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PART I

Oxytocin and vasopressin systems

Anatomy, function, and development

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1

Oxytocin, vasopressin, and their interplay with gonadal steroids

Monica B. Dhakar, Erica L. Stevenson, and Heather K. Caldwell

1.1 Overview

The neuropeptides oxytocin (OT) and vasopressin (AVP) are two evolutionarily ancient neurohormones known to influence mammalian sex-specific and species-specific behaviors. The gonadal steroids are also important modulators of many mammalian behaviors. Thus, it is not surprising that there are profound and complex interactions between these two systems. This chapter will provide an overview of the OT and AVP systems, including their interactions with gonadal steroids.

1.2 Oxytocin and vasopressin

OT and AVP are composed of nine amino acids and differ from one another by only two amino acid residues, specifically those in the third and eighth positions (as reviewed in Hara, 1990; Burbach et al., 2001; Young and Gainer, 2003; Caldwell et al., 2008; Donaldson and Young, 2008; Lee et al., 2009). Their gene structures are also similar as they are the result of a gene duplication of the ancestral vasotocin gene, which occurred approximately 700 million years ago (Acher and Chauvet, 1995). While OT and AVP are prominent only in mammals, they are a part of a peptide family that is conserved across phyla (as reviewed in Caldwell, 2008; Lee, 2009). Both OT and AVP amino acids sequences are largely conserved across mammalian species, with a notable exception in OT in some species of New World primates. This novel OT was dubbed [P8] OT due to the substitution of a proline for a leucine in the eighth position (Lee, 2011). Across species, OT and AVP are important to the regulation of social interactions, with OT being mostly identified with bonding between individuals, and AVP being mostly identified with the regulation of aggression and male parental care; though, their roles are not nearly so restricted as these generalizations.

1.2.1 Oxytocin and vasopressin gene and protein structures

Within a species, the *OT* and *AVP* genes are located on the same chromosome (i.e., chromosome 2 in mice, 20 in humans, and 3 in rats) and contain three exons and two introns. The genes are oriented in opposing transcriptional direction on the chromosome and are separated by a region of DNA referred to as the intergenic region (IGR). The IGR is highly variable across species, being approximately 11 kbp in rat (Mohr et al., 1988) and human (Gainer et al., 2001) and approximately 3.6 kbp in mouse (Hara et al., 1990). The significance of the IGR is not completely understood, but in the hypothalamus, portions of the IGR appear to be critical for the normal expression of OT and AVP (Fields et al., 2003; Young and Gainer, 2003).

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OT and AVP are each synthesized as part of a larger preprohormone, which contains a signal peptide, the biologically active peptide, a neurophysin, and a glycoprotein. The first exon encodes the 5′ non-coding promoter region, the nonapeptide, the tripeptide processing signal, and the first nine residues of the neurophysin. The second exon encodes for the bulk of the neurophysin molecule (residues 10–76), and the third exon encodes the remainder of the neurophysin (77–93/95 residues), including the COOH terminal, as well as the glycopeptide of the AVP preprohormone (Gainer et al., 2001; Gimpl and Fahrenholz, 2001). While neurophysin does not possess biological activity, it is thought to play a role in protecting OT and AVP from enzymatic degradation (de Bree, 2000). Also, as AVP is considered less biologically stable than OT, it has been proposed that the glycopeptide portion of the AVP preprohormone may be important for folding of the AVP precursor (Barat et al., 2004).

Based on the sequence analysis of *OT* and *AVP* in several species, including rat (Ivell and Richter, 1984), human (Sausville et al., 1985), cow (Ruppert et al., 1984), sheep (Ivell et al., 1990), and mouse (Hara et al., 1990), the transcriptional start site of the genes are found downstream of a TATA-like sequence in the 5′ flanking region (Hara, 1990; Gainer et al., 1994). Upstream of this start site, within the putative promoter, there are several regulatory elements that provide an opportunity for gonadal steroids to affect the transcription of *OT* and *AVP*. There are estrogen response elements (EREs), which allow estrogens bound to estrogen receptor alpha (ERα) or estrogen receptor beta (ERβ) to directly affect transcription, as well as a highly conserved DNA segment called the multiple hormone response element (HRE), which binds to multiple members of the retinoic acid and thyroid hormone receptor superfamily (Mohr et al., 1988; Adan et al., 1991; Richard and Zingg, 1991). There are also identified promoter regions that allow for the more “indirect” action of gonadal steroids, including activator protein-1 (AP-1) and activator protein-2 (AP-2) sites. The details of these regulatory elements will be detailed below (Section 1.4).

1.2.2 Distribution of oxytocin and vasopressin neurons and fibers

OT and AVP are primarily synthesized in the magnocellular neurons of the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus. The axons of these neurons project to the posterior pituitary, ultimately releasing OT and AVP into the bloodstream where their peripheral effects can be exerted. OT and AVP fibers are widely distributed within the central nervous system (CNS) and originate from other neurons, either within the PVN, SON, or elsewhere. It is the actions of OT and AVP in many of the subcortical regions, described below, that are involved in the regulation of aspects of social and sexual behavior in mammals.

1.2.2.1 Oxytocin

In most species, central OT production is limited to the PVN and SON. However, in mice, smaller quantities of OT appear to be produced by neurons in the bed nucleus of stria terminalis (BNST), the medial preoptic area (MPOA), and the amygdala (Castel and Morris, 1988; Jirikowski et al., 1990; Wang et al., 1996). There are also subtle species differences found in the extrahypothalamic distribution of OT-immunoreactive (OT-ir) neurons. In rats and humans, the parvocellular neurons of the PVN provide robust projections to the olfactory bulb, the dorsal and ventral hippocampus, the amygdala, the substantia nigra and substantia grisea, the nucleus of solitary tract and the nucleus ambiguous of the brainstem, and to the substantia gelatinosa of the spinal cord (Buijs, 1978; Sofroniew, 1980; Swanson and Kuypers, 1980; Rinaman, 1998). In contrast to the rat, the guinea pig (*Cavia porcellus*) shows prominent OT-ir neurons in the visual pathway, the retrochiasmatic and subchiasmatic areas, and in the medial preoptic nucleus (MPN) (Dubois-Dauphin et al., 1989b), and in the garden mouse (*Eliomys quercinus L.*), there are OT-ir neurons in the prefrontal cortex, the claustrum, and the septum (Hermes et al., 1988). Species differences have also been

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detected in the distribution of OT-ir cells within four closely related species of voles (Wang et al., 1996).

1.2.2.2 Vasopressin

Unlike OT, central AVP is produced in a variety of brain areas other than the PVN and SON. In all mammals studied to date, there are AVP-producing neurons found in the preoptic area (POA) and anterior hypothalamus (AH), and in many species there are AVP-immunoreactive (AVP-ir) cells in the BNST and medial amygdala (MeA). These latter two sites send projections to the lateral septum (LS) and lateral habenular nucleus (LHb). The suprachiasmatic nucleus (SCN), the primary mammalian circadian clock, is also an area rich in AVP-producing neurons (Buijs et al., 1978; Castel et al., 1990).

The AVP system has been examined in many species, including: marmosets (*Callithrix jacchus*) (Wang et al., 1997), golden hamsters (*Mesocricetus auratus*) (Delville et al., 1994), prairie voles (*Microtus ochrogaster*) (Wang and De Vries, 1993; Wang, 1995), meadow voles (*Microtus pennsylvanicus*) (Wang, 1995), Djungarian hamsters (*Phosdopus sungorus*) (Dubois-Dauphin et al., 1994), European hamsters (*Cricetus cricetus*) (Buijs et al., 1986), garden dormouse (*Eliomys quercinus*) (Hermes et al., 1990), and a variety of laboratory rat and mouse strains (Sofroniew, 1983; De Vries and al-Shamma, 1990). In most mammalian species, AVP-ir fibers can be found in the POA, anterior and lateral hypothalamic areas, midbrain tegmentum, periaquaductal grey, locus coeruleus (LC), LS, LHb, nucleus of the solitary tract, and area postrema (Moore and Lowry, 1998).

1.3 Oxytocin and vasopressin receptors

To date, only one receptor subtype has been identified for OT, the oxytocin receptor (OTR). Whereas three receptor subtypes have been identified for AVP: the vasopressin 1a receptor (AVPR1a), the vasopressin 1b receptor (AVPR1b), and the vasopressin 2 receptor (AVPR2). As the AVPR2 is not found centrally it will not be discussed in this chapter, but is reviewed in (Barberis et al., 1998; Foletta et al., 2002; Bankir et al., 2010). While there are instances of dramatic gonadal steroid-dependent species and sex differences in OT and AVP, gonadal steroid-dependent changes in distributions of the OTR and the AVPR1a seem to contribute greatly to behavioral differences within and between species.

1.3.1 Oxytocin receptor structure and distribution

The OTR was first sequenced from human myometrium by Kimura and colleagues (1992). Subsequently, the OTR has been cloned and sequenced in a variety of species, including rat (Rozen et al., 1995), vole (Young et al., 1996), mouse (Kubota et al., 1996), rhesus monkey (Salvatore et al., 1998), cow (Bathgate et al., 1995), and pig (Gorbulev et al., 1993). The OTR is a member of the G protein-coupled receptor family and signals via activation of $G_{q/11\alpha}$ class GTP binding proteins and generation of inositol triphosphate and 1,2-diacylglycerol (for review, see Gimpl and Fahrenholz, 2001; Young and Gainer, 2003).

In humans, the chromosomal location of the OTR has been mapped to gene locus 3p25–3p26.2, using fluorescence *in situ* hybridization, (Inoue et al., 1994; Michelini et al., 1995; Simmons et al., 1995). The OTR gene contains 4 exons and 3 introns and spans approximately 17 kbp and encodes approximately 389 amino acids. Exon 1 and 2 encode the 5′ non-coding region and exons 3 and 4 encode the receptor protein. The transcription start site lays 618–621bp upstream of the methionine initiation codon. Twenty-eight to 31bp upstream of the transcription start site is a TATA-like motif and 65bp upstream is a potential specific protein-1 (SP-1) binding site. The 5′ flanking region, while lacking a classic ERE, has binding sites for other transcription regulating factors such as AP-1, AP-2, GATA-1, and c-Myb. It also contains two half-palindromic 5′-GGTCA-3′ and one half-palindromic 5′-TGACC-3′ ERE motifs (Inoue et al., 1994).

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The amino acid sequence of the mouse and the rat OTR have 91% and 93% homology with the human OTR, respectively (Rozen et al., 1995; Kubota et al., 1996). The gene structure in mouse is similar to that in humans, except that the promoter region lacks an apparent TATA box, but does contain putative interleukin-response elements and an ERE (Kubota et al., 1996). The rat OTR, on the other hand, spans more than 20 kbp and contains 3 exons. The promoter region contains multiple putative interleukin-response elements but lacks an apparent TATA or CCATT box (Rozen et al., 1995). The 5′-flanking region has been shown to contain a palindromic ERE within 4 kbp of the translational start site (Bale and Dorsa, 1997).

The OTR is widely distributed throughout the central nervous system, however regional distribution shows marked species and sex differences. The distribution of the OTR has been extensively studied in the rat brain using two radiolabeled ligands: 1) a tritiated OT ([^{3}H]OT) and 2) an iodinated OT antagonist ([^{125}I]OTA) (Tribollet et al., 1992). Specific OT binding sites are found in numerous areas including anterior olfactory nucleus, cell groups of the olfactory tubercle, LS, BNST, hypothalamic ventromedial nucleus (VMH), PVN, central amygdala (CeA), MeA, shell of the nucleus accumbens, ventral subiculum of the hippocampus, and the caudoputamen region (Brinton et al., 1984; De Kloet et al., 1985; Tribollet et al., 1988; Tribollet et al., 1990). In the rat brain, the expression of OTR mRNA, as detected by *in situ* hybridization, corresponds to the location of OT binding sites (Yoshimura et al., 1993). This suggests that the site of synthesis of protein is the same as the location of the OTR in the central nervous system.

1.3.2 Vasopressin receptor structure and distribution

The AVPR1 was initially characterized by Jard and colleagues (1983) and was later broken down into subtypes, AVPR1a and AVPR1b (Antoni, 1984; Jard et al., 1986). Both receptor subtypes are G protein-coupled receptors that activate $G_{\alpha q/11}$ GTP binding proteins, which in turn activate phospholipase C with the help of $G_{\beta\lambda}$ (Michell et al., 1979; Jard et al., 1987). The *AVPR1a* gene is made up of two exons divided by one intron (~1.8 kbp) and spans 3.8 kbp total. Suggestive of a common ancestry, most of the AVP/OT receptor family has six of the seven transmembrane domains encoded by a single exon, and the seventh transmembrane domain encoded by a separate exon. The cDNA is made up of 1354 nucleotides that produce 394 amino acids (Morel et al., 1992); with the rat and human AVPR1a sharing 72% of their amino acid sequence (Thibonnier et al., 1994). The AVPR1a gene promoter contains three transcriptional initiation sites at –405, –243 and –236bp upstream of the start codon, the major sites are at –243 and –236bp (Murasawa et al., 1995). The promoter for the *AVPR1a* contains no TATA or CCAAT promoter elements and has a high G and C content (~62%) (Murasawa et al., 1995). The AVPR1a gene promoter also contains several regulatory elements, including AP-1, AP-2, and SP-1 binding sequences (Murasawa et al., 1995).

Unlike the *AVPR1a*, the *AVPR1b* is made up of three exons and two introns in mouse and rat strains. Exon 2 encodes six of the seven transmembrane domains, while exon 3 encodes for the seventh domain. There are two transcription start sites at –861 and –830bp relative to the start codon. The *AVPR1b*, like the *AVPR1a*, does not contain a proximal TATA box. However, there is a CACA box and an inverted GAGA box present in the promoter, which is unusual for G protein-coupled receptors. There is no sequence homology between the *AVPR1b* promoter regions of the mouse and rat except for the location of the CACA and inverted GAGA boxes, suggestive that these are the sequences conserved in the *AVPR1b*. The promoter also contains several regulatory elements, including three AP-1, five AP-2, three SP-1, and two CCAAT-enhancer-binding-protein (C/EBP) sites as well as a glucocorticoid response element (GRE) (Rabadan-Diehl et al., 2000).

The AVPR1a is widely distributed throughout the central nervous system, but its localization

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is fairly conserved across mouse and rat strains (Johnson et al., 1993; Tribollet et al., 1997; Young et al., 2000). Radiolabeled receptor autoradiography reveals AVPR1a binding in the LS, neocortical layer IV, hippocampal formation, amygdalostriatal area, BNST, hypothalamus, ventral tegmental area (VTA), substantia nigra, superior colliculus, dorsal raphe, nucleus of the solitary tract, and superior olive (Johnson et al., 1993). *In situ* hybridization histochemistry shows prominent AVPR1a mRNA in the olfactory bulb, hippocampal formation, LS, SCN, PVN, AH, arcuate nucleus, LHb, VTA, substantia nigra, LC, inferior olive, area postrema, and nucleus of the solitary tract (Ostrowski et al., 1994; Szot et al., 1994).

The AVPR1b appears not to be as widely distributed as the AVPR1a. It is highly abundant in the anterior pituitary, where it is found on the corticotrophes (Antoni, 1984), In the brain, *in situ* hybridization histochemistry and immunocytochemistry have localized the AVPR1b to the olfactory bulb, piriform cortical layer II, septum, cerebral cortex, hippocampus, PVN, SCN, cerebellum, and red nucleus (Lolait et al., 1995; Saito et al., 1995; Vaccari et al., 1998; Hernando et al., 2001; Stemmelin et al., 2005; Young et al., 2006). However, a later study found that the distribution of the AVPR1b in rat, mouse, and human was limited, with prominence in the anterior pituitary, the CA2 region of hippocampus, and a few cells in anterior amygdala (Young et al., 2006). This latter study may better reflect the distribution of the AVPR1b, as the earlier studies by Lolait et al. (1995) and Vaccari et al. (1998) used sequences that had significant identity with the AVPR1a and the OTR.

1.4 The gonadal steroids

The two primary classes of gonadal steroids are the androgens and the estrogens. While predominately synthesized in the testes and ovaries, they can also be generated in other tissues such as adrenal glands, liver, and fat, or locally synthesized in the brain. Like all steroids, the gonadal steroids are derived from cholesterol through the process of steroidogenesis. The common precursor hormone for all androgens and estrogens is dehydroepiandrosterone (DHEA), which is a "weak androgen" produced primarily in the adrenal cortex. For a summary of gonadal steroid synthesis, starting with DHEA, see Figure 1.1 (Torn et al., 2003; Steckelbroeck et al., 2004; Bauman et al., 2006). Interestingly, all of the enzymes necessary to metabolize testosterone into other androgens, or estradiol, can be found in the brain (Guennoun et al., 1995). Thus, in the brain, depending on what enzymes are locally available, the presence of testosterone can ultimately result in either, or both, androgenic and estrogenic effects. As many behaviors are associated with an animal's reproductive status, it is not surprising that gonadal steroids are important regulators of the OT and AVP systems.

1.4.1 Possible interactions among oxytocin, vasopressin, and gonadal steroids

So, how do the gonadal steroids interact with the OT and AVP systems? There are numerous ways for this to occur. The most direct "classical" action is through the binding of gonadal steroids to their respective intracellular receptors such as ERα and ERβ for the estrogens, and the androgen receptor (AR) for the androgens. These hormone-receptor complexes dimerize and are then translocated from the cytosol to the nucleus where they can directly interact with the DNA through response elements (i.e., an ERE or an androgen response element (ARE) in the promoter regions of the *OT* and *AVP* genes or the genes for their receptors. While there can be crosstalk between the estrogens and androgens and their respective receptors, this is limited due to differences in their affinity for the receptors. Even within a given class of gonadal steroids, different estrogens or androgens may have more or less affinity for their ERs or AR, respectively. For example, dihydrotestosterone (DHT) has a higher affinity for the AR compared to testosterone (3–5 times), but does not bind well with ERα or ERβ (Handa et al., 1987; Kuiper et al., 1998). Similarly, estradiol binds to ERα and ERβ with equal affinity (Kuiper et al., 1998),

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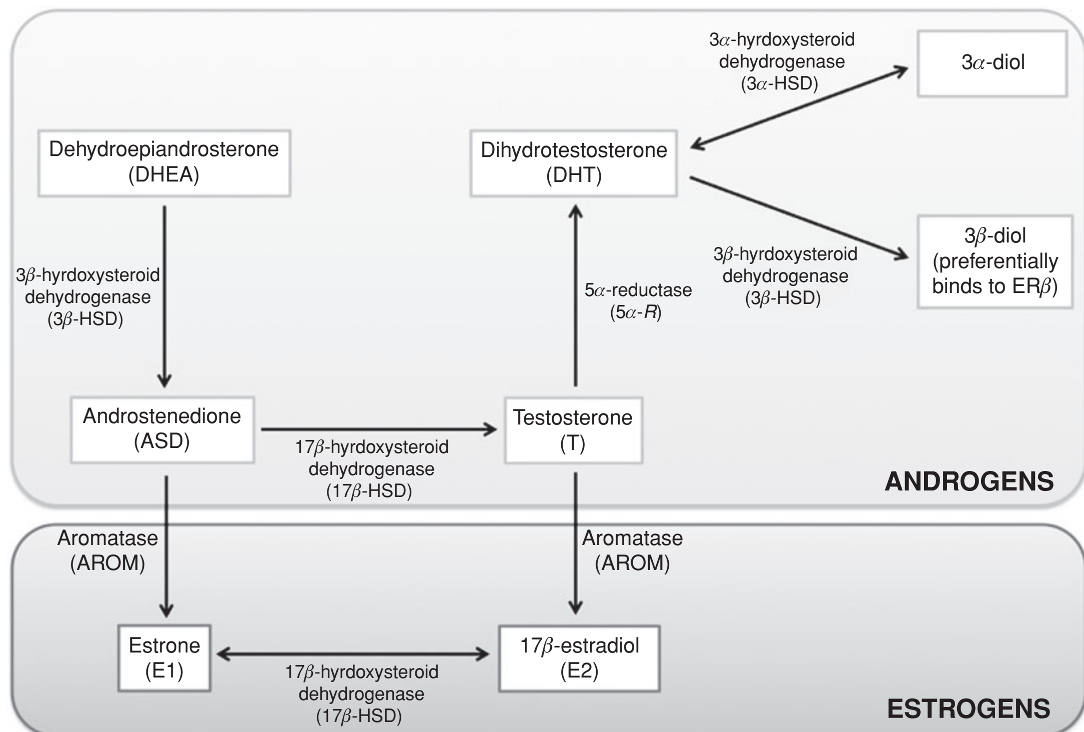

Figure 1.1 Pathway showing the synthesis of the key androgens and estrogens that regulate the expression of OXT and AVP peptides and receptors. Adapted and modified from Handa et al., 2009. See color version in plates section.

but has a very low affinity for the AR (Handa et al., 1987).

It is also important to note that there are numerous subtypes of intracellular receptors. The originally described ERβ would now be better termed ERβ1 due to the discovery of at least five different splice variants of the receptor. These variants include one lacking the third exon (δ3), one lacking the fourth exon (δ4), one with an insert between exons 5 and 6 (β2), and combinations of the three (Peterson, 1998; Hanstein et al., 1999; Price et al., 2000; Price et al., 2001). There are also interactions between estrogenic and androgenic pathways. For example, 5α-androstane-3β,17β-diol (3β-diol), an androgen metabolite, has estrogen-like effects since it preferentially binds and activates ERβ but has a low binding affinity for the AR (Weihua et al., 2002). 5α-androstane-3α,17β-diol (3α-diol) on the other hand has little to no affinity for either ER but preferentially binds to the AR (Kuiper et al., 1998).

Estrogens and androgens bound to receptors can also exert effects by interacting with other regulatory sites on the DNA, such as AP-2 sites, which stimulate transcription via protein kinase A and C pathways (Imagawa, 1987); AP-1-like sequences, the sole function of which is to regulate gene transcription in response to immediate early gene induction (e.g., Fos and Jun); and a SP-1 transcription factor binding site, which directly binds to DNA to enhance gene expression during early development (Webb et al., 1995; Marin et al., 1997; Price et al., 2001; Kim et al., 2005). The presence of one or more of these regulatory elements in the promoters of the

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OT, *AVP*, or their receptor genes allows for myriad types of transcriptional regulation.

1.5 Gonadal steroid regulation of oxytocin and vasopressin

1.5.1 Oxytocin

There is ample evidence suggesting that gonadal steroids regulate various aspects of the OT system in both males and females. In rats, increased expression of OT mRNA in certain areas of the hypothalamus are coincident with the onset of puberty and vary across the estrous cycle; both of these events are associated with increased concentrations of circulating estrogens (Van Tol et al., 1988; Zingg and Lefebvre, 1988; Miller et al., 1989a). Gonadectomy of prepubertal and adult female rats decreases OT mRNA, and estradiol replacement during puberty can increase OT mRNA (Miller et al., 1989a; Chibbar et al., 1990). Similarly, the expression of OT, as measured by mRNA levels in the SON and PVN, mirrors the fluctuations in estradiol and progesterone concentrations that are found during pregnancy and lactation (Van Tol et al., 1988; Miller et al., 1989a; Crowley et al., 1993). In a study by Crowley and colleagues (1995), which mimicked the hormone levels of pregnancy in rats, treatment with estradiol and progesterone followed by progesterone withdrawal increases OT mRNA in the hypothalamus (Crowley et al., 1995). Apart from regulating the transcription of OT, estrogens also affect serum and pituitary levels of OT, axonal and dendritic release of OT, as well as the electrical activity of OT neurons (Yamaguchi et al., 1979; Akaishi and Sakuma, 1985; Skowronski et al., 1987; Van Tol et al., 1988; Wang and De Vries, 1995).

The actions of estrogens on OT are primarily mediated via ERα and ERβ. These receptors are often overlapping in their CNS distribution; though, the PVN is a pronounced exception. Within the PVN ERα is found at low or undetectable levels (Shughrue et al., 1997; Simonian and Herbison, 1997; Alves et al., 1998; Hrabovszky et al., 1998; Greco et al., 2001), suggesting that any effects of estrogens on PVN-derived OT may be mediated solely by ERβ. Support for a modulatory role of ERβ on OT neurons comes from immunocytochemical and *in situ* hybridization studies. These studies have found that ERβ is expressed in OT producing neurons of the SON and PVN of rats, being most abundant in the caudal regions of the PVN (Alves et al., 1998; Hrabovszky et al., 1998; Shughrue et al., 2002; Hrabovszky et al., 2004), in the SON of mice (Sar and Stumpf, 1980), and in the PVN of guinea pigs (Warembourg and Poulain, 1991). Further, studies conducted in ERβ knockout (ERβKO) mice have found that treatment with estradiol has no effect on OT mRNA expression compared to wild-type (WT) mice, suggesting that the estrogenic regulation of OT is likely via ERβ (Nomura et al., 2002; Patisaul et al., 2003).

The aforementioned *in vivo* effects of estradiol on OT expression prompted a series of *in vitro* studies designed to examine whether estrogen-dependent activation of the OT system could be attributed to the *direct* interaction of ERβ with the OT promoter. As the OT promoter in human and rat contains a highly conserved DNA binding site approximately –160 nucleotides from the transcription start site, which is homologous to the classic ERE palindromic sequence, there is the potential for direct genomic effects by estradiol on *OT* transcription (Mohr and Schmitz, 1991). Co-transfection studies in a heterologous cell culture system using promoter-reporter constructs confirm that the human and rat OT promoters are activated by estradiol (Peter et al., 1990; Richard and Zingg, 1990; Adan et al., 1993). Though, based on *in vitro* work in bovine and human this -160 HRE does not appear to directly interact with estradiol bound to ERβ, indicating that estrogens might regulate the *OT* gene via some indirect mechanism (Stedronsky et al., 2002). One possible mechanism for the *indirect* effects of estradiol on the *OT* gene is through the interaction of the –160 HRE with nuclear orphan receptors. Recently, the human OT –160 HRE has been found to have a strong affinity for the nuclear orphan receptors, steroidogenic

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factor-1 (SF-1), and chicken ovalbumin upstream promoter transcription factor I (COUP-TF I) (Wehrenberg et al., 1994a; Wehrenberg et al., 1994b; Stedronsky et al., 2002). Ligand-activated estrogen receptors likely bind to these nuclear orphan receptors, thus facilitating their binding to the OT promoter, ultimately causing estrogen-dependent upregulation of OT gene expression (Giguere et al., 1988; Giguere, 2002; Sanchez et al., 2002; Koohi et al., 2005).

Yet another way that estrogens can indirectly alter OT gene transcription is through a non-genomic mechanism. Recently, a membrane-bound estrogen receptor, the GPCR-30 (recently renamed GPER), was characterized. The GPER is a member of the 7 transmembrane G-protein coupled superfamily of receptors. It is found in OT producing neurons of the SON and PVN, providing a means by which estrogens could affect oxytocinergic cells. It is speculated that the GPER mediates the rapid actions of estrogens on the OT system through activation of adenylyl cyclase, intracellular calcium mobilization, and generation of phosphatidylinositol-3,4,5-triphosphate (Revankar et al., 2005; Sakamoto et al., 2007).

1.5.1.1 Sexual dimorphisms

Sexual dimorphisms in the distribution of OT are not as common as with AVP (detailed below), with differences being species specific (Buijs et al., 1978; Haussler et al., 1990; Wang et al., 1996; Rosen et al., 2008). In studies where sex differences have been found, the number of OT-ir neurons, as well as the amount of OT as measured by immunoassay, tends to be higher in females compared to males (Haussler et al., 1990). A summary of changes in OT-ir that are thought to be gonadal steroid-dependent can be found in Table 1.1.

1.5.2 Vasopressin

In vivo, the AR and ERβ have been localized to brain regions rich in AVP or AVP receptors, especially the PVN, SON, and BNST (Laflamme et al., 1998; Suzuki and Handa, 2005). Further, the AVP promoter contains at least one ERE and several AP-1 and HRE sites (Shapiro et al., 2000). Work *in vitro* has found that gonadal steroids can have either facilitatory or inhibitory effects on AVP gene transcription, depending on what hormone/receptor complex binds to the DNA. Facilitation of AVP gene transcription occurs through the activation of ERα and ERβ (Pak et al., 2005; Pak et al., 2007). Estradiol and DHT, both metabolites of testosterone (Naftolin et al., 1975; Selmanoff et al., 1977), are known to affect AVP expression. Castrated rats treated with estradiol and DHT have a full restoration of AVP-ir and mRNA expression within the BNST and MeA. However, estradiol administered alone only partially restores AVP-ir and mRNA expression in these brain areas (de Vries et al., 1986; De Vries et al., 1994; Wang and de Vries, 1995). Interestingly, there is also evidence that AVP transcription can be inhibited by DHT when it is bound to the AR (Pak et al., 2007). However, the mechanism by which this occurs is not yet understood. With a lack of an apparent ARE in the AVP promoter it is unclear how DHT is influencing the transcription of the *AVP* gene.

There is evidence that a metabolite of DHT, 3β-diol, interacts with the AVP promoter. While 3β-diol is derived from an androgen, it preferentially binds to ERβ and has been found to stimulate AVP promoter activity though ERβ1 and ERβ2 (Pak et al., 2007). In culture, 3β-diol significantly increases ERE-mediated promoter activity to levels greater than that achieved by estradiol. This effect is specific to the presence of the ERE, as there is no effect on AVP promoter activity in the presence of only an AP-1 site (Pak et al., 2005). When ERβ splice variants are cotransfected with a firefly luciferase reporter construct containing the AVP promoter in the presence of 3β-diol, it was determined that 3β-diol increases AVP promoter activity in the presence of the ERβ1 and ERβ2 splice variants, but not the ERβ1δ3 splice variant (Pak et al., 2007). (This latter finding was not surprising since the ERβ1δ3 splice variant lacks exon 3, which encodes for the second finger of the

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