (530). Conclusively, cholesterol and Mg^{2+} are essential allosteric modulators of the

OT receptor and may be involved in the regulation of OT-mediated signaling functions (see Fig. 6).

Do these allosteric modulators play a role for the regulation of OT-related physiological processes? Some reports suggest that particularly in reproductive tissues, the cholesterol concentrations may be highly dynamic. With the use of freeze-fracture cytochemistry with the cholesterol-binding filipin, marked increases in cholesterol have been found in rat uterine epithelial cells at the time of blastocyst implantation (400). In the human placental syncytiotrophoblast basal membrane, Sen et al. (512) observed a steady decrease in cholesterol-to-phospholipid ratio in correlation with an increase in membrane fluidity during placental development. At term however, the cholesterol-to-phospholipids ratio in syncy-
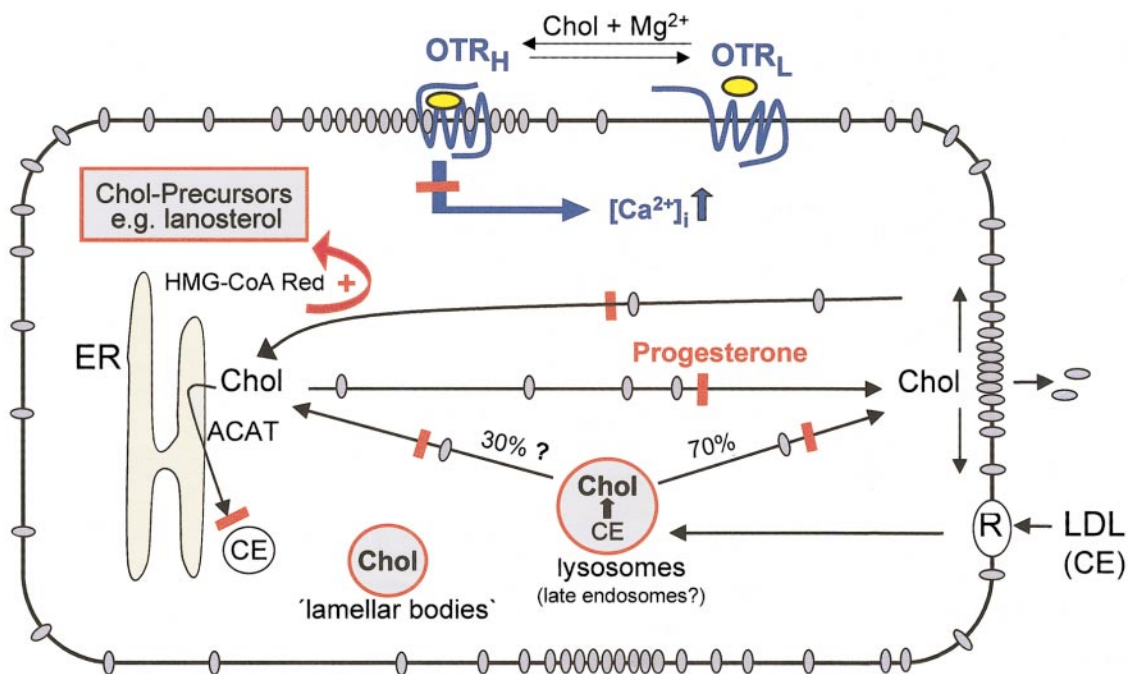

FIG. 6. Schematic model of nongenomic inhibitory effects of progesterone. Progesterone inhibits both the signal transduction of G_q coupled receptors (as shown here for the OT receptor) and the intracellular trafficking of cholesterol. Principally, eukaryotic cells can obtain the required cholesterol (Chol, gray ellipses) by two sources: endogenously by de novo synthesis of cholesterol and exogenously by uptake of cholesteryl ester (CE)-rich low-density lipoprotein (LDL) particles via receptor-mediated (R) endocytosis. De novo synthesized cholesterol first arrives at cholesterol-rich domains in the plasma membrane (caveolae and/or "lipid rafts") that may function as cholesterol "sorting centers" within the plasma membrane, where most of the cellular cholesterol resides. Progesterone blocks several intracellular transport pathways of cholesterol (red bars) except for the LDL receptor-mediated uptake of cholesterol. Moreover, cholesterol esterification does not occur in the presence of progesterone, presumably due to the lack of cholesterol substrate for acyl-CoA:cholesterol acetyltransferase (ACAT). As a consequence, unesterified cholesterol accumulates in lysosomes (or late endosomes) and lysosome-like compartments (designated as "lamellar bodies") (marked by red background). The key enzyme for the cholesterol de novo synthesis, 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA Red), is stimulated in the presence of progesterone (red arrow), but the cholesterol biosynthesis stops at the level of precursors (e.g., lanosterol). Enzymes involved in the conversion of cholesterol precursors reside in the endoplasmic reticulum (ER), and progesterone most likely prevents sterol precursors localized in the plasma membrane from reaching the ER-resident enzymes, thereby preventing their conversion to cholesterol. Overall, progesterone induces a state of cholesterol auxotrophy (383). However, after progesterone withdrawal, the accumulated precursors will be rapidly converted to cholesterol. Thus cells will become overloaded with cholesterol for a certain period of time, after which the cholesterol homeostasis will be reestablished. We hypothesize that these reversible progesterone-induced changes of the cholesterol trafficking could have a strong influence on signal transduction processes, particularly in case of the OT receptor (OTR$_H$ and OTR$_L$, high-affinity and low-affinity OT receptor, respectively; receptor in blue; OT in yellow) (for further details see sect. III*F*).

tiotrophoblast membranes was found to be increased compared with the cholesterol-to-phospholipid ratio in early placentas (365). Moreover, cholesterol-enriched caveolae structures are a conspicuous feature in the rat myometrium at term (70). We have provided evidence that cholesterol can modulate receptor function by both changes of the membrane fluidity and direct binding effects, e.g., in case of the OT receptor (209). Plasma membranes with lowered cholesterol content showed a decreased capacity (B_{max}) of binding sites and/or a decreased affinity (K_d) of ligand-receptor binding. Interestingly, Lopez et al. (345) reported that pregnancy in humans was associated with increases in both density and affinity of OT receptors. To draw further conclusions, correlation studies are required using tissues in which both the membrane cholesterol content and the OT receptor activity will be measured at the same time.

2. Progesterone

Progesterone is considered to be essential to maintain the uterine quiescence. Grazzini et al. (218) recently postulated that progesterone specifically binds to the rat OT receptor with high affinity (K_d ~20 nM) and thereby inhibits the receptor function. In case of the human OT receptor, a direct inhibitory interaction [inhibitory constant (K_i) ~30 nM] with a progesterone metabolite, 5β-pregnane-3,20-dione, has been reported by the same authors. They claimed that progesterone could act as a negative modulator of the OT receptor and thus offered a plausible mechanism of how progesterone could contribute to uterine quiescence. However, these findings could not be reproduced in several other laboratories including our own (81). Instead, we found that high concentrations of progesterone (>10 μM) attenuated or blocked the signaling of several GPCRs, including the OT receptor. The progesterone effects occurred within minutes, were reversible, and could not be blocked by a protein synthesis inhibitor (81). Overall, the action of progesterone was more cell type specific than receptor specific. The progesterone doses that are required to affect the signaling function of receptors are much higher than the progesterone levels found in plasma or in nonsteroidogenic tissues such as the myometrium. In steroidogenic tissues, however, huge amounts of progesterone have been measured. Near term, the human placenta secretes upward of 300 mg of progesterone daily. The progesterone content of this organ was shown to be 7 μg/g wet tissue (520). In human corpus luteum, progesterone concentrations reached peak levels of ~25 μg/g tissue shortly after ovulation and in the early luteal phase (545). These values are within the range of the progesterone concentrations that were effective in our study (81). Thus, in steroidogenic cells as well as in their environment, progesterone might nongenomically influence the signaling of receptors. The molecular

mechanisms underlying this progesterone action are not understood. A well-known progesterone binding protein is the multidrug resistance P-glycoprotein (471). In addition to their role in detoxification, P-glycoproteins are involved in intracellular cholesterol transport. It is known that progesterone markedly interferes with the intracellular transport (and metabolism?) of cholesterol (see model in Fig. 6). At concentrations in the micromolar range, it inhibits both the cholesterol esterification and the transport of cholesterol to and from the plasma membrane (343). In particular, progesterone reduces the cholesterol pool residing in caveolae (521). Paradoxically, at the same time, progesterone stimulates the activity of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, the key enzyme of de novo cholesterol biosynthesis. Hence, cholesterol precursors like lanosterol begin to enrich in the membranes of the cell (383). As mentioned above, the OT receptor needs a cholesterol-rich microenvironment to become stabilized in its high-affinity state (210). Because the cholesterol precursors, particularly lanosterol, are completely inactive to support the OT receptor in its high-affinity state (209), the responsiveness of the OT system may not be fully operative during the continuous presence of high progesterone concentrations. According to this scenario, progesterone withdrawal would restore the cholesterol transport so that the highly enriched amounts of cholesterol precursors would now become rapidly converted to cholesterol. This would lead to a sudden rise of cholesterol and should push the responsiveness to OT since low-affinity OT receptors could now be converted into their high-affinity state (302). According to this postulated mechanism, progesterone could affect the signaling of all those receptors that are functionally dependent on cholesterol. It is important to note that the nongenomic actions of progesterone including its influence on the cholesterol transport require progesterone concentrations in the micromolar range. This suggests that the described effects may be limited to the steroidogenic tissues and to their environment. Most likely, progesterone acts in these tissues via both genomic and nongenomic pathways (summarized in Fig. 6) together with other steroids to control receptor activity.

IV. THE PERIPHERAL OXYTOCIN SYSTEM

A. Female Reproductive System

1. Uterus

The pregnant uterus is one of the traditional targets of OT. OT is one of the most potent uterotonic agents and is clinically used to induce labor. Accordingly, the development of highly specific OT antagonists may be of therapeutic value for the prevention of preterm labor and the regulation of dysmenorrhea (358, 594).

The OT gene was found to be expressed in the rat uterine epithelium at term. The estrogen-induced elevation of OT mRNA levels was restricted to 3 days and reached peak levels that exceeded hypothalamic OT mRNA levels by a factor of 70 (327). In rats, OT gene expression was shown to be present in placenta and amnion (328) and in humans in amnion, chorion, and decidua (104). However, in most studies, significant increases of OT before the onset of labor have not been detected, neither in maternal plasma nor in intrauterine tissues. On the other hand, some findings suggest that there is a relationship between the pattern of OT secretion and advancing pregnancy. In rhesus monkeys, it was shown that maternal but not fetal OT concentrations were positively correlated with nocturnal uterine activity and progressively increased during late pregnancy and delivery (239).

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 strong increase in the density of myometrial OT receptors, reaching a peak during early labor (188, 299). This has been demonstrated both in the rat and in the human species, in which receptor levels rise during early labor to 200 times that in the nonpregnant state (189). Thus, at the onset of labor, OT can stimulate uterine contractions at levels that are ineffective in the nonpregnant state. After parturition, the concentrations of OT receptors rapidly decline. In rats, the uterine OT receptor mRNA levels decreased more than sevenfold within 24 h (624). Possibly, the downregulation of the OT receptors may be necessary to avoid unwanted contractile responses during lactation when OT levels are raised.

Gonadal steroids play an important role in the regulation of uterine OT receptors. In the days preceding birth, the ratio of plasma progesterone to estrogen falls. These changes in the steroid concentrations may occur in most mammals. At least in humans, progesterone withdrawal has not been determined. The steep drop of circulating progesterone occurs after placental delivery. Alteration of sex steroid metabolism in the fetoplacental unit appears to occur in women and primates (374). As shown in bovine and in sheep, maturation of the fetal hypothalamus leads to an increased secretion of CRH, which in turn stimulates the pituitary to secrete ACTH (188). Subsequently, ACTH stimulates the fetal adrenal to release cortisol. Additionally, OT-induced contractures of the myometrium in late pregnancy could lead to temporary decreases in blood flow and transient episodes of fetal hypoxia, which also may provoke a fetal stress response. Cortisol then increases the activity of the key enzyme cyctochrome P-450c17α, which promotes placental pregnenolone turnover into estrogens. In primates, CRH is additionally synthesized by the placenta and stimulates the fetal adrenal to secrete dehydroepiandrosterone sul-

fate (DHEAS), the precursor of placental estrogen (374, 522). In each case, the ratio of estrogen to progesterone increases in the maternal plasma concomitantly with increases in the synthesis of connexin-43, formation of gap junctions, increased production of prostaglandins from intrauterine tissues, and an upregulation of OT receptors (188, 388). Finally, the uterine quiescence that was maintained by the high progesterone level ceases, and parturition can occur. Upregulation of OT receptors and an increased expression of gap junctions also occur in the hours or days preceding human labor onset (191, 546). Obviously, estrogens and progesterone act in opposing fashion on the function, expression, and/or regulation of OT receptors. The mechanisms for the sudden and sometimes unpredictable responsiveness to OT of the myometrium is still mysterious (298). As Kimura (293) pointed out, the reaction of the uterus to OT in the same patient could vary from day to day. To induce labor at term, in some patients, OT is completely ineffective even at high doses, whereas in others a minimal dose can induce hypertonus of the uterus (293). However, despite the striking steroid dependence of the OT system, the promoter of the human OT receptor gene does not contain a classical steroid responsive element (see sect. III*A*). Several observations indicated that progesterone promotes uterine relaxation and inhibits the function of the OT receptor system by both genomic and nongenomic mechanisms. Even in the absence of protein synthesis, progesterone induces a reduction in uterine OT binding (528). Moreover, progesterone-mediated downregulation of OT receptors was not accompanied by a decrease in OT receptor gene expression (323). The mechanisms of how progesterone acts nongenomically are still unknown but are of fundamental importance (see sect. III*F*).

The increase in OT receptors before labor is not confined to the myometrium. It is also found in the decidua. During the course of parturition, the OT receptor gene expression in human chorio-decidual tissue was fivefold increased (547). In the decidua, OT has a separate action of stimulating the release of prostaglandin $PGF_{2\alpha}$. At the end of pregnancy, an increased secretion of $PGF_{2\alpha}$ drives luteolysis and thus leads to progesterone withdrawal and labor initiation in rodents. Mice lacking the gene encoding the $PGF_{2\alpha}$ receptor developed normally but were unable to deliver normal fetuses at term. These knock-out mice neither responded to OT nor did they show an induction of OT receptors. Furthermore, the normal decline of serum progesterone concentrations that precedes parturition did not occur. Ovariectomy restored induction of OT receptors and permitted successful delivery in the $PGF_{2\alpha}$ receptor-deficient mice. This indicates that $PGF_{2\alpha}$ acts upstream of OT to induce luteolysis, i.e., production of progesterone (543). The OT system may be regarded as a key regulator of parturition, with the induction of OT receptors as a trigger event.

However, in OT-deficient mice, parturition remained unaffected (416). This suggests that at least in mice, uterotonins other than OT are (additionally?) operative as myometrial contractants to initiate labor. A recent study with mice deficient in both OT and cyclooxygenase-1 shed more light for OT's role at the onset of labor (219). Cyclooxygenase-1 is involved in the synthesis of prostaglandins, and as expected, mice lacking this enzyme showed reduced levels of $PGF_{2\alpha}$, impaired luteolysis, a less pronounced fall in progesterone in late gestation, and finally a delayed initiation of labor. Surprisingly, mice deficient in both OT and cyclooxygenase-1 initiated labor at the normal time. Thus OT and prostaglandins had apparently opposing actions on the onset of murine parturition: a luteotrophic function of OT versus a luteolytic action of prostaglandins. So, OT stabilizes the progesterone synthesis before parturition. For the normal timing of labor it might be essential to generate sufficient $PGF_{2\alpha}$ to overcome the luteotrophic action of OT in late gestation. These findings might explain why the upregulation of OT receptor occurs without an increased expression of OT shortly before the onset of labor. The induction of uterine OT receptor expression allows OT to act as a potent uterotonic agent, whereas an increase in OT expression could prolong gestation due its trophic effects on the corpus luteum (219). Fuchs et al. (195) reported that OT may also be involved in induction of cyclooxygenase-2 expression and $PGF_{2\alpha}$ release at term and during parturition in cows.

In ruminants, the endometrial OT receptor exhibits a cycle-dependent regulation and plays a crucial role in reproduction (518). In the bovine endometrium, OT receptor mRNA levels can exceed the levels in the myometrium at the same stage of the cycle (272). Within 2–3 days around estrus, the endometrial OT receptors increase to a level similar to that observed at term of pregnancy. For the remainder of the cycle, OT receptor concentrations are almost undetectable. Pulsatile secretion of endometrial $PGF_{2\alpha}$ is stimulated by OT during late diestrus in domestic ruminants and results in regression of the corpus luteum leading to the onset of a new estrous cycle. Thus the uterus influences the length of the luteal phase via the expression of the endometrial OT receptor. The administration of an OT antagonist or the continuous administration of OT, which downregulates the OT receptor, delays the onset of luteolysis and lengthens the cycle (178, 517). A steep rise in endometrial OT receptors also precedes the onset of labor in ruminants. In cows, the receptor density increases almost 200-fold and remains at high levels during labor (192). Suppression of the endometrial OT receptor gene expression was shown to be caused by antiluteolytic proteins, e.g., interferon-γ secreted by the trophoblast of the developing blastocyste (267, 483). In primates, the endometrium is not essen-

tial for luteolysis, since hysterectomy does not abolish cyclic ovarian function. In humans, Northern blotting revealed the presence of OT receptor mRNA in the pregnant myometrium, in the endometrium, and in the ovary. In the nonpregnant human uterus, the OT receptor mRNA is mainly expressed in the glandular epithelial cells of the endometrium with highest expression level occurring at ovulation (548). However, its physiological role is unclear.

One of the highest concentrations of OT receptors (~10 pmol/mg protein) has been measured in rabbit amnion at term (234). At the end of gestation, rabbit amnion OT receptors increase more than 200-fold. This receptor upregulation is associated with the release of PGE_2 from cultured amnion cells and suggests an important role for the amnion and OT in the initiation of labor in rabbits (234). In the same system, cortisol and cAMP caused a marked physiological upregulation of OT receptors as well as an increased PGE_2 response to OT. Addition of cortisol to amnion cells increased OT-stimulated PGE_2 release almost 100-fold, whereas the combination of forskolin and cortisol increased the PGE_2 response to OT ~5,600 times (236). OT receptors in human amnion were also associated with PGE_2 release and were found to be upregulated in early and advanced labor, albeit to a much lower degree (49, 393). In contrast, no OT binding sites were observed in amniotic/chorionic or placental membranes from rats (328).

Many studies have supported the view that OT is synthesized in a paracrine system operating within human intrauterine tissues (389). This includes the fetal membranes amnion and chorion and the maternal decidua. These tissue layers are continuously bathed in amniotic fluid and could transmit signals of maternal or fetal origin to the myometrium. Because fetal membranes are capable of steroidogenesis, they may contribute to local changes in the estrogen-to-progesterone ratio. Although there is evidence that during late gestation OT gene expression is upregulated in intrauterine tissues, the OT peptide itself was not found at concentrations significantly higher than in the circulating blood. This could be due to the high activity of oxytocinases, but that still remains to be shown (388). The possibility of local, paracrine effects of OT was also suggested to be present within the intrauterine tissues of the rat and bovine (272, 327). In the pregnant cow, OT mRNA levels were found to be very low except for the corpus luteum. After the onset of labor, both OT gene and OT peptide were expressed at significant levels in the corpus luteum. This rescue of luteal OT at term could act to supplement the circulating hormone of pituitary origin (272). Thus OT may act primarily as a local mediator and not as a circulating hormone during parturition. In an autocrine/paracrine system within the uterus, significant changes of OT, prostaglandins, and sex steroids could

occur without being reflected in the maternal circulation (389).

2. Ovary and corpus luteum

In several species, the ovary has been shown to contain OT and may be a site of local OT production (271). In the marmoset monkey *(Callithrix jacchus)*, in vivo and in vitro studies suggested that OT is a follicular luteinization factor. After human chorionic gonadotropin treatment, almost all granulosa cell layers in antral follicles showed immunoreactivity for both OT and OT receptor. OT was produced only by granulosa cells derived from preovulatory follicles, and after application of OT, only the granulosa cells cultured from preovulatory follicles elicited an increase in progesterone production (146, 149). Functional OT receptors have been detected in bovine granulosa cells, suggesting that OT may be an autocrine factor during follicular growth (423). OT also increased the rate of mouse blastocyst development and might therefore play some role in the early stage of development of fertilized oocytes (199). 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 (198).

The interaction of neurohypophysial OT with endometrial OT receptors evokes the secretion of luteolytic pulses of uterine $PGF_{2\alpha}$. McCracken et al. (372) have postulated the concept of a central OT pulse generator that functions as pacemaker for luteolysis. According to this concept, the uterus transduces hypothalamic signals in the form of episodic OT secretion, into luteolytic pulses of uterine $PGF_{2\alpha}$. In ruminants, luteal OT is released synchronously by uterine $PGF_{2\alpha}$ pulses. Thus a positive-feedback loop is established that amplifies neural OT signals. The onset of this feedback loop and episodic $PGF_{2\alpha}$ secretion is controlled by the appearance of OT receptors in the endometrium. During pregnancy, the developing conceptuses must prevent endometrial $PGF_{2\alpha}$ from being released into the uterine vasculature to prevent corpus luteum regression and progesterone withdrawal. The continued progesterone secretion is required for establishment and maintenance of pregnancy. Luteal cells produce OT, but they can also be target cells for OT action. OT receptors have been characterized on both steroidogenic cell types of the corpus luteum, small and large cells (454). In several species, OT release was highest in the young corpus luteum (148, 277). In the pig, the density of OT receptors was also highest during the early luteal phase, suggesting that autocrine/paracrine actions of OT may occur primarily in the young corpus luteum (454). The effects of OT on cultivated luteal cells were also strongly dependent on the age of the corpus luteum. In the pig, a function for OT in the ovary has been sug-gested in both a luteotrophic and luteolytic context (604). Luteal OT and progesterone release occurs in tightly coupled pulses. In vivo, OT and $PGF_{2\alpha}$ stimulate estradiol and progesterone release, and estradiol itself further stimulates progesterone release. Thus there may be an intraluteal circuit that involves paracrine effects of estradiol, OT, and $PGF_{2\alpha}$.

B. Male Reproductive Tract

1. Testis

In several species, a pulse of systemic OT, presumably of hypothalamic origin, appears to be associated with ejaculation. The systemic hormone could act peripherally stimulating smooth muscle cells of the male reproductive tract, but could also reflect central effects in the brain modulating sexual behavior (see sect. VIA).

OT has been identified in the testes from various mammalian species, and a mesotocin-like peptide has been demonstrated in the testes of birds (453) and marsupials (47). There is evidence that OT is made locally within the testis, and possibly also the epididymis and prostate (265). Concerning the localization of the OT system in the male reproductive tract, species-specific differences are important to note. For example, mice do not have detectable levels of OT mRNA in their testes, whereas cattle have relatively high levels of testicular OT mRNA (16). In contrast to the eutherian mammals, in the tammar wallaby (*Macropus eugenii*), the mesotocin receptor gene and protein, which are highly homologous to the eutherian OT receptor, are expressed in the prostate gland, but not in the testis (437). In the human, the complete OT system appears to be present in testis, epididymis, and prostate (182).

In the rat testis, OT concentrations are higher than those in the peripheral circulation. OT gene transcripts could be detected using PCR analysis but not by Northern hybridization (180). Within the rat testes, OT is present in the interstitial Leydig cells, the cells which provide the main source of testosterone in the male (412). Testicular OT levels are not constant but vary in dependence of the level of gonadotrophins and the activity of the seminiferous epithelium. OT production is increased in vitro by luteinizing hormone (LH), whereas testosterone itself has no effect on the secretion of OT. Notably, OT was only detectable during active spermatogenesis (413). Primarily two functions have been ascribed to testicular OT, namely, the regulation of seminiferous tubule contractility and the modulation of steroidogenesis. In the testis, the seminiferous tubules are surrounded by smooth muscle-like cells, the myoid cells. OT has been shown to enhance the contractility of the tubules; therefore, the responsiveness to OT was higher at a certain stage of the spermatogenic cycle, around the time when the sperm are

shed into the lumen (413). Obviously, OT promotes the spermiation and the subsequent transport of the immotile spermatozoa to the epididymis. However, there is no clear evidence for the expression of OT receptors on myoid cells, the cells which are mainly responsible for the contractile activity of the tubules (246). Because Sertoli cells exhibit the components of a local OT system under some circumstances, they may also be involved in the contractile activity of seminiferous tubules. On the other hand, it was suggested that the contractility effects are mediated by V1a receptors that are present in testes at severalfold higher densities compared with OT receptors (227).

Uterine-type OT receptors have been identified in the interstitial spaces in the rat testis consistent with binding to Leydig cells (45). Also in the male marmoset monkey (*Callithrix jacchus*), OT and its receptor have been detected predominantly within the Leydig cells (145). The Leydig cells drive spermatogenesis via the secretion of testosterone, which acts on the Sertoli and/or peritubular cells to create an environment that enables normal progression of germ cells through the spermatogenic cycle. Daily subcutaneous injections of OT led to an increase in the plasma and testicular levels of testosterone. In contrast, continuous administration of the peptide into the testis, whether by implants or in the transgenic mouse which overexpresses the bovine OT gene in its testes, produced a decrease in testosterone but an increase in dihydrotestosterone concentrations (15, 411). Although in the testis the predominant androgen is testosterone, elsewhere in the male reproductive tract testosterone acts as a prohormone and is converted to its active metabolite dihydrotestosterone by the enzyme 5α-reductase. OT was shown to increase the activity of 5α-reductase in both testis and epididymis and may thus have an autocrine/paracrine role modulating steroid metabolism in these tissues (413).

2. Prostate gland

The prostate is an androgen-dependent gland. Testosterone enters the cell and is converted to dihydrotestosterone, which then modulates growth and prostatic functions. OT is present in the prostate at concentrations higher than in the plasma and can increase the resting tone of prostatic tissue from guinea pig, rat, dog, and human (58, 182, 265). OT also evoked contractile activity on mammalian prostates in vitro (58). Thus it was suggested that OT is involved in the contraction of the prostate and the resulting expulsion of prostatic secretions at ejaculation (413).

OT may also be involved in the pathophysiology of benign prostatic hyperplasia (410). In humans, benign prostatic hyperplasia occurs spontaneously, is often associated with bladder outlet obstruction, and affects 50% of men over the age of 60 (51). However, obstructive symp-

toms may not only be caused by an enlargement of the gland but also by an increase in smooth muscle tone. So, in the nonoperative management of prostatic hyperplasia, α_1-adrenergic blockers are presently used. Because OT is even more potent at increasing smooth muscle tone than norepinephrine, OT antagonists may be potentially useful in the treatment of this disease (413).

OT can stimulate growth of the prostate in the rat, particularly the mitotic activity in the glandular epithelium (455). OT treatment elevated the testosterone levels of plasma, testes, and prostate. Conversely, prostatic OT concentrations are found to be decreased by testosterone and increased after castration. In rats, OT treatment only transiently increased 5α-reductase activity in the prostate, consistent with the short-lived increase of dihydrotestosterone in this tissue. These observations suggest that local feedback mechanisms act to control prostatic levels of dihydrotestosterone and prostatic growth (413). The findings in rats were somewhat different to those in dogs, which unlike rats can spontaneously develop prostatic hyperplasia. Interestingly, old dogs with established prostatic hyperplasia showed raised OT levels accompanied by elevated 5α-reductase activity within their prostatic tissues. It is hypothesized that OT acts as a paracrine factor to regulate cell growth via its influence on the enzyme 5α-reductase (410).

C. Mammary Tissues

1. Milk ejection

One of the classical roles assigned to OT is milk ejection from the mammary gland. The secretion of the mammary glands is triggered when the infant begins to suck on the nipple. The stimulation of tactile receptors at that site generates sensory impulses that are transmitted from the nipples to the spinal cord and then to the secretory oxytocinergic neurons in the hypothalamus. These neurons display a synchronized high-frequency bursting activity, consisting of a brief (3–4 s) high-frequency discharge of action potentials recurring every 5–15 min. Each burst leads to massive release of OT into the bloodstream by which OT is carried to the lactating breasts. There it causes contraction of the myoepithelial cells in the walls of the lactiferous ducts, sinuses, and breast tissue alveoli. In humans, within 30 s to 1 min after a baby begins to suckle the breast, milk begins to flow. This process is called milk ejection or milk let-down reflex and continues to function until weaning.

As mentioned above, central oxytocinergic neurons are decisive components for the initiation and maintenance of successful lactation. So, the reflex that elicits OT release in women is activated before the tactile stimulus of suckling occurs and is related to such factors as the baby crying (373). The essential role of OT for the milk

let-down reflex has been confirmed in OT-deficient mice. In fact, the major deficit of these female OT knock-out mice was the failure to nurse their offspring (416). In addition, the presence of OT in conjunction with continued milk removal was also required for postpartum alveolar proliferation and mammary gland function. Alveolar density and mammary epithelial cell differentiation at parturition were similar in wild-type and OT-deficient dams. However, within 12 h after parturition, ~2% of the alveolar cells in wild-type dams incorporated DNA and proliferated, whereas no proliferation was detected in OT-deficient dams. Continuous suckling of pups led to the expansion of lobulo-alveolar units in wild-type but not in OT-deficient dams. Despite suckling and the presence of systemic lactogenic hormones, mammary tissue in OT-deficient dams partially involuted (581). Continuously elevated OT concentrations such as those during infusion or during normal milking are also necessary for complete milk removal in diary cows (75).

Biochemical studies showed that the mammary OT receptor is a 65-kDa protein (in rats), coupled to phosphoinositide turnover, and is most likely the same as the uterine-type OT receptor (399, 448). In rats, Soloff and Wieder (533) reported an ~100-fold increase in the number of OT receptors per mammary gland between the first day of pregnancy and late lactation. A very strong increase in OT binding sites toward term was also observed in porcine mammary tissues (351). In humans, however, Kimura et al. (294) did not find an elevated expression level of the OT receptor mRNA during lactation. As judged by OT receptor immunoreactivity, unexpectedly, the ductal/glandular epithelium revealed a higher density of OT receptors compared with myoepithelial cells in both the lactating and nonlactating breast in humans and in the common marmoset (*Callithrix jacchus*) (294).

2. Breast cancer and tumor cells

Breast cancer is the leading cause of death in women between ages 35 and 45, but it is most common in women over age 50. It is unknown why mothers who breast-fed their babies have a 20% lower incidence of breast cancer after menopause than mothers who did not. Murrell (403) proposed a hypothesis that partially links breast cancer to the activation of the OT system. Accordingly, carcinogens in the breast may be generated by the action of superoxide free radicals released when acinal gland distension causes microvessel ischemia. Thus inadequate nipple care in the at-risk years could lead to ductal obstruction preventing the elimination of carcinogens from the breast. The regular production of OT from nipple stimulation would cause contraction of the myoepithelial cells, relieving acinal gland distension and aiding the active elimination of carcinogenic fluid from the breast. Thus OT production was suggested as a preventative factor in the

development of breast cancer both pre- and postmenopausally (403).

OT receptors have been described in a number of human breast tumors and breast cell lines, e.g., the human tumor cell lines MCF-7 or Hs578T. Copland et al. (118) recently studied the behavior of OT receptors in dependence of culture conditions in Hs578 cells. Hs578 cells responded to OT by an increase of intracellular Ca^{2+} and stimulation of ERK-2 phosphorylation and PGE_2 synthesis. OT receptors in these cells were strongly downregulated by serum starvation. Conversely, restoration of serum and addition of dexamethasone (1 μM) increased the OT receptor levels by nearly 10-fold and also led to an elevation of the steady-state OT receptor mRNA level. These cells may be good models for future studies with respect to OT receptor regulation in dependence of steroids (118).

Controversial results have been published concerning a proliferative action of OT on breast tumor cells. Ito et al. (263) found that OT had no effect on the growth of different breast cell lines cultured for 7 days. The OT receptor was expressed in breast cancer derived not from the myoepithelium but from the glandular or ductal epithelium. According to Sapino et al. (497), OT exerts a trophic effect on myoepithelial cells in the mammary gland. In organotypic cultures of the mouse mammary gland, OT induced differentiation and proliferation of myoepithelial and, to a lesser extent, luminal epithelial cells. On the other hand, OT inhibited cell proliferation and tumor growth of rat and mouse mammary carcinomas. In MCF7 cells for instance, OT inhibited estrogen-induced cell growth and enhanced the inhibitory effect of tamoxifen on cell proliferation. Antiproliferative effects of OT have also been observed in various breast carcinoma cell lines as well as in human neuroblastoma and astrocytoma cells, and these effects were accompanied by the activation of the cAMP/protein kinase A pathway (97, 98). In vivo, OT reduced the growth of certain mammary tumors. With the use of immunohistochemistry and RT-PCR, OT receptors and its corresponding mRNA were detected in normal and pathological breast tissues. Interestingly, the expression of OT receptors was positively correlated with the progesterone level of these tissues (496). Further studies are required to clarify the role of the OT system concerning the long-term effects in mammary tissues and tumor cell lines derived from them.

D. Kidney

The kidney is one of the peripheral target tissues for neurohypophysial hormones that exercise control over hydromineral excretion. The hormones are released into the blood by stimulations, such as hypovolemia or hyperosmolarity, that generally activate the OT and AVP neu-

rons. When plasma sodium concentration exceeds 130 mM, the levels of both hormones increase as an exponential function of plasma sodium concentration (575). OT is a nonhypertensive natriuretic agent. It is involved in normal osmolar regulation, which is presumably different from the volume regulatory components of Na^+ homeostasis. Acute administration of OT to conscious rats produced a modest increase in the glomerular filtration rate and effective filtration fraction. The natriuretic effect of OT is mainly due to a reduction in tubular Na^+ reabsorption, probably in the terminal distal tubule or the collecting duct (116, 247, 426).

Autoradiographical analysis showed the existence and precise localization of OT receptors in the rat kidney. Interestingly, the distribution of OT binding sites undergoes reshaping during postnatal development as it was similarly observed in the rat brain during maturation (see sect. v*A* and Table 4) (504, 560). Specific OT binding sites have been first detected at embryonic *day 17* in the cortex. In the medulla, OT binding sites were first detected at embryonic *day 19* when this region is forming. In the adult rat, OT binding sites were exclusively localized in the cortex, mainly concentrated in the macula densa. In the inner medulla, the OT binding sites were found on the loops of Henle of the juxtamedullary nephrons. Interestingly, at all stages examined, cortical OT-binding sites had a higher selectivity for OT versus vasopressin compared with the medullary sites. It was therefore suggested that OT binding sites of the macula densa and thin Henle's loop could represent two subtypes of OT receptors (33, 34). The location profile of the receptors suggests a possible role for OT in the regulation of tubuloglomerular feedback and solute transport.

Estrogen induced OT receptor gene expression in the outer medulla region and increased expression of OT receptors in macula densa cells of ovariectomized female and adrenalectomized male rats (426). Experiments with the antiestrogen tamoxifen suggested cell-specific regulation of OT receptor expression in macula densa and proximal tubule cells. Thus OT receptors may mediate estrogen-induced alterations in renal fluid dynamics (426). OT receptor mRNA levels have been measured in kidneys of late-pregnant, peri-parturient, and lactating rats. Of interest, OT receptor transcripts could not be detected in renal tissues of peri-parturient females, at times when OT receptor mRNA levels were highest in uterus. However, OT receptor gene expression in macula densa cells gradually reappeared and again achieved control levels by *day 20* post partum (426). Breton et al. (67) observed that both the OT receptor mRNA levels as well as the OT binding capacity of rat renal extracts were significantly lower at term. Although in the rat kidney there was no indication for another than the uterine-type OT receptor gene expression, the mechanisms controlling the expression of OT receptor genes in uterus, pituitary, and kidney are obviously different (67).

OT injected intraperitoneally caused dose-dependent increases in urinary osmolality, natriuresis, and kaliuresis in the rat. Moreover, the injection of OT evoked concomitant release of ANP and natriuresis (222) (see sect. IV*E*). Soares et al. (523) recently provided evidence for the hypothesis that OT acts in concert with ANP to induce natriuresis and kaliuresis via the common mediator cGMP. In rats, the OT-induced natriuresis was caused mainly by decreased tubular Na^+ reabsorption and was accompanied by increases in both urinary cGMP and NO_3^- excretion (523). Although the natriuretic action of ANP is mediated by activation of renal guanylyl cyclase A (GC_A) receptors localized in glomeruli, their afferent and efferent arterioles, and the tubules, OT is hypothesized to induce natriuresis by activation of renal NO synthase localized in macula densa cells (591). Presumably, binding of OT to their receptors on NOergic cells in the proximal tubules and macula densa leads to an increase of intracellular $[Ca^{2+}]$, followed by Ca^{2+}/calmodulin-induced stimulation of NO synthase and activation of the soluble guanylate cyclase by NO. The consequent release of cGMP could then mediate natriuresis and kaliuresis via closure of Na^+ and K^+ channels. In cortical collecting duct cells, it has been shown that the NO-inhibited Na^+ transport is associated with increased cGMP content (538). Overall in rats, OT as well as ANP induce a concomitant reduction of both extracellular and intracellular fluid volume in states of increased body fluid volume.

The signal transduction of the renal OT receptor has been evaluated in various kidney epithelial cells in culture, e.g., LLC-PK_1. In these cells, OT induced stimulated phosphoinositide hydrolysis and transiently increased cytosolic $[Ca^{2+}]$. OT was also able to stimulate the soluble guanylate cyclase and increased the intracellular level of cGMP in LLC-PK_1 cells (334, 534). Moreover, OT was found to stimulate the synthesis of prostaglandins in a dose-dependent manner in kidney homogenates (100).

What is known about the OT effects on kidney function in species other than rats? In rabbits, OT was shown to affect apical sodium conductance of the microperfused cortical collecting duct through OT receptors distinct from the vasopressin receptors (255). The effects were inhibited by the addition of an OT antagonist. Although the natriuretic response to OT has also been described for conscious dogs, it probably does not occur in humans and nonhuman primates. Thus the contribution of OT to renal physiology in primates including humans, if any, remains uncertain (116).

E. Heart and Cardiovascular System

Peripherally injected OT decreases mean arterial pressure in rats (445, 449). Even in the absence of a

central control mechanism, OT is able to reduce the heart rate and the force of atrial contractions in isolated atria from perfused rat hearts (167). An OT antagonist reversed the bradycardia caused by OT. Moreover, administration of OT at high concentrations (1 μM) leads to the stimulation of ANP release (221). An OT antagonist first inhibited this ANP release, and after prolonged perfusion, it finally decreased the OT-induced ANP release below that of control hearts. This suggested that intracardial OT stimulates ANP release in the heart. Gutkowska et al. (221) proposed that OT and ANP act in concert in the control of body fluid and in cardiovascular homeostasis. In favor of this hypothesis, OT receptor transcripts and OT binding sites were shown to be present on atrial and ventricular sections as detected by in situ hybridization and autoradiography, respectively. Furthermore, the OT receptor gene is expressed in all chambers of the rat heart, and the analysis of the RT-PCR products indicated the presence of the uterine-type OT receptors in the heart (221). Although the OT receptor mRNA levels in the atria were found to be higher than in the ventricles, overall the OT receptor mRNA levels were calculated to be at least 10 times lower than the OT receptor mRNA level present in the uterus of a nonpregnant rat.

The rat heart was also shown to be a site of OT synthesis. OT was detected in the effluent of isolated heart perfusates as well as in the medium of cultured atrial myocytes. OT concentrations were found to be higher in the atria than in the ventricles. In the right atrium, OT concentrations $\sim$20-fold higher than those in the rat uterus have been measured. In contrast, the OT mRNA level in the heart tissues was found to be lower than that in the rat uterus. This discrepancy argues against the postulated abundant biosynthesis of cardiac OT (274). The relatively low concentrations of OT in the heart chambers and the high OT doses required for ANP release would be more compatible with paracrine or autocrine effects of cardiac OT, particularly in the right atrium. Although the OT quantities released from the perfused heart are not sufficient to substantially change the plasma concentrations of OT, they may contribute to the natriuretic action via stimulation of ANP release (274). Presumably, blood volume expansion via baroreceptor input to the brain causes the release of OT that circulates to the heart. OT-induced ANP release in the heart may be achieved after activation of OT receptors and subsequent elevation of intracellular [Ca^{2+}], which in turn could stimulate exocytosis and ANP secretion (221, 274). ANP then exerts a negative chrono- and inotropic effect via activation of guanylyl cyclase and release of cGMP. Finally, a rapid reduction in the effective circulating blood volume is produced by an acute reduction in cardiac output, coupled with ANP's peripheral vasodilating actions. The ANP released would also act on the kidneys to cause natriuresis, and ANP acts within the brain to inhibit water and salt intake, leading to a gradual

recovery of circulating blood volume to normal (167). Since the plasma concentration of both OT and ANP were found to be increased after parturition, the OT-stimulated ANP release might be at least partly responsible for the massive diuresis observed postpartum.

The effects of repeated subcutaneous OT injections on blood pressure and heart rate were investigated in spontaneously hypertensive rats. Surprisingly, when OT were given for 5 days, a sustained decrease in blood pressure was observed in male but not female rats, whereas the heart rate was unaffected (446). Moreover, acute versus chronic OT treatments caused opposite effects on blood pressure, and these effects were modified by female sex hormones (447).

It appears that the complete OT system is present in the vasculature of the rat. The OT concentrations in the aorta and vena cava were reported to be even higher than those in the right atrium of the heart. Thus OT might play a direct role in volume and pressure regulation in a paracrine/autocrine manner (275). In the case of the umbilical-placental vasculature, OT was reported to be a far more potent vasoconstrictor than vasopressin. OT was therefore proposed to be involved in the closure of the umbilical vessels at birth (9). A uterine-type OT receptor could also mediate vasodilatory responses in human vascular endothelical cells (555).

OT-induced cardiac effects were also reported for some other species. The cardiac effects of neurohypophysial hormones were studied in the frog, *Rana tigrina*, and in the snake, *Ptyas mucosa*. In both species, OT produced dose-related cardiac effects in isolated atrial preparations. In the frog, vasotocin was the most potent hormone, whereas in the snake, OT produced the most potent dose-related positive inotropic changes (110). Furthermore, OT was found to modulate the intrathoracic sympathetic ganglionic neurons regulating the canine heart. When OT was injected into right atrial ganglionated plexi, heart rate and atrial forces were reduced in 5 of 10 dogs studied. However, no cardiac changes occurred when OT was injected into left atrial or ventricular ganglionated plexi. In this study, the connectivity with the central nervous system (CNS) was found to be necessary to elicit consistent OT-induced responses (32). Although OT elicited neuronal and concomitant cardiovascular responses in most dogs when administered adjacent to spontaneously active intrinsic cardiac neurons, OT injection into the superior vena cava only evoked a slight systemic hypotension in two of seven dogs (31).

F. Other Localizations

1. Thymus

The thymus is the primary lymphoid organ reponsible for the selection of the peripheral T-cell repertoire. Neuropeptides and their receptors have been described in

the thymus, supporting the concept of a close dialogue between the neuroendocrine and the immune systems at the level of early T-cell differentiation. The neurohypophysial peptides have been shown to trigger thymocyte proliferation and could induce immune tolerance of this conserved neuroendocrine family. OT has been identified in human thymus by immunoreactivity and at the transcriptional level. OT was present in the thymus in surprisingly large amounts and was found to be restricted to certain subtypes of thymic epithelial cells (205, 392). Due to its higher expression level in the thymic epithelium, OT was proposed as the self-antigen of the neurohypophysial family (204). OT was found to be colocalized with the cytokeratin network of thymic epithelial cells and not within secretory granules. The peptide is therefore not secreted but behaves like an antigen presented at the outer surface of the cell. Thymic OT also behaves as a cryptocrine signal targeted at the epithelium membrane from where it is able to interact with neurohypophysial peptide receptors expressed by pre-T cells. OT receptors are predominantly expressed by cytotoxic CD8$^+$ lymphocytes, and they transduce signals via the phosphoinositide pathway. In pre-T cells, OT was found to induce the phosphorylation of focal adhesion kinase (360). Thus it was suggested that OT actively intervenes in the program of T-cell differentiation both as a neuroendocrine self-antigen and as a promoter of T-cell focal adhesion following a cryptocrine pathway.

OT receptors detected in thymic membranes or on thymocytes revealed a ligand selectivity similar to that of uterine OT receptors (153). Caldwell et al. (86) reported that sexual activity leads to decreases of OT receptor densities in the thymus of rats. In human thymus extracts, the content of the OT immunoreactivity declined with increasing age, whereas in rat thymic extracts, it was reported to increase during aging. The age-dependent changes might be linked to thymic involution (381). Some immune pathologies in humans may be explained by thymic OT being involved in T-cell-positive selection and activation.

2. Fat cells

In adipocytes, OT has a so-called insulin-like activity in that it stimulates glucose oxidation and lipogenesis and increases pyruvate dehydrogenase activity (223). Treatment of rat adipocytes with lipolytic stimuli such as glucagon or isoprenaline increased the conversion of choline into phosphatidylcholine, and this lipolytic effect was antagonized by OT (285). In rat adipocytes, OT at a concentration of 1 nM markedly increased phosphoinositide breakdown (159). OT also elicited a transient increase in intracellular [Ca^{2+}] and stimulated the protein kinase C activity (144, 510). Moreover, human fat cells possess a plasma membrane-bound H$_2$O$_2$-generating system that is sensitive to extracellular stimuli. OT as well as insulin

were able to activate this system. It was suggested that the H$_2$O$_2$ produced might participate in the regulation of fat cell differentiation and/or maintenance of the differentiated state (312). Populations of low- and high-affinity OT receptors have been described in rat adipocytes (61).

3. Pancreas

OT and AVP have been identified in human and rat pancreatic extracts at higher concentrations than those found in the peripheral plasma (10). However, a local synthesis of these peptides within this organ has not yet been established. According to most studies in several species, the neurohypophysial hormones induce the release of glucagon and, to a lesser extent, insulin from the pancreas. During in situ perfusion of the rat pancreas with OT, a marked stimulation of glucagon release and a modest stimulation of insulin release were observed (142). With the use of isolated islets from rat pancreas, OT elicited a stimulation of glucagon release but failed to influence insulin release in a culture medium with low glucose content. The glucagonotrophic action of OT was greatly diminished in the presence of a higher concentration of glucose (141). Similar findings were reported in studies with conscious dogs (141, 578). With the use of the microdialysis technique, OT administered directly in the pancreas of the rat stimulates the release of both insulin and glucagon. OT binding sites were identified in the periphery of the islets of Langerhans, corresponding to the localization of the glucagon-producing α-cells (535). In isolated mouse islets, OT produced an increase in somatostatin, glucagon, and insulin release, and it amplified the glucose-induced insulin release probably via stimulation of the phosphoinositide metabolism (201). In humans, OT evoked a rapid surge in plasma glucose and glucagon levels followed by a later increase in plasma insulin and epinephrine levels. The effects of OT on plasma glucagon and epinephrine levels were potentiated by hypoglycemia. OT was also found to potentiate glucose-induced insulin secretion (434). In contrast, Page et al. (432) observed no effects of OT on the decline or recovery of blood glucose concentrations or on the plasma glucagon response to insulin-induced hypoglycemia in humans.

Impaired glucose tolerance and hyperinsulinemia are common features of obesity. Interestingly, the plasma levels of OT were found to be fourfold higher in male and female obese subjects compared with control subjects (536). OT rises in hypoglycemia, and this response is partially inhibited by dexamethasone (108). The OT rise in response to insulin-induced hypoglycemia was reduced in obese men. Pretreatment with the opioid antagonist naloxone enhanced the OT response to hypoglycemia in obese men and suggested an abnormal activity of endogenous opioids in obesity (115). In women, unlike men,

endogenous opioids did not modulate OT release during insulin-induced hypoglycemia (282).

A pancreatic OT receptor was cloned from the rat insulinoma cell line RINm5F (279). CHO cells that have been transfected with this receptor responded to OT with an increase in cytosolic Ca^{2+} concentration and inositol phosphate levels as well as arachidonic acid release and PGE_2 synthesis. Amino acid sequence homology, binding specificity, and the ligand-activated signal transduction pathways suggested that the pancreatic OT receptors expressed in RINm5F cells are indistinguishable from the uterine-type OT receptors that have been described in other tissues (279).

4. Adrenal gland

Ang and Jenkins (17) first identified immunoreactive OT and AVP in human and rat adrenal glands. OT predominanted over AVP, and both peptides occurred at concentrations far greater than those found in plasma. Immunohistochemical studies in adrenal glands from rat, cow, hamster, and guinea pig showed that OT was localized in both the cortex and medulla in all these species. In the cortex, the OT immunoreactivity was most intense in the zona glomerulosa, whereas in the medulla, the OT staining was higher but revealed more species variation (231). Bovine adrenal medulla membranes revealed high-affinity low-capacity OT binding sites (420). OT binding was also detected on primary monolayer cultures of bovine adrenal chromaffin cells. However, the capacity of these sites was near the detection limit, and OT concentrations in the micromolar range were necessary to stimulate the phosphoinositide pathway (550). From the few studies that have addressed the role of OT in the adrenal gland, no clear picture has emerged. Perfusion of the isolated rat adrenal gland with OT at 100 nM inhibited the acetylcholine-stimulated aldosterone secretion (462), whereas smaller doses of OT given as a bolus were able to stimulate aldosterone secretion in the intact perfused rat adrenal gland, but not in superfused adrenal cells (238). Legros et al. (331) hypothesized that OT acts also at the adrenal gland level to decrease cortisol release and/or synthesis in humans. In addition, a proliferative effect of OT was suggested by the observation that the number of chromaffin cells in the adrenal medulla of rats was increased in response to OT (461).

The list of peripheral tissues containing OT receptors is certainly not complete. Recently, functional OT receptors have also been discovered in primary cultures of human osteoblasts and in a human epithelial osteosarcoma cell line (Saos-2) (117). Further studies are required to show whether OT is a bone anabolic agent as suggested by the authors.

Table 3 gives an overview of the expression pattern of the OT system in different species.

V. THE CENTRAL OXYTOCIN SYSTEM

A. Localization Profile

1. Localization of OT

In the CNS, the OT gene is primarily expressed in magnocellular neurons in the hypothalamic PVN and SON. Action potentials in these neurosecretory cells trigger the release of OT from their axon terminals in the neurohypophysis (464). In the PVN, two populations of OT-staining neurons have been identified: magnocellular neurons that terminate in the neurohypophysis and parvocellular neurons that terminate elsewhere in the CNS. Only a small fraction (0.2%) of the OT neurons were calculated to possess axon collaterals to both the neurohypophysis and the extrahypothalamic areas. Although few OT perikarya were observed other than in the magnocellular nuclei, OT fibers and endings have been described in various brain areas in the rat: the dorsomedial hypothalamic nucleus, several thalamic nuclei, the dorsal and ventral hippocampus, subiculum, entorhinal cortex, medial and lateral septal nuclei, amygdala, olfactory bulbs, mesencephalic central gray nucleus, substantia nigra, locus coeruleus, raphe nucleus, the nucleus of the solitary tract, and the dorsal motor nucleus of the vagus nerve. OT fibers also run toward the pineal gland and the cerebellum, with most of them continuing toward the spinal cord. A few OT fibers end on the portal capillaries in the median eminence (see references in Refs. 78, 309, 476, 501, 524).

OT concentrations in the extracellular fluid of the SON were calculated to be >100- to 1,000-fold higher than the basal OT concentration in plasma, i.e., more than 1–10 nM. High-frequency electrical discharges of OT neurons as they occur, e.g., during the milk ejection reflex, might release even higher local OT concentrations (321).

Plasma OT does not readily cross the blood-brain barrier, and there is no relationship between the release of OT into the blood by the neurohypophysis and the variations in OT concentrations in the cerebrospinal fluid (CSF). Peripheral stimulations such as suckling or vaginal dilation that elicit large increases in plasma OT may or may not change the concentration of OT in the CSF. As shown in rats, electrical stimulation of the neurohypophysis only evokes the release of OT into the blood, whereas stimulation of the PVN elicits a release of OT into the blood and into the CSF (228). After hypophysectomy, OT disappears from the blood, whereas its concentration increases in the CSF (139). The OT in the CSF is probably derived from neurons that extend to the third ventricle, the limbic system, the brain stem, and the spinal cord. In the CSF, OT is normally present at concentrations of 10–50 pM, and its half-life is much longer (28 min) than in the blood (1–2 min) (283, 384). In humans and in mon-

TABLE 3. *Expression of oxytocin and oxytocin receptors in the peripheral system*

Tissue	Species	OT Gene	OT Binding	OTR mRNA	Reference Nos.
Female reproductive system					
Myometrium*	Hum bov she		+	+	44, 191, 299, 482, 529, 531
	Rat	ND	+	+	7, 120, 194, 323, 624
	Rab gp		+		164, 479, 531
	Pig	+	+		63, 351
Endometrium†	Hum		+	+	299
	Rat	+	+	ND	101, 323, 327, 624
	Bov she		+	+	44, 480, 482, 529
	Pig	+	+		63, 351, 387, 388
	Rab		+		355
Amnion	Hum	+	+	+	104
	Rat	+	ND		328
	Bov		+	ND	193, 272
	Rab		+		234
Chorion	Hum	+	+	+	49, 104, 547
	Rat	+	ND		328
Decidua	Hum	+	+	+	104, 190, 547
	Rat gp rab		+		190, 196, 479, 547, 550
Ovary	Hum mar	+	+	+	147, 187, 198, 269, 299
	Mou	+		+	199
	Bov	+	+		268, 271, 187
	Gp pos		+		46, 619
Corpus luteum‡	Hum pig mac	+	+		126, 150, 269, 291, 292, 454, 585
	Bov	+	+	ND	173, 187, 272, 584
	She	+	+		513, 554, 583
	Rat bab goa	+			197, 243, 269
Male reproductive system					
Testis	Hum rat	+	+		45, 180, 269, 414
	Mar	+		+	145
	Mac	+	(+)		182
	Mou	(+)			16, 415
	Bov she ban	+			16, 47, 207, 563
	Pig		+		354
Epididymis	Hum	+	(+)		182
	Mar	(+)		(+)	145
	Wal		+	+	437
	Pig		+		354
	Rat	+			226
Prostate gland	Hum	+	(+)		58, 182
	Mar	ND		(+)	145
	Mar mac		(+)		145, 182
	Wal		+	+	437
	Bov	ND			502
	Rat gp dog		(+)		58
Mammary gland*	Hum		(+)	+	293, 294
	Rat mar bov pig		+		294, 351, 399, 531, 533, 620
Kidney	Rat		+	+	33, 34, 67, 353, 427, 504, 551
	Rab		(+)		14, 255
	Pos		+		46
Heart	Rat	+	+	+	221, 274
Vascular endothelium	Hum		+	+	555
Thymus	Hum	+	+		205, 206
	Rat		+		153
	Mou	+			203
Adipocytes	Rat		+		36, 61, 62, 144
Pancreas	Hum	(+)			10
	Rat	(+)	+		10, 535, 608
Adrenal gland	Hum rat ham gp	+			17, 180, 231, 419, 473
	Bov	+	+		420, 550
Osteoblasts	Hum		+	+	117
Cell lines					
Osteosarcoma Saos-2	Hum		+	+	117
Myometrial PHM1-41	Hum		+		138
Pancreatic RINm5F	Rat		+		279
Gonadotrope-derived αT3-1	Mou		+		161
Mammary tumor Hs578T	Hum		+		242
Breast cancer lines (e.g., MCF7)	Hum		(+)	+	40, 168, 263, 496, 549
Vascular endothelial ECV304	Hum		+		555
Vascular smooth muscle (HVSMC)	Hum		+		607
Sweat gland NCL-SG3	Hum		(+)		481
Immature thymic pre-T RL12-NP	Mou		+		360
Kidney epithelial LLC-PK₁	Pig		+	+	215

+, Detectable signal; (+), weak or unclear signal (e.g., based on poorly characterized antireceptor antibodies) and/or contradictory reports have been published (for details, see the references); ND, not detected. Indicated species are as follows: bab, baboon; ban, bandicoot; bov, bovine; goa, goat; gp, guinea pig; ham, hamster; hum, human; mac, macaque monkey; mar, marmoset monkey; mou, mouse; pos, brushtail possum; rab, rabbit; she, sheep; wal, wallaby. * Oxytocin (OT) receptors are present in myometrial and mammary myoepithelial cells of all mammalians. † OT receptors are probably present in endometrium of all ruminants. ‡ OT has been found to be synthesized in the luteal cells of all species so far studied.

keys, a circadian rhythm in the OT concentrations in the CSF has been found with peak values at midday. No such circadian rhythms have been observed in the CSF of rats, cats, guinea pigs, or goats. Circadian rhythms have never been observed in plasma OT concentrations (12).

Further complicating factors are the presence of an OT-like peptide and the appearance of conversion products of OT that possibly exert their effects exclusively on the brain, e.g., to influence learning and memory processes. Moreover, OT fragments such as OT-(1—6) or OT-(7—9) could cross the blood-brain barrier more easily (132).

2. *Localization of OT receptors*

Experiments with primary cell cultures showed that OT receptors are localized both on hypothalamic neurons and astrocytes (136). Concerning the regional distribution of OT binding sites in the brain, marked species differences have been observed. In rats, OT receptors are abundantly present in several brain regions, i.e., some cortical areas, the olfactory system, the basal ganglia, the limbic system, the thalamus, the hypothalamus, the brain stem, and the spinal cord (see Table 4). In the adult rat, a high density of OT receptors is found in the dorsal peduncular cortex, the anterior olfactory nucleus, the islands of Calleja and ventral pallidum cell groups, the limbic system (bed nucleus of the stria terminalis, central amygdaloid nucleus, ventral subiculum), and the hypothalamic ventromedial nucleus (43, 561). OT receptor mRNA was detected in brain areas mostly coinciding with the occurrence of OT binding sites (610). OT receptors were detectable in all spinal segments, but in low amounts and restricted to the superficial layers of the dorsal horn (557). No major differences in receptor distribution were observed between male and female brains. Notably, the distribution pattern of OT binding sites is markedly different from that of binding sites for AVP. Whenever present in the same area, OT and AVP binding sites were located in different parts of the area.

The distribution and numbers of OT binding sites undergo major changes during development. As shown by Tribollet et al. (561), only a fraction of OT receptors is constantly present during the development. Some OT receptors are only transiently expressed on neurons, e.g., during infancy or during maturation (Table 4). In male and in female rats, two critical periods during the development were recognized: the third postnatal week, which precedes weaning, and puberty. For example, in the infant brain, the cingulate and retrosplenial cortex as well as the substantia gelatinosa of the spinal cord contained the highest densities of OT binding sites, whereas low or undetectable numbers of OT receptors were observed in these areas in the adult rat brain. Conversely, OT receptors in some other areas were abundant in the adult brain

but undetectable before puberty, e.g., in the olfactory tubercle (Calleja islands and ventral pallidum cell groups) and in the hypothalamic ventromedial nucleus (561) (Table 4). During aging, however, the number of OT binding sites decreased again in the latter areas. This was probably mediated by the markedly lower level of plasma testosterone in aged rats. In fact, the expression of OT receptors in the olfactory tubercle and in the ventromedial hypothalamic nucleus was shown to be dependent on gonadal steroids, and testosterone treatment of aging rats could restore normal adult levels of OT receptors in these areas (35).

As already mentioned, there is a high diversity of OT receptor distribution between different species. For example, the ventral subiculum in the hippocampus contains high densities of OT binding sites in the rat, whereas in the guinea pig, the hamster, the rabbit, and the marmoset, no OT binding sites were detected in this area. In the rabbit, no OT receptors were found in the hypothalamic ventromedial nucleus. In monogamous versus polygamous voles, OT receptor distribution was shown to reflect social organization (258). In human brains, dense OT receptor binding sites were visualized in the pars compacta of the substantia nigra unlike in several other species examined so far. Thus, in humans, nigrostriatal dopamine neurons could be a target for OT, and the OT system may be involved in motor and other basal ganglia-related functions. Strong OT binding intensity was also observed in the basal nucleus of Meynert, but OT binding sites were lacking in the hippocampus, amygdala, entorhinal cortex, and olfactory bulb of human brains (346) (Table 4).

In a few brain areas such as the ventral hippocampus and the bed nucleus of the stria terminalis (BNST), the reactivity of neurons to OT could be related with OT axon endings and OT receptors. The distribution of OT binding sites in the rat spinal cord was shown to coincide with that of the OT innervation, suggesting that OT is involved in sensory and autonomic functions (474). In most other brain areas, it was not possible to identify a clear relationship between the presence of the whole OT system and the physiological data. So, in male rats, Hosono et al. (244) identified a functional OT receptor system in the subfornical organ, where in binding studies the expression is almost neglected. Marked receptor-peptide mismatches exist in some brain regions, for example, in the amygdala, where very few OT afferents but highest OT binding activities have been found. On the other hand, one has to recognize that OT binding sites in regions with high OT concentrations may be downregulated to a level that may not be detectable by autoradiography using radioligands. In the lactating rat, for instance, OT binding sites have been found to be nearly undetectable in den PVN and SON. As soon as 5–20 min after intracerebroventricular injection of an OT antagonist, a strong increase of OT

TABLE 4. *Distribution of oxytocin receptors in the central nervous system*

Brain Regions	Rat			Human
	mRNA	OT binding (infant)*	OT binding (adult)*	OT binding
Olfactory system				
Olfactory bulb	+	?	?	ND
Anterior olfactory nucleus	+++	++	++	?
Olfactory tubercle	+++	?	++	?
Islands of Calleja	ND	ND	+++	+
Piriform cortex	++	?	?	?
Entorhinal/perirhinal area	+	+	+	ND
Cortical areas				
Peduncular cortex	?	++	+++	(+)
Insular cortex	?	+	+	?
Cingulate cortex	+	+++	ND	?
Retrosplenial cortex	?	+++	ND	?
Frontal cortex	++	?	(+)	ND
Temporal cortex	(+)	?	+	ND
Taenia tecta	+++	?	(+)	?
Diagonal band of Broca	+	?	?	+
Basal nucleus of Meynert	ND	ND	ND	+++
Basal ganglia				
Caudoputamen	+++	+++	++	ND
Ventral pallidum cell groups	++	ND	+++	++
Globus pallidus	ND	+++	ND	++
Nucleus accumbens	+	?	+	ND
Limbic system				
Lateral septal nucleus	+	+	+	+++
Bed nucleus of stria terminalis (BNST)	+++	++	+++	ND
Amygdaloid-hippocampal area	+++	+	+	ND
Central amygdaloid nucleus	+++	++	+++	ND
Medial amygdaloid nucleus	++	+	+	ND
Basolateral amygdaloid nucleus	+++	+	+	ND
Parasubiculum and presubiculum	ND	++	++	ND
Dorsal subiculum	+++	+++	(+)	ND
Ventral subiculum	+++	+	+++	ND
Thalamus and hypothalamus				
Anteroventral thalamic nucleus	ND	+	ND	ND
Paraventricular thalamic nucleus	++	++	+	+
Ventromedial hypothalamic nucleus[b]	+++	ND	++	ND
Anterior medial preoptic area	+++	ND	ND	++
Supraoptic nucleus (SON)	+++	ND	(+)	ND
Paraventricular nucleus (PVN)	++	ND	(+)	ND
Medial tuberal nucleus	ND	++	++	+
Posterior hypothalamic area	+	ND	ND	++
Supramammillary nucleus	++	+	+	ND
Lateral mammillary nucleus	ND	+++	+	++
Medial mammillary nucleus	ND	+++	ND	+
Brain stem				
Substantia nigra pars compacta	++	ND	ND	+++
Ventral and dorsal tegmental area	++	ND	ND	ND
Central gray	+	ND	ND	+
Dorsal raphe nucleus	+	ND	ND	+
Reticular nuclei	+	ND	ND	ND
Medial vestibular nucleus	+	ND	ND	ND
Hypoglossus nucleus	++	ND	ND	++
Nucleus of the solitary tract	ND	ND	(+)	+++
Dorsal motor nucleus of the vagus nerve	+++	+	+	+
Inferior olive nucleus	ND	+	+	(+)
Substantia gelatinosa of trigeminal nucleus	+	+++	+	+++
Pituitary gland	ND	+	+	ND

Expression levels of the OT receptors are indicated by the following symbols: +, low; ++, moderate; +++, high; ND, not detectable; (+), at the detection limit and/or not observed by all investigators; ?, not recorded. The data are derived from References 5, 152, 184–186, 303, 311, 346, 347, 514, 556–561, 609, 610. * The relative densities of OT binding sites in adult (postnatal *day 90*) versus infant (postnatal *day 10*) rat brain has been reported by Tribollet et al. (561). † OT receptor density is strongly regulated by estrogen.

binding sites was noticed in the magnocellular nuclei. In this case, the presence of the OT antagonist may prevent the downregulation of OT receptors induced by the high concentrations of OT that are released into the magnocellular nuclei during lactation (183).

In the brain, estrogen has only a modest influence on the synthesis of OT, but it has a pronounced effect on the regulation of the OT receptor. OT receptors (but not AVP receptors) are regulated by gonadal steroids in the rat brain in a complex fashion (368, 556). Castration and inhibition of aromatase activity reduced, whereas estradiol and testosterone increased OT binding, particularly in regions of the brain assumed to be involved in reproductive functions, such as the ventrolateral part of the hypothalamic ventromedial nucleus (VMN) and the islands of Calleja and neighboring cell groups (556). Estrogen treatment increased the affinity of OT receptors in the medial preoptic area-anterior hypothalamus (88) and increased both the density and the area of OT binding in the rat VMN (114). Progesterone caused a further increase in receptor binding and was required for a maximal extension of the area covered by OT receptors (114, 509). In another study, chronic progesterone treatment increased basal OT receptor density in the limbic structures, decreased it in the ventromedial nucleus, and prevented estrogen-induced increases in ligand binding in all areas studied with the exception of the medial preoptic area (439). Glucocorticoids (340) have also been reported to modulate cerebral OT receptors (439, 509). The brain OT receptor varies across species (compare rat versus human in Table 4) not only in its distribution but also in its regional regulation by gonadal steroids. For example, estrogen increased OT receptor binding in the rat brain but reduced OT receptor binding in the homologous regions of the mouse brain (262).

B. Hypothalamus-Neurohypophysis

The magnocellular neurons also release OT and VP from their perikarya, dendrites, and/or axon collaterals (335, 467). Although the amount of release is small compared with the amount released from the neurohypophysis, the concentration of OT and VP in the extracellular fluid of the SON resulting from this somatodendritic release has been calculated to be 100- to 1,000-fold higher than the basal plasma concentration (319). Intranuclear release of these peptides occurs in response to a wide variety of stimuli, including suckling; parturation; hemorrhage; certain kinds of stress such as fever, physical restraint, and pain; mating and territorial marking behaviors; dehydration; administration of hypertonic solutions; and a range of pharmacological stimuli (318, 349, 476). The magnocellular neurons display characteristic activity patterns that are associated with particular secretion patterns for the peptide (475). For example, OT neurons respond to such stimuli as hyperosmolarity with small increases in spontaneous firing rate, whereas during lactation the same cells display explosive synchronized bursts of activity associated with a pulsatile release of OT into the circulation to cause contraction of mammary smooth muscle and milk let-down (475). PVN neurons can be identified as either AVP or OT secreting on the basis of their spontaneous discharge patterns. Interestingly, in most cases, central release patterns of OT are accompanied by peripheral ones, whereas release of AVP is not. In the rat, OT is released into the plasma without AVP, e.g., by suckling, parturition, stress, and nausea (603).

OT is required for successfull milk ejection in response to suckling as confirmed by the phenotype of OT knock-out mice (see sect. IV*C*). All offspring of these mice died shortly after birth because of the dam's inability to nurse. Postpartum injections of OT to the OT-deficient mothers restored milk ejection and rescued the offspring (416). OT released into the SON probably plays an essential role in the milk-ejection reflex, facilitating suckling-induced electrical activation of OT neurons (395). So, the injection of an OT antagonist into the SON prevented the OT-induced facilitation and completely interrupted the milk-ejection reflex (317). During parturition and suckling, OT is released within the SON (394, 408) and apparently excites via a short positive-feedback loop the same cells by which it is produced and secreted (406, 407). This autoexcitatory mechanism leads to further amplification of local and/or neurohypophysial OT release and ensures the synchrony of firing activity among oxytocinergic neurons. The underlying molecular mechanism of this putative autoexcitation is unclear. It was suggested that OT depresses the synaptic GABAergic input from perinuclear interneurons projecting into the SON (77). In addition to the magnocellular nuclei, the BNST may participate in the control of neuroendocrine responses during lactation (250). Injection of OT into the BNST increases the frequency of milk ejections, and electrophysiological recordings showed increased activity of BNST neurons coincident with this facilitatory effect (316).

All the physiological situations during which large amounts of OT are released into the blood are characterized by ultrastructural changes in the magnocellular nuclei, e.g., reduced astrocytic coverage of oxytocinergic somata and dendrites, increases in GABAergic synapses, increases in the juxtaposition of the membranes of the perikarya and of the dendrites between adjacents neurons, and increases in the contact area between neurosecretory terminals and the perivascular space. These morphological changes are reversible with cessation of stimulation, affect exclusively oxytocinergic neurons, and may serve to facilitate and maintain the characteristic synchronized electrical activity of these neurons at milk ejection (552, 553). Neuronal network rearrangement may

also occur after behavioral experience. So, in maternally experienced ewes, parturition-induced increases in the expression of OT receptor mRNA in the PVN and the islands of Calleja were potentiated (69).

C. Adenohypophysis

It has been suggested that some hypothalamic OT reaches the anterior pituitary lobe via the hypothalamo-pituitary portal vasculature. OT might thus be able to influence anterior pituitary hormones as a hypothalamic regulating factor. OT is present in nerve terminals in the median eminence. It was found to be released into the portal vessels, and specific OT receptors are present in the rat adenohypopophysis (18, 494). Another pathway for OT delivery to the adenohypophysis might be the short portal vessels connecting the posterior and anterior lobes. OT may participate in the physiological regulation of the adenohypophysial hormones prolactin (433), ACTH (332), and the gonadotropins (484).

There was a long controversy on whether OT released in response to suckling was responsible for the concomitant secretion of prolactin from the adenohypophysis. During suckling and under stress, both hormones are quantitatively predominant among the factors released. OT could only act as prolactin releasing factor when the dopamine levels are low, e.g., during the brief periods of dopamine withdrawal that characterize the onset of prolactin secretion under various physiological stimuli (494). With the use of a cell-specific targeting approach, it was shown that a subpopulation of lactotrophs respond to OT (493). Breton et al. (68) demonstrated that the pituitary OT receptor gene expression is restricted to lactotrophs and dramatically increases at the end of gestation or after estrogen treatment. These findings question a direct function of OT on pituitary cells other than lactotrophs and suggest that OT might exert its full potential as a physiological prolactin-releasing factor only toward the end of gestation (68).

The major endocrine response to stress is via activation of the hypothalamic-pituitary-adrenal axis. ACTH secretion from the anterior pituitary is primarily regulated by CRH and AVP synthesized in neurons of the PVN. Unlike ACTH, plasma OT does not increase in response to all kinds of stress. In rats, OT can potentiate the release of ACTH induced by CRH. OT was also reported to stimulate ACTH secretion from corticotrophs in fetal and adult sheep (363). CRH is responsible for the immediate secretion of ACTH in response to stress. However, when the CRH levels are decreased following prolonged stress, the persistent level of OT in the median eminence could become important for the delayed ACTH response and for the generation of pulsatile ACTH secretory bursts (65).

In contrast, OT infusion into human volunteers actu-ally inhibited the plasma ACTH responses to CRH (433). Suckling and breast stimulation in humans produced an increase in plasma OT and a decrease in plasma ACTH level. The observed negative correlation of both hormones indicates an inhibitory influence of OT on ACTH/cortisol secretion under a certain physiological condition in humans (11, 107). Conclusively, OT might control ACTH release under some physiological conditions in a species-specific manner.

Gonadotropes in the adenohypophysis synthesize and secrete the two gonadotropin hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Although gonadotropin-releasing hormone (GnRH) is believed to be the primary secretagogue for LH, OT has also been shown to stimulate LH release. OT administered to proestrous rats caused advancement of the LH surge and earlier ovulation. In addition, OT antagonists inhibited the peak of LH at proestrous (484). On the other hand, OT receptors have not been detected on gonadotropes but were identified on a gonadotrope-derived cell line (162). Indirect effects of OT on LH release have also been discussed (160). OT has been observed to synergistically enhance GnRH-stimulated LH release. OT may sensitize the pituitary before full GnRH stimulation. In human females, preovulatory OT administration promoted the onset of the mid-cycle LH surge (248). Overall, the physiological connection between OT and LH release has yet to be definitively established (160).

D. Centrally Mediated Autonomic and Somatic Effects

1. Cardiovascular regulation

In the rat, OT-containing axons terminate in several brain stem nuclei known to be involved in cardiovascular control. High concentrations of OT are found in the nucleus of the solitary tract, which receives sensory inputs from the viscera, including baroreceptors of the cardiovascular system. Nevertheless, microinjection of OT into the nucleus tractus solitarius did not change the mean arterial pressure or the heart rate in the rat (567). In contrast, injection of OT into the dorsal motor nucleus of the vagus reduced the heart rate, and this effect was eliminated by an OT antagonist (485). In humans and rats, the bolus intravenous administration of OT is often associated with a decrease in blood pressure (445).

A role of central OT with respect to cardiovascular control has been established in many studies. Central pretreatment of rats with an OT antisense oligodeoxynucleotide attenuated the mean arterial pressure and heart rate responses induced by substance P. This suggests that OT neurons in the PVN mediate the increases in blood pressure and heart rate induced by stimulation of substance P receptors in the forebrain (357). An OT anti-

sense oligonucleotide injected into the PVN abolished the tachycardia produced by shaker stress in rats, indicating that OT may act as mediator of stress-induced tachycardia (397). In addition, neuropeptide Y and peptide YY, which are central and peripheral modulators of cardiovascular and neuroendocrine functions, triggered OT release from perfused isolated terminals of the rat neurohypophysis (515). There is further evidence for an interaction between the central oxytocinergic and vasopressinergic systems in cardiovascular control. So, central injection of AVP (1–10 pmol) increased the mean arterial pressure and heart rate, and both responses were found to be enhanced in rats pretreated with OT (465). Recent findings suggested a putative interaction of OT with the ANPergic system. Although atrial stretch releases ANP from cardiac myocytes, the response to acute blood volume expansion was found to be markedly reduced after elimination of neural control (19). Volume expansion distends baroreceptors in the right atria, carotid-aortic sinuses, and kidney and in turn alters afferent input to the brain stem and the hypothalamus. Because axons of the ANP neurons contained only low amounts of ANP (1,000 times less than in right atrium of the heart), it was hypothesized that hypothalamic ANP neurons cause release of OT, which circulates to the right atrium to stimulate ANP release. Because ANP has a negative ino- and chronotropic effect in the atrium, this would produce an acute reduction in cardiac output and, coupled with peripheral vasodilating actions, causes a rapid reduction in effective circulating blood volume (220).

2. Analgesia

Analgesic effects of OT have been reported in most but not all studies in mice, rats, dogs, and humans (73, 85, 121, 307). Certain stimulations such as vaginal dilation led not only to a rise in plasma OT concentrations but also to an increase of the pain threshold (121). Dogs with neck and back pain caused by spinal cord compression had significantly more OT in their CSF than clinically normal dogs (73). Analgesia was observed in rats after injecting OT into the lateral ventricles (307). On the other hand, systemic OT did not produce analgesia in rats. Thus it was concluded that the observed increase in response latency in the hot-plate test may result from the sedative and vasoconstrictive effects of OT. Because the OT antagonist did not alter response latency on the hot-plate test, it seems unclear whether endogenous OT exerts a tonic effect on the pain threshold in rats (605).

In humans, intrathecal injection of OT was effective in treating low back pain for up to 5 h. An OT antagonist and the opiate receptor-blocker naloxone could reverse OT-induced analgesia. OT also increased β-endorphin and L-encephalin contents in the spinal cord, whereas an OT antagonist caused a decrease in the concentration of these opioids. Moreover, OT levels were elevated in the CSF of patients with chronic low back pain, perhaps a compensatory response to the painful condition (606). In a clinical case study, a high concentration (300 μg) of OT injected intravenously was reported to evoke strong analgesic effects lasting more than 70 min in a patient with intractable cancer pain at a time when opiates were no longer effective (352, 476).

3. Motor activity

Centrally administered OT can induce or modify several forms of behavior together with the associated motor sequences. OT increased general motor activity, and OT antisera decreased this hyperactivity and seizures in a complementary fashion. In this context, OT may possibly act at the spinal level (59). OT changed the spontaneous motor activity in female rats in strong dependence on steroid hormones (444). Treatment with low OT doses led to a decrease in peripheral locomotor activity, whereas increasing doses of OT provoked sedative effects as indicated by a suppression of locomotor activity and rearing. A maximal effect was obtained within 1 h and thereafter, the behavior gradually returned to normal within 24 h (566). During static muscle contraction, blood pressure and heart rate reflexly increase as shown in anesthetized cats. In this respect, OT may participate to regulate cardiovascular responses elicited by contraction of skeletal muscle (338, 476).

4. Thermoregulation

In contrast to centrally administered AVP, for which antipyretic actions have been well documented, OT evoked, if at all, mostly weak antipyretic effects at higher concentrations (404). In rabbits, intracerebroventricular administered OT produced small but long-lasting hyperthermias that did not exceed 0.4°C (342). Intracisternally injected OT to adult male mice significantly increased colonic temperatures, with the maximal rise in temperature occurring 30 min after administration of the peptide. OT also antagonized the hypothermia produced by other peptides such as bombesin or neurotensin (362, 476). Conversely, whenever the body temperature was elevated in urethan-anesthetized rats, e.g., in response to prostaglandin fever, OT was released into the ventral septal area, but not into the hippocampus (320). OT could also modulate the antipyretic action of AVP in rats (466). Possibly, the effects of OT with respect to thermoregulation may be physiologically significant during parturition and lactation.

5. Gastric motility

In conjunction with the natriuretic effects of circulating OT, Verbalis et al. (573) suggested the concept of a

coordinated central and peripheral OT secretion (603) as a mechanism for regulating body solute homeostasis in rats. The following findings support this concept. OT inhibits food intake in fasted rats and NaCl intake in hypovolemic rats. Inhibition of food and/or NaCl intake stimulates expression of c-*fos* in parvocellular and magnocellular OT neurons and indicates simultaneous activation of centrally projecting and pituitary-projecting OT neurons. Selective inactivation of brain cells containing OT-receptive elements leads to a disinhibition of NaCl intake similar to that produced by OT antagonists (573). In particular, gastric motility is inhibited by microinjection of OT into the dorsal motor nucleus or by intracerebroventricular administration of OT in rats. An OT antagonist administered intracerebroventricularly alone caused an increase in baseline gastric motility (175). In addition, stimulation of gastric vagal afferents by systemic administration of cholecystokinin (CCK) inhibited gastric motility, reduced food intake, and stimulated pituitary secretion of OT in rats. McCann and Rogers (366) provided evidence that central OTergic neurons influence gastric motility and secretion by increasing the excitability of brain stem vagal neurons, i.e., sensory neurons in the nucleus tractus solitarius and motor neurons in the dorsal motor nucleus, which are both related to gastric function. According to Esplugues et al. (157), the inhibitory effect of OT on the distension-stimulated gastric acid secretion in the rat perfused stomach is mediated by a nervous reflex involving a neuronal pathway that includes NO synthesis in the dorsal motor nucleus of the vagus.

For OT to have physiological relevance for maintenance of solute homeostasis, a coactivation of magnocellular and parvocellular OTergic systems has been postulated: central, i.e., parvocellular OT as inhibitor of solute ingestion, and peripheral, i.e., pituitary secreted OT to enhance solute excretion via renal sodium excretion. However, pituitary OT secretion from magnocellular neurons is not always linked to decreased gastric motility in rats. For example, nipple attachment and sucking by pups, a well-known stimulus for neurohypophysial secretion of OT, did not decrease gastric motility in lactating rats (232). In view of the fact that OT promotes reproductive behaviors, it appears quite plausible that OT simultaneously acts as inhibitor for food intake, since both behavioral responses would conflict with each other. However, some findings do not fully support this concept or need to be clarified. First, central administration of an OT antagonist did not block the effects of CCK and LiCl to inhibit gastric motility. This suggests that the parvocellular OT projections modulate the activity of intrinsic brain stem reflex arcs and do not exert a direct control over vagal efferent outputs (175). Second, OT receptors and mRNA localizations have been reported to occur predominantly on vagal neurons of the dorsal motor nucleus, which are generally excitatory to gastric motility, whereas

OT is known to inhibit motility via neurons of the nucleus tractus solitarius. Finally, marked interspecies differences have been reported with respect to OT's relevance for maintenance of solute homeostasis. Whereas in rats nausea-producing agents and CCK each stimulated OT rather than AVP release, in humans and monkeys vasopressin but not OT secretion was provoked in response to these agents (385, 577). In humans, nausea is a powerful stimulator of VP release induced by centrally acting emetics such as apomorphine, whereas OT is only weakly stimulated by apomorphine. In the rat, the converse is true. OT is stimulated by emetic agents, and VP shows little change. Therefore, we have little evidence that OT is involved in the physiology of emesis in humans. Intracisternal injection of OT also failed to induce emesis in dogs (337).

6. Hydromineral regulation and osmoregulation

In rats, the concerted release of OT and AVP plays a key role in osmoregulation through the effects of natriuresis and diuresis. Osmoreceptors are located in systemic viscera and in central structures that lack the blood-brain barrier. The nucleus tractus solitarius and lateral parabrachial nucleus receive neural signals from baroreceptors and are responsible for inhibiting the ingestion of fluids under conditions of increased volume and pressure and for stimulating thirst under conditions of hypovolemia and hypotension. Osmosensitive neurons combine with endogenously generated osmoreceptor potentials to modulate the firing rate of magnocellular neurons. In magnocellular neurons isolated from the SON of the adult rat, stretch-inactivated cationic channels transduce osmotically evoked changes in cell volume into functionally relevant changes in membrane potential (64, 281). As judged from the distribution of Fos immunoreactivity, a fraction of oxytocinergic neurons in the subnuclei of the PVN were found to be activated during the satiety process of sodium appetite (181). Blackburn et al. (54) provided evidence that salt appetite is regulated by both sodium- and osmolality-sensing mechanisms in rats. Using a selective receptor inactivation approach, these authors concluded that in contrast to the central ANP receptors that mediate both sodium- and osmolality-induced inhibition of NaCl ingestion, the central OT receptors primarily mediate osmolality-induced inhibition of NaCl ingestion (54).

In anesthetized rats, electrical stimulation of renal afferents provided evidence that sensory information originating in renal receptors changes the activity of oxytocinergic neurons in the PVN. Thus renal receptors could contribute to the hypothalamic control of AVP and OT release into the circulation (113) (see sect. IV*D*). In humans, there is neither an OT response to changes in

plasma osmolality nor is there a natriuretic response to large doses of OT (418).

VI. CENTRAL BEHAVIORAL EFFECTS

A. Sexual Behavior

The "classical" peripheral target tissues for OT, uterus and mammary glands, are both organs linked to reproduction. There is also evidence that OT homologs in fish, amphibians, and reptiles as well as in molluscs and annelids are important in the control of reproductive behaviors. In the mollusc *Lymnaea stagnalis*, the conopressin gene encoding the putative ancestral receptor to the VP/OT receptor family is expressed in neurons that control male sexual behavior, and its gene products are present in the penis nerve and the vas deferens (569). Thus a phylogenetically old connection may exist between systems controlling reproductive hormone release and reproductive behaviors. Moreover, the neurohypophysial release of OT into the circulation is most efficiently provoked by various kinds of stimulations of genitals and the breast in different mammalian species (94, 369).

Unlike humans, the majority of animals breed only during certain times of the year, and the expression of sexual behavior is under strict endocrine regulation. Male and female animals mate only when circulating steroid hormone levels are at appropriate concentrations, and to influence behavior, the steroid hormones must profoundly affect the neurotransmission in the brain. In contrast, humans and other primates display a phenomenon called "concealed ovulation" that may have played a role in the evolution of social structures (369).

1. Female sexual behavior in animals

In the female rat, the sexual behavior is generally divided into proceptive (soliciting) and receptive (lordosis) responses, both of which are under the control of gonadal steroids. Many studies have shown that intracerebral infusion of OT into the hypothalamus facilitates both aspects of the sexual behavior in estrogen-primed female rats (82, 256). In many but not in all studies, progesterone administration was necessary for the OT-induced facilitation of lordosis behavior (29, 87, 216, 508). In particular, prolonged (3 days) priming with estradiol induced lordosis, even in the absence of progesterone (87). Furthermore, the frequency and/or duration of lordosis was differentially affected, dependent on the site of OT administration. It was therefore suggested that OT may control different aspects of lordosis (506). Surprisingly, low concentrations of OT could even suppress lordosis when infused into the lateral ventricle of female rats (507).

On the other hand, several experiments with OT antagonists confirmed that endogenous OT is essential for the expression of sexual behavior. So, infusion of an OT antagonist into the medial preoptic area before progesterone inhibited sexual receptivity and increased rejection in female rats (84). Additionally, in nonovariectomized female rats in spontaneous behavioral estrus, intracerebroventricular injection of OT increased lordosis quotient and lordosis duration, whereas an OT antagonist prevented this OT-induced effect (50). Witt and Insel (601) observed that significant OT antagonist effects were absent in females primed with estradiol alone, suggesting that the OT antagonist attenuates progesterone facilitation of female sexual behavior. Surprisingly, these behavioral effects of OT antagonist administration did not appear immediately, but emerged only when antagonist was given with progesterone 4–6 h before behavioral testing (601). Two other observations support further evidence for the involvement of endogenous OT in the expression of female sexual behavior. First, the immunoreactivity of the immediate early gene product Fos was induced in OT neurons after sexual activity in female rats (176). Second, intrahypothalamic infusions of antisense oligonucleotides directed against the OT receptor mRNA reduced lordosis behavior as well as OTR in the VMN of estrogen-primed rats (370).

How could OT act to induce the expression of lordosis? Vincent and Etgen (579) showed that steroid priming promotes an OT-induced norepinephrine release in the ventromedial hypothalamus of female rats. This occurs most probably by a peripheral mechanism, e.g., vaginocervical contraction, leading to increased excitability of ventromedial hypothalamus neuronal activity and the expression of lordosis (158, 579).

With respect to OT effects on sexual behavior, only few studies were performed in species other than rats (for more details, see Table 5). In ovariectomized hamsters (*Mesocricetus auratus*) primed with estradiol, microinjection of OT into the medial preoptic area-anterior hypothalamus or the ventromedial hypothalamus induced sexual receptivity, whereas injection of a selective OT antagonist reduced the levels of sexual receptivity (589). OT injection into the hypothalamus also increased ultrasonic vocalizations that female hamsters use to alert and attract potential mates (179).

2. Male sexual behavior

Male sexual behaviors and performances have also been mostly studied in rats and are measured, e.g., by changes in the frequency or durations of mounts, penile erections, intromissions, ejaculations, and yawnings. Yawning is considered to be a phylogenetically old, stereotyped event that occurs alone or associated with

TABLE 5. *Actions of oxytocin on behaviors in different species*

Behaviors	Rat	Mouse	Prairie vole	Sheep	Human	Reference Nos.
Maternal behavior	↑	(↑)	?	↑	?	163, 256, 289, 367, 440, 441, 599, 616
Female sexual behavior	↑	↔	↑ or ↓	↓	↑	13, 87, 122, 256, 288, 416, 599, 601
Male sexual behavior	↑ or ↓	↔	↓	?	↑	21, 24, 90–92, 286, 356, 416, 537, 599
Female affiliative behavior	?	↔	↑	↑	?	256, 286, 416, 593
Male affiliative behavior	↑	↔	↑ or ↔	?	?	111, 256, 416, 602, 613
(Auto)grooming	↑	↑	(↑)	?	(↑)	83, 127, 140, 324, 356, 375, 568, 600, 602
Social memory	↑	↑	↑	↑	?	94, 125, 137, 154, 256, 286, 459, 598
Male aggression	?	↑	↑ or ↔	?	?	130, 286, 356, 597, 613
Female aggression	↑ or ↓	?	↓	?	?	172, 213, 286, 600
Nociception	↓	↓	?	?	↓	27, 350, 606
Anxiety	↓	↓	?	?	?	368, 371, 566, 595
Feeding	↓	?	?	?	?	25, 26, 382, 574, 576
Memory and learning	↓	↑ or ↓	?	?	↓	56, 60, 76, 133, 134, 155, 170, 310, 431
Tolerance to opiates	↓	↓	?	?	?	313, 498

The effects of oxytocin on behaviors are indicated as follows: ↑, increase; ↔, no effect; ↓, decrease; ?, unknown. Symbols in parentheses indicate a weak effect or a slight tendency to a decrease (↓) or increase (↑) (for details, see the indicated references and additional references in the text). [Modified from Kendrick (286).]

stretching and/or penile erection under different conditions (22).

OT was found to be one of the most potent agents to induce penile erection in rat, rabbits, and monkeys (21). Melis et al. (378) reported that low doses of OT ($\geq$3 ng) can elicit penile erection and yawning in rats following site-specific injection into the PVN, i.e., the nucleus which contains cell bodies of the majority of oxytocinergic neurons projecting to extrahypothalamic brain areas. Both OT effects were prevented by intracerebroventricular injection of OT antagonists as well as by electric lesions of the PVN (23, 379). According to Argiolas and Melis (22), the oxytocinergic neurons facilitate yawning by releasing OT at sites distant from the PVN, i.e., the hippocampus, the pons, and/or the medulla oblongata when activated by the D1/D2 dopamine agonist apomorphine, *N*-methyl-D-aspartate, or OT itself. This activation was antagonized by opioid peptides that prevent the yawning response (22). Central administration of OT also significantly reduced the latency to achieve ejaculation and shortened the postejaculatory interval. This effect was more pronounced in aged male rats and might be related to decreases in circulating testosterone (441). Apomorphine- and OT-induced penile erection and yawning were endocrine dependent and are differentially modulated by sexual steroids (380).

It was hypothesized that OT induces sexual behavioral responses by increasing NO synthase activity in the cell bodies of oxytocinergic neurons projecting to extrahypothalamic brain areas (22), e.g., via intracellular [Ca^{2+}] increase and stimulation of the Ca^{2+}/calmodulin-dependent NO synthase (66). In agreement with this suggested mechanism, OT-induced penile erections were prevented by PVN injections of ω-conotoxin, a blocker of N-type voltage-dependent calcium channels (542). The PVN is one of the richest brain areas containing NO synthase (580), and NO is a well-known mediator of penile erection, both peripherally and centrally. Accordingly, injections of NO donors into the PVN induced penile erections, and this response was prevented by the intracerebroventricular administration of an OT antagonist (377). Conclusively, OT may induce male sexual behaviors by activating NO synthase in the PVN. The concomitant production of NO could cause the activation of oxytocinergic neurons projecting to extrahypothalamic brain areas such as the hippocampus and/or spinal cord and control the male sexual function.

However, not all studies are in favor of a central facilitatory effect of OT on male sexual activity. OT produced an immediate cessation in sexual activity of male prairie voles and remained so for at least 24 h (356). In another study, centrally injected OT prolonged the postejaculatory refractory periods (537). These and some other findings are consistent with the hypothesis that OT could also play a role in sexual satiety. OT-immunoreactive neurons in the parvocellular subnuclei of the PVN project to a sexually dimorphic motor nucleus in the lower spinal cord which innervates the striated bulbocavernosus muscle. This muscle is responsible for penile reflexes, and it was suggested that this pathway is involved in the integration of penile reflexes with other aspects of male copulatory behavior that are under hypothalamic control (2) (Table 5).

3. Sexual behavior in humans

It is well documented that levels of circulating OT increase during sexual stimulation and arousal and peak

during orgasm in both men and women (90, 92, 401). Murphy et al. (402) measured plasma OT and AVP concentrations in men during sexual arousal and ejaculation and found that plasma AVP but not OT significantly increased during arousal. However, at ejaculation, mean plasma OT rose about fivefold and fell back to basal concentrations within 30 min, while AVP had already returned to basal levels at the time of ejaculation and remained stable thereafter (402). Men who took the opioid antagonist naloxone before self-stimulation had reductions in both OT secretion and the degree of arousal and orgasm. A recent study confirmed that also in women peak levels of serum OT were measured at or shortly after orgasm (55). Carmichael et al. (91) reported that the intensity of muscular contractions during orgasm in both men and women were highly correlated with OT plasma level. This suggests that some of OT's effects may be related to its ability to stimulate the contraction of smooth muscles in the genital-pelvic area. Enhanced sexual arousal and orgasm intensity was reported in a woman during intranasal administration of OT. This response could be elicited only while she was taking daily doses of an oral contraceptive with estrogenic and progestogenic actions and might be caused through direct effects on sexual organs or sensory nerve sensitivity (13). Overall, beyond its peripheral effects on reproductive organs, OT might affect or sensitize cerebral neurons responsible for the cognitive feelings of orgasm and could thus serve as a physiological substrate for both sexual behavior and performances in humans as well as in animals (Table 5).

B. Maternal Behavior

The development of maternal care has been well studied in rats. Nulliparous females display little interest in infants and when presented with foster young will either avoid or cannibalize them. At parturition, however, a dramatic shift in motivation occurs and maternal behaviors such as nest building and retrieval of pups became established. Pedersen and Prange (442) first demonstrated that injection of OT into the lateral ventricles of nulliparous ovariectomized rats induces maternal behavior. The rapid onset of maternal behavior in response to OT has been confirmed in several studies (256, 287, 405, 476, 592). However, it is important to note that OT is effective only to initiate the maternal behavior, but not for the performance of maternal behavior per se. So, when the females become maternal or enter into estrus, an OT antagonist had no effect (601). Moreover, steroid priming was found to be essential for the initiation of maternal behaviors in all cases so far studied. Subcutaneous injection of the NO donor sodium nitroprusside was shown to prolong parturition and to inhibit maternal behavior in rats. Because OT was able to restore intrapartum mater-

nal behavior, NO was suggested to interfere with the initiation of maternal behavior via blocking or diminishing the release of OT (422). Genital stimulations that are known to activate the oxytocinergic system could also provoke maternal behaviors as shown in ewes and rats. Genital stimulations may also influence olfactory function through the activation of afferent noradrenergic pathways in the olfactory bulbs. The olfactory bulb is in fact regarded as a critical site where OT may induce onset of maternal behavior. OT originating in the hypothalamic PVN possibly acts there to decrease olfactory processing (618). This is in line with earlier findings according to which anosmia favors the establishment of maternal behavior (582). However, the female gets a variety of sensory stimulations from contact with the pups so that the neural circuits responsible for postpartum maternal behavior are rather complex. In addition, recent studies demonstrate that the paternal genome has aquired the ability to regulate maternal behavior by a epigenetic mechanism called genomic imprinting, which results in the parent-of-origin-specific expression of only one allele of a gene. A striking impairment of maternal behavior was observed in mice in which neurally expressed imprinted genes of paternal origin (e.g., *Peg3* or *Mest*) were mutated or deficient (329, 339). One hypothesis to explain this phenomenon is that the paternal interest is best served by prolonged care and feeding of his progeny through maternal lactation.

The role of OT for maternal behavior became questionable by studies with transgenic mice, since female mice genetically deficient in OT displayed an apparently normal maternal behavior (416). This clearly demonstrates that OT itself is not essential for maternal behaviors in mice. Of course, this does not rule out that other OT-like ligands are present to activate the OT receptors or that in other species than mice, OT is essential to develop normal maternal behaviors.

In humans, OT-related maternal behaviors have not been the subject of any systematic studies so far. In earlier reports it was shown that breast-feeding within 1 h of birth, when OT levels are very high, supports a long-lasting mother-infant bond and has a beneficial effect on the development of the child (290).

C. Social Behavior

Parental care, nursing, social interaction, pair bonding, and mutual defense are prototypical mammalian species behaviors and have been important for the successfull evolution of mammals. Love and social attachments function to facilitate reproduction, provide a sense of safety, and reduce anxiety or stress. These social behaviors are clearly opposed to the ancient self-preservative behaviors. When these balanced social interactions are

disturbed, e.g., by a stressor, the self-preservative, fight-flight response pattern takes priority (233). Recent studies in rodents suggest that the neurohypophysial hormones in concert with steroids are key components in the central mediation of complex social behaviors, including affiliation, parental care, sexual behavior, mate guarding, and territorial aggression (256, 441, 613). OT has been reported to mediate aggressive and affiliative behaviors in several species (see Table 5). Moreover, disruption of the OT gene in mice showed reduced aggression behaviors in some tests (130, 617).

Social behaviors have been intensively studied in North American species within the genus *Microtus* (voles), which exhibit diverse forms of social organization ranging from minimally parental and promiscous (e.g., montane vole, *M. montanus*) to biparental and monogamous (e.g., prairie vole, *M. ochrogaster*) social structures (93). The highly social prairie voles usually form long-term monogamous relationships as a consequence of mating and thereby develop a variety of affiliative behaviors, such as parental care, increased physical contacts with each other, and the attacking of adult intruders as a putative mate guarding response. Because vaginocervical stimulation concomitant with the intense mating of the prairie voles activates the oxytocinergic system, OT was suggested as a good candidate for the facilitation of pair bonding in prairie voles. Moreover, in females that did not mate, intracerebroventricular but not peripheral administration of OT facilitated the formation of a pair bond (593). Conversely, intracerebroventricular injection of an OT antagonist before mating prevented partner preference formation in female prairie voles (593). This suggests that OT, released in response to mating behavior, is sufficient and necessary for the female to form a pair bond to her mate. In males, however, AVP, but not OT, was shown to be critically involved in partner preference and selective aggression (596). In contrast, neither peptide appears to induce pair bonding in the promiscuos montane voles. Whereas OT had little effect on the social behavior of montane voles, the same dose of AVP that induced aggression in the prairie vole increased self-grooming in the montane vole (597, 614, 615). Since the *Microtus* species share similar OT and AVP immunoreactive patterns but respond differently to exogenous peptide, the neuroendocrine differences between monogamous and polygamous species probably reside on the corresponding receptor molecules. In addition, the neuroanatomical distribution of OT and AVP receptors in several *Microtus* species suggested that the pattern of OT and AVP receptor binding is associated with social organization (613). High OT receptor densities were found in the prelimbic cortex and nucleus accumbens of prairie voles, but not montane voles. Because these brain areas are involved in the mesolim-

bic dopamine reward pathway, it was hypothesized that in the female prairie voles OT released in response to mating activates this reward pathway and may condition the female to the odor of her mate (613). Furthermore, in the montane vole, which shows little affiliative behavior except during the postpartum period, brain OT receptor distribution changed within 24 h of parturition, concurrent with the onset of maternal behavior. This suggests that variable expression of the OT receptor in brain could be an important mechanism in evolution of species-typical differences in social bonding and affiliative behavior (258).

The complexity of the neuropeptide's actions on social behavior is illustrated by studies in the squirrel monkey, in which the effects of exogeneous OT depended on the prior social status of individuals. Dominant males responded to OT administration with increased sexual and aggressive behavior, whereas subordinate males displayed more associative behaviors. The differential behaviors might be due to the higher serum testosterone levels in dominant animals (260).

Transgenic mice created with the 5′-flanking region of the prairie vole OT receptor gene demonstrated that sequencing in this region influences the pattern of expression within the brain. Promoter sequences of the prairie vole OT receptor and V1a receptor genes and the resulting species-specific pattern of regional expression provide a potential molecular mechanism for the evolution of pair-bonding behaviors and a cellular basis for monogamy (259). Recently, Young et al. (611) reported that an AVP-induced increase in affiliative behaviors could be observed in mice that were transgenic for the prairie vole V1a receptor gene.

Interactions among OT, AVP, and glucocorticoids could provide substrates for dynamic changes in social behaviors, including those required in the development and expression of monogamy. Results from research with voles suggest that the behaviors characteristics of monogamy may be modified by hormones during development and may be regulated by different mechanisms in males and females (94). Nonnoxious sensory stimulation associated with friendly social interaction induces a response pattern involving sedation, relaxation, and decreased sympathoadrenal activity. It is suggested that OT released from parvocellular neurons in the PVN in response to nonnoxious stimulation integrates this response pattern at the hypothalamic level. The health-promoting aspect of friendly and supportive relationships might be a consequence of repetitive exposure to nonnoxious sensory stimulation (564). On the other hand, OT and AVP could also be involved in the pathophysiology of clinical disorders, such as autism, which is characterized by an inability to form normal social attachments (257).

D. Stress-Related Behavior

In both male and female rats, OT exerts potent anti-stress effects such as decreases in blood pressure, corticosterone/cortisone level, and increases in insulin and CCK level. After repeated OT treatment, weight gain could be promoted, and the healing rate of wounds increased (565). Furthermore, acute exposure of rats to immobilization stress resulted in an increase in OT mRNA level (280). OT secretion was also increased by forced swimming in virgin and early pregnant rats (409). In addition, microdialysis experiments on male rats showed specific increases in both central and plasma OT in response to forced swimming (603) and shaker stress (417). Thus the stimulated release of OT could function to facilitate the activation of the hypothalamic-pituitary-adrenal axis and increase glucocorticoid release. In contrast to most other OT-induced behavioral and physiological effects, the antistress effects could not be blocked by OT antagonists, suggesting that yet unidentified OT receptors may exist (565).

It is also well documented that stress-induced central release of OT can ameliorate the stress-associated symptoms such as anxiety. So, OT has anxiolytic properties in estrogen-treated females in mice and in rats (371) (Table 5) (595). Estrogen-induced increases in OT binding density in the lateral septum may contribute to the facilitation of social interactions (371). Like many other anxiolytic compounds, OT acts as an antidepressant as shown in two animal models of depression (30). Moreover, the antinociceptive action of OT is consistent with its antidepressant action (Table 5). Some of these effects may be mediated by an influence of OT on dopaminergic neurotransmission in limbic brain regions (368).

Because stress and anxiety can impair maternal caretaking and reduce milk ejection, reduced stress responsiveness during lactation is adaptive for both mother and infant. Accordingly, lactating women had reduced hormonal responses to exercise stress when compared with postpartum women who bottle-feed their infants (8). In addition, women with panic disorder can experience a relief of symptoms during lactation (300). Because OT levels increase in blood and CSF after nonnoxious sensory stimulation such as touch, light pressure, and warm temperature in both female and male rats, OT could not only be responsible for the antistress effects occurring during lactation but may also be involved in health-promoting effects of relationships, social contacts, and networks (564).

Some of the OT-induced effects may be explainable by the anxiolytic actions of OT (368) (Table 5). For example, OT acting as an anxiolytic may reduce the inhibition inherent in social encounters. Also, behavioral tests in the laboratory frequently involve the exposure of the animal to a novel environment, combined with exposure to an unfamiliar conspecific. These stimuli are likely to induce a stress response, and perhaps this anxiety is reduced by OT. So, a unifying principal in OT action in the brain may be to facilitate social encounters by reducing the associated anxiety (368). However, more studies are necessary to clearly distinguish between physiological and pharmacological effects.

E. Feeding and Grooming

OT acts as a "satiety hormone" in animals since both peripherally and centrally administered OT reduces feeding. In addition, food and anorexia-inducing agents, such as CCK, lead to pituitary OT secretion and subsequently to reduced food intake. This suggests that both nausea and satiety activate a common hypothalamic oxytocinergic pathway that controls the inhibition of digestion (425). Arletti et al. (25) reported that OT, whether given intraperitoneally or intracerebroventricularly, reduced food consumption and the time spent eating, and it increased the latency to the first meal in fasted rats. Pretreatment with an OT antagonist completely prevented the feeding inhibitory effect of OT, and per se increased food intake. In particular, hyperosmolality is a very potent stimulus for OT release (541), and central OT was observed to mediate osmolality-related inhibition of salt appetite but was not essential for Na^+-sensing mechanism in salt appetite in rats (53). While Arletti et al. (26) reported that OT directly inhibited both food and water intake in rats, Olsen et al. (425) observed a relatively small effect of OT on water intake when given in doses that significantly inhibited food intake. How could OT mediate its influence on the ingestive behaviors? PVN neurons in general, and OT projections in particular, could either act to modulate the activity of intrinsic brain stem reflex arcs or exert a direct control over vagal efferents that project to the gut and inhibit the gastric motility.

In mice, central administration of OT elicits dramatic behavioral excitation such as stress-induced escape, scratching, and grooming, particularly a pronounced self-grooming (376). Slow infusion of OT into the PVN also initiates self-grooming in rats (568). OT activates several aspects of grooming, preferentially genital grooming (570) (Table 5). The ability of OT to facilitate grooming involves activation of dopamine D1 receptor-mediated neurotransmission in the mesolimbic pathway as shown with knockout mice lacking the D1 dopamine receptor. However, neurotransmitters other than dopamine (e.g., endorphins) may also play a supplementary role in neuropeptide-enhanced grooming in mice (140). Grooming is a behavioral response to stressors, and the induction of grooming might be part of a homeostatic mechanism for reducing anxiety and facilitation of pair bonds, whether between mother and infant or between mating partners, by increased transfer of odors between individuals (94).

F. Memory and Learning

OT and AVP are considered to play a pivotal role in various aspects of learned behavior (131). Overall, the concept emerged that AVP reinforces memory, whereas OT has just the opposite effect, namely, the attenuation of learning processes and memory (Table 5). In particular, OT was shown to facilitate the extinction of avoidance reaction (134, 249) and to attenuate the storage of verbal memory (76). In cultured neurons, OT at concentrations over 1 μM reduces the activity of NMDA receptors, thus impairing one of the major substrates for the induction of learning and memory (96). Regarding its impairing effects on some memory-related tasks, OT could possibly be involved in the forgetting of delivery pain in mothers (161). On the other hand, studies on rodents indicated that socially relevant behaviors are controlled in a more complex way by both AVP and OT released intracerebrally and argued against a simplistic view with respect to the memory-attenuating effects of OT (155). In this context, reports on the social memory in rats provide a good example. Social recognition can be measured in rats as the specific decrease in social investigation of a juvenile conspecies during the second encounter of the same individual. This social memory is based on the olfactory characteristics of the conspecies and is short lasting (<40 min). OT had an impairing effect on the social memory in male rats (125). In another study, intraperitoneally injected OT to male rats significantly improved social memory (28). Depending on the dose, social memory was shown to be attenuated or facilitated by OT and derivatives as reported by Popik et al. (458). Interestingly, the active moieties for attenuation and facilitation of social memory reside in different parts of the OT molecule, e.g., the 5–7 and 8–9 region, respectively (460). However, the wide variety of observed effects has also led to the suggestion that OT has a more general effect on the cortical arousal rather than a specific effect limited to a certain stage of information processing (169).

G. Tolerance and Dependence to Opioids

Drug tolerance, dependence, and addiction may involve neuroadaptive mechanisms related to learning and memory at cellular and systems levels. Chronic morphine administration alters the brain OT system, which suggests that OT might contribute to the behavioral, emotional, and neuroendocrine responses to opioids (322). Moreover, OT has been shown to inhibit the development of tolerance to morphine and to attenuate various symptoms of morphine withdrawal in mice (Table 5). OT also decreased cocaine-induced locomotor hyperactivity and stereotyped behavior in rodents (308). Dopaminergic neurotransmission and central OT receptors located in limbic and basal forebrain structures are probably responsible for mediating these various effects of OT in the opiate- and cocaine-addicted organism.

The influence of opioids on the secretory activity of OT neurons in the SON was studied in rats in more detail. The brain stem noradrenergic system that is activated, e.g., by peripheral administration of CCK, represents a major excitatory input to OT neurons (348). Morphine inhibits the OT neurons presynaptically via inhibition of the afferent noradrenergic input. When the opiate antagonist naloxone is administered locally in the SON, the inhibitory effect of morphine on CCK-stimulated norepinephrine release is reversed, and the firing rate of OT cells markedly increases (71). However, noradrenergic systems are not essential for the expression of morphine withdrawal excitation, since chronic neurotoxic destruction of the noradrenergic inputs to the hypothalamus did not affect the magnitude of withdrawal-induced OT secretion (72). Opioid receptors on OT neurons and/or on their afferent input may be activated by the endogenous opioids pro-dynorphin and pro-enkephalin and are involved to restrain the secretory activity of OT cells (490). Interestingly, the efficiency of the endogenous opioid restraint changes during pregnancy in rats (336). Therefore, Russell et al. (490) have speculated that central opioid mechanisms could control OT neurons during parturition and can interrupt established parturition by inhibiting OT neuron-firing rate in disadvantageous environmental circumstances.

H. Central Disorders in Humans

1. Obsessive-compulsive disorder

Obsessive-compulsive disorder (OCD) includes cognitive and behavioral symptoms that are related to OT-associated behaviors. A possible role for OT in the neurobiology of a subtype of the OCD was suggested by the elevated CSF levels of OT and by the correlation between CSF OT levels and the severity of the disorder (325). Excessive grooming is often a component of OCD, and central OT administration induces high levels of self-grooming in animals. Periods of increased gonadal steroid secretion such as puberty and pregnancy are associated with a heightened risk for onset of OCD. Perhaps, gonadal steroids activate OT receptor during these periods. However, the results of another study do not support the hypothesis that OT might be a potential anticompulsive agent (129).

2. Eating disorders: anorexia and Prader-Willi syndrome

The OT neurons of the PVN are good candidates for playing a physiological role in ingestive behavior as "sa-

tiety neurons" in the human hypothalamus. In humans, OT appears not to be released in response to a meal. In women with anorexia nervosa, CSF levels of OT are reduced during the starvation phase of the illness and return to normal as the patients increase their food intake (128). Both hypoglycemia- and estrogen-induced OT increases were lower in underweight anorectic patients compared with normal controls. Anorectic subjects regained normal OT responsiveness to both stimuli after complete weight recovery (109). Reduced brain and plasma levels of OT during the starvation phase of the illness might contribute to the symptom profile of anxiety, loss of libido, amenorrhea, and increased activation of the hypothalamic-pituitary-adrenal hormonal stress axis.

In Prader-Willi syndrome, a genetic disorder characterized by gross obesity, insatiable hunger, hypotonia, hypogonadism, mental retardation, and a high risk for OCD, a 42% decrease in the number of hypothalamic OT neurons was observed. Moreover, there was a reduction in total cell number in the PVN, and the volume of the PVN-containing OT-expressing neurons was decreased by 54% (544). The lower number of OT neurons in the hypothalamic PVN is presumed to be the basis of the insatiable hunger and obesity of patients with the syndrome. Further evidence for hypothalamic and oxytocinergic dysfunction in Prader-Willi syndrome was provided by the finding that CSF OT was nearly twofold higher in patients with Prader-Willi syndrome compared with control subjects (361).

3. Depression and schizophrenia

In postmortem samples from patients with depression, a 23% increase in the number of OT-immunoreactive neurons in the PVN of the hypothalamus was found (469). The putative higher rate of oxytocinergic activity in these patients is possibly associated with activation of the hypothalamic-pituitary-adrenal axis in these patients, since OT is known to potentiate the effects of CRH.

Increased concentrations of OT were observed in the CSF of schizophrenic patients, and they were higher in patients receiving neuroleptic treatment (48). However, Glovinsky et al. (214) found no changes of the OT concentration in the CSF of schizophrenic patients either with or without neuroleptic medication. In another study, the basal level of OT-neurophysin was found to be threefold higher in the plasma of schizophrenics (333). So, impairments in the function of the OT system could play a role in the social deficits of schizophrenic patients, and OT is a candidate novel endogenous antipsychotic (171).

4. Neurodegenerative diseases

OT injected into the hippocampus is reported to interfere with the formation of memory in experimental animals. In humans, high density of OT binding sites was observed in the nucleus basalis of Meynert, which is the major nucleus for cortical cholinergic neurons and degenerates in Alzheimer's disease (346). It has been reported that OT binding site density in the basal nuclei of Meynert decreases in the brains of patients suffering from Alzheimer-type senile dementia (186). OT concentration was found to be increased 33% in the hippocampus and temporal cortex of Alzheimer brains but was normal in all other regions examined (364). In two other sets of patients with Alzheimer dementia, the number of hypothalamic OT-expressing cells remained unaltered with aging (177, 590). A slightly decreased number of OT-immunoreactive neurons was reported in the PVN of the hypothalamus in Parkinson's disease (468). However, more studies are needed to evaluate the role of OT for neurodegenerative disorders.

VII. CONCLUDING REMARKS

In the past decade, the OT receptor structure has been elucidated, and considerable advances have been made in understanding the structure and function of the OT receptor system that has now been detected in many different tissues. Is there a further OT receptor subtype as suggested by some findings? It is certainly too early to exclude this possibility. On the other hand, many of the unexplained observations in the OT receptor field may result from complex cross-talks to poorly defined signaling cascades, interactions with receptor modulators like Mg^{2+} and cholesterol, and/or regulation by steroids via genomic and nongenomic pathways. Particularly, the functional dependence on steroids, a characteristic feature of the OT receptor system, is among the least understood. This is not surprising in view of the multiple targets of steroids and the novel facets of steroid receptor actions. For example, recent observations suggest that several steroid receptors can be activated by various agents in the absence of cognate hormone (99). To clarify the underlying mechanisms for both genomic and nongenomic steroid actions will therefore be of fundamental importance to understand the physiological regulation of the OT receptor system.

To some extent, most of the different actions of OT can be integrated in a concept according to which OT supports and facilitates the reproduction at several levels. In social living species like humans, affiliative behaviors are an essential component of reproduction. It will be therefore interesting to see whether in humans, mislocations of the OT receptor, naturally occurring mutations, or polymorphisms in the OT receptor gene can be correlated with (gender-specific?) physiological or behavioral deficits. In view of the widespread OT-related actions, OT antagonists may not only be regarded as promising candidates to prevent preterm labor and dysmenorrhea, but

may together with OT agonists also prove useful for treatment of psychiatric illnesses such as anxiety, drug abuse, sexual dysfunctions, eating disorders, or autism.

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