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# Ovarian Steroids and Serotonin Neural Function

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## Abstract

The serotonin neural system originates from ten nuclei in the mid- and hindbrain regions. The cells of the rostral nuclei project to almost every area of the forebrain, including the hypothalamus, limbic regions, basal ganglia, thalamic nuclei, and cortex. The caudal nuclei project to the spinal cord and interact with numerous autonomic and sensory systems. This article reviews much of the available literature from basic research and relevant clinical research that indicates that ovarian steroid hormones, estrogens and progestins, affect the function of the serotonin neural system. Experimental results in nonhuman primates from this laboratory are contrasted with studies in rodents and humans. The sites of action of ovarian hormones on the serotonin neural system include effects within serotonin neurons as well as effects on serotonin afferent neurons and serotonin target neurons. Therefore, information on estrogen and progestin receptor-containing neurons was synthesized with information on serotonin afferent and efferent circuits. The ability of estrogens and progestins to alter the function of the serotonin neural system at various levels provides a cellular mechanism whereby ovarian hormones can impact mood, cognition, pain, and numerous other autonomic functions.

**Index Entries:** Ovarian steroids; serotonin neural function; estrogen; progestin; afferent neurons; target neurons; afferent circuits; efferent circuits.

## Introduction

Serotonin (5-hydroxytryptamine, 5HT) is involved in a wide variety of complex physiological, behavioral, and emotional processes, such as autonomic functions, satiety, vigilance states, mood, affect, learning, memory, sexual behavior, and both inwardly and outwardly directed aggression each of which may be a consequence of the state of arousal (Van de

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Kar, 1991; Jacobs and Azmitia, 1992; Wang and Nakai, 1994). Furthermore, it has been suggested that one function of the serotonergic system is to enable the organism to ward off feelings of fear, helplessness, and depression (Graeff et al., 1996). Recent evidence suggests that ovarian steroids affect the serotonin neural system, which establishes a link between estrogens, progestins, and mental health. This article will examine the effects of ovarian steroids on the serotonergic neural system as well as estrogen and progestin receptors (ER, PR) expression in serotonin neurons and terminal fields.

A gender difference in the occurrence of major depression and dysthymia is well recognized. Twice as many women suffer from depression as men (Weissman and Klerman, 1985; Williams et al., 1995). Thus, a relationship between ovarian hormones and depression has been postulated for decades. The decline in estrogens and progestins prior to parturition and at the onset of natural or surgical menopause has been correlated with an increase in negative affect (Gitlin and Pasnau, 1989). Moreover, E replacement therapy can in some cases alleviate depression or anxiety in women (Oppenheim, 1983; Gregoire et al., 1996). It has been suggested that depression associated with changes in the levels of ovarian steroids is related to serotonin neural function (Halbreich and Tworek, 1993; Mortola, 1994; Eriksson et al., 1995, Steiner et al., 1995; Parry, 1997; Su et al., 1997). Ovarian steroids may affect serotonin synthesis, content, release, reuptake, degradation, binding sites, and intracelluar receptor-coupling mechanisms. We will attempt to collate the available information in each of these areas.

The dorsal raphe nucleus located in the pontine midbrain is the largest single collection of neurons containing serotonin in the brain (Steinbusch, 1981; Felten and Sladek, 1983; Tork, 1985; Azmitia and Gannon, 1986; Tork and Hornung, 1990; Jacobs and Azmitia, 1992). The dorsal raphe and the adjacent median raphe nucleus provide major serotonergic innervation to the cerebral cortex, the basal ganglia, the hypothalamus, and thalamus (Koler and Steinbusch, 1982; Steinbusch and Nieuwenhuys, 1983; Jacobs and Azmitia, 1992; Kitzman and Bishop, 1994; Gonzalo et al., 1995). Therefore, elucidation of the factors that regulate serotonin neurotransmission, including regulation of the many different serotonin receptors, is critically important for understanding functional aspects of the central nervous system (CNS).

The hormones estradiol 17-β (E) and progesterone (P) are steroids produced by the ovary and the placenta. E and P bind to protein receptors (ER and PR, respectively) located largely in the cell nucleus (Greene et al. 1984, 1988; Press and Greene, 1988). These receptors function as transcription factors and interact with DNA to modulate gene expression (Tsai and O'Malley, 1994). The dorsal raphe contains ER and PR, as do the hypothalamus and limbic areas. Thus, the potential exists for ovarian steroid hormone regulation of gene expression related to serotonin and serotonin receptors.

## Nuclear ER and PR Receptors in Serotonin Neurons, Afferent Neurons, and Terminal Fields

### *ER and PR in Serotonin Neurons*

The presence of nuclear ER or PR defines the cell as a target for the cognate hormones, E and P. ER and PR are nuclear transcription factors, and as such, they interact with steroid response elements on genes to promote or inhibit gene transcription. The location of ER or PR in the CNS has been mapped in chicken (Sterling et al., 1987), mouse (Shughrue et al., 1992), rat (Romano et al., 1989; Fox et al., 1990; Simerly et al., 1990), guinea pig (DonCarlos et al., 1989; Warembourg et al., 1989; Brown et al., 1990), cat (Bayliss et al., 1991), and primate (Pfaff et al., 1976; Bethea et al., 1992) with receptor autoradiography, immunocytochemistry, and/or with *in situ* hybridization (ISH).

We demonstrated (Fig. 1) with double immunocytochemistry for PR and the trans-

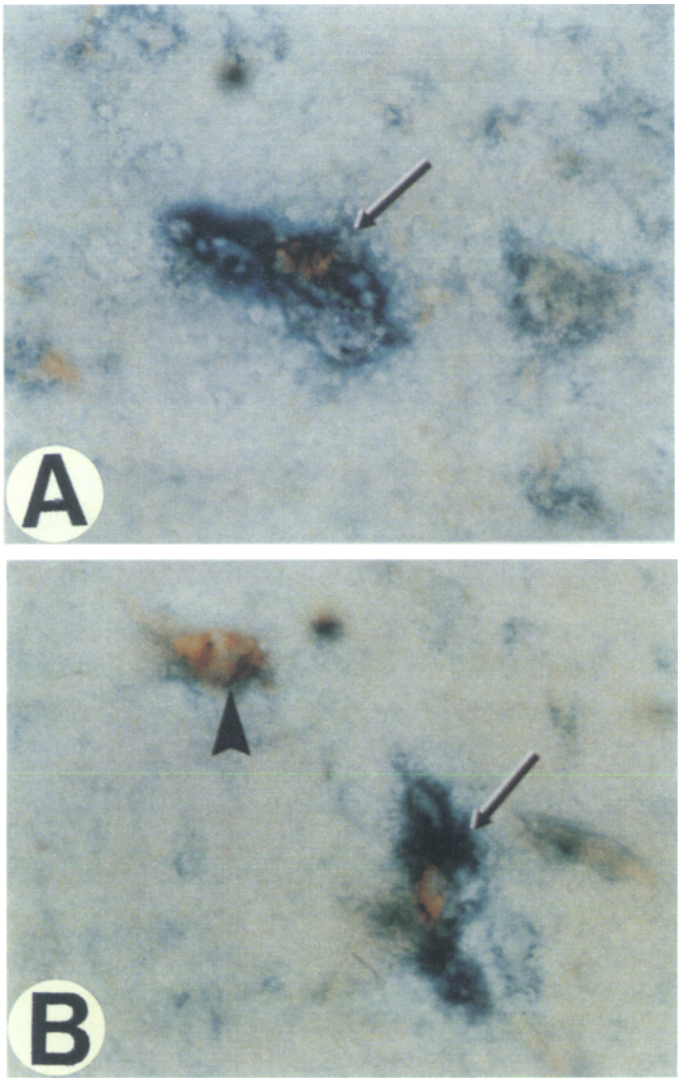


Fig. 1. Photomicrographs of neurons in the dorsal raphe of the rhesus monkey, which were double immunostained for PR and serotonin. The cytoplasm, containing 5HT, is developed with an avidin–biotin–alkaline phosphatase bridge and substrate III from Vector Laboratories that yields a dark blue reaction product. The nucleus, containing PR, is developed with an avidin – biotin horseradish peroxidase bridge and diaminobenzadine that yields a brown reaction product. Panel **A** contains a serotonin neuron with a PR-positive nucleus (black and white arrow). Adjacent neurons are unidentified. Panel **B** also contains a serotonin neuron with a PR-positive nucleus (black and white arrow) and an adjacent neuron in which the nucleus is labeled for PR, but the cytoplasm does not contain 5HT (Reprinted from Bethea, C. L. [1993] *Neuroendocrinology* **57:** 1–6.

mitter, 5HT, that nuclear PR is found within dorsal and median raphe serotonin neurons of the nonhuman primate (Bethea, 1993). However, the steroid receptors were not detected within serotonin neurons of rat dorsal raphe with double immunocytochemistry for ER or PR, and the rate-limiting enzyme in serotonin synthesis, tryptophan hydroxylase (TPH) (Alves et al., 1996). Rather, ER and PR were present in neurons adjacent to the TPH-con-

taining neurons. This suggests that there may be a significant difference in the mechanism of action of ovarian steroids on serotonin neural function in rats and primates. Nonetheless, E treatment induces PR expression in the dorsal raphe of both rats and primates, and PR is not detected in the absence of E (Bethea, 1994). ER has not been sought in serotonin neurons of macaques, but the marked induction of PR by E treatment strongly suggests that ER is present within primate serotonin neurons. A different form of ER, called ER-β, has been recently described in addition to the classical ER, now known as ERα (Kuiper et al., 1996; Tremblay et al., 1997; Couse et al., 1997). ERβ mRNA has been localized in the dorsal raphe of both the rat (Shughrue et al., 1997a) and the ERα knockout mouse with *in situ* hybridization (Shughrue et al., 1997b). ERβ induces PR in the brain (Shughrue et al., 1997c) and the possibility exists that serotonin neurons may express ERα or ERβ, or both.

The observations that E induces PR in unidentified neurons in the rat dorsal raphe (Alves et al., 1996) and that E induces PR in serotonin neurons in the monkey dorsal raphe are consistent with the biological action of E in many other tissues (Katzenellenbogen, 1996). In addition, we observed in monkeys that supplementation of the E treatment with P had no effect on PR expression in the dorsal raphe (Bethea, 1994). Although this differs from certain cell types of the uterus (Clarke, 1990), it means that PR is present and able to transduce the action of P as long as adequate levels of E are maintained. In summary, the serotonin neurons of primate contain nuclear receptors for ovarian steroids and, thus, are direct targets of ovarian steroids that act to modify gene expression. A steroid-responsive interneuron may be involved in the rat dorsal raphe.

## Afferent Innervation

The serotonin system receives afferent innervation from neurons in various brain regions. Of interest is whether any of these are derived from hormone-responsive neurons that contain ER or PR. Afferent input to the serotonergic system can be divided into projections to the three major cell body regions: the dorsal raphe nucleus, the median raphe nucleus, and the medullary nuclei. The majority of incoming axons originate in the cortex, subcortical regions, and within the brainstem. The dorsal raphe contains predominantly serotonin cells and GABAergic interneurons, as well as some enkephalin and neurotensin neurons. Therefore, afferent information may be transmitted directly to the 5HT neurons or indirectly via GABA or peptidergic interneurons.

## Afferents to Dorsal Raphe

Significant projections from the brainstem to the dorsal raphe originate in the nucleus tractus solitarius (visceral sensory information), the locus ceruleus (homeostatic control in stress, sensory input), the dorsomedial medulla (vasopressor control), the lateral parabrachial nucleus (autonomic cardiovascular information), and the raphe magnus (nociception). The nucleus tractus solitarius in rat contains ERα mRNA (Simerly et al., 1990) and ERβ mRNA (Shughrue et al., 1997a). Therefore, E may modify the visceral sensory information that the nucleus tractus solitarius provides to the dorsal raphe. We were unable to detect ERα or PR in the primate locus ceruleus (Schutzer and Bethea, 1997). However, in rat locus ceruleus, 30–40% of cells positive for dopamine-βhydroxylase, the enzyme responsible for conversion of dopamine to norepinephrine, concentrated $^{3}$[H]-E (Sar and Stumpf, 1981). Also in rats, the locus ceruleus contained a low density of cells positive for ERα mRNA, but none were positive for PR mRNA with *in situ* hybridization (Simerly et al., 1990; Simerly, 1993). A small amount of ERβ was detected in the rat locus ceruleus, but this has not been examined in primate (Shughrue et al., 1997a). Thus, in rodents E and P may affect the noradrenergic transmission from the locus ceruleus to the dorsal raphe, but this may not operate in

primates in the same manner. Small amounts of ERα mRNA (Simerly, 1990) and ERβ mRNA (Shughrue et al., 1997a) were detected in the lateral parabrachial nucleus of rat, suggesting that E could modulate the autonomic cardiovascular information directed to the dorsal raphe. ERβ has been recently detected in the raphe magnus (Shughrue et al., 1997a), but there have been no reports of steroid hormone receptors in the medulla.

The GABA efferents from the lateral habenula of the thalamus form a significant afferent projection to the dorsal raphe (Wang and Aghajanian, 1997; Descarries et al., 1982). This area is involved in the hormonal onset of maternal behavior and exhibits an increase in c-fos expression following sexual stimulation (Pfaus et al., 1993; Matthews-Felton et al., 1995). The lateral habenula in the rat contains a dense population of neurons that are positive for ERα mRNA (Simerly et al., 1990) and minor levels of ERβ (Shughrue et al., 1997a). Therefore, there is potential for E modulation of this important link between the forebrain and the raphe.

Minor hypothalamic projections to the dorsal raphe also exist, but in general, there is a paucity of direct projections to the dorsal raphe from hypothalamic regions that contain a high density of ovarian steroid receptors. The dorsal raphe receives input from the suprachiasmatic nucleus, which in the rat contains ERα and ERβ mRNA (Simerly et al., 1990; Shughrue et al., 1997a). This nucleus plays a pivotal role in the regulation of circadian rhythms in rodents. However, neither ERα nor PR mRNA was detected in primate suprachiasmatic nucleus, which is more diffuse than in rat (Bethea et al., 1996). The preoptic area, periventricular area of the paraventricular nucleus, and the lateral and dorsal hypothalamus appear to communicate with the dorsal raphe through the periaqueductal gray (Osborne and Hamon, 1988). There is a high density of ERα and PR in the preoptic area, in the periventricular area of the paraventricular nucleus, and in the ventromedial nucleus extending laterally into the ventrolateral hypothalamus in guinea pigs (DonCarlos et al., 1989). Approximately 26–36% of E-concentrating neurons in the ventrolateral-ventromedial hypothalamus send axons to the periaqueductal gray as determined with a combined retrograde tracer and [$^3$H]-E autoradiography. However, in the nearby arcuate nucleus, only 1% of the E-concentrating neurons project to the periaqueductal gray (Morrell et al., 1984). In addition, the rat periaqueductal gray contains a moderate concentration of ERα and ERβ mRNA (Simerly et al., 1990; Shughrue et al., 1997a). Thus, E and P may act via their nuclear receptors in hypothalamic areas or in the periaqueductal gray *per se* and modify periaqueductal gray transmission to the dorsal raphe; however, the functional relevance of this pathway remains to be determined. Studies in which the steroid receptors are colocalized with a retrograde label from the dorsal raphe serotonergic neurons would be helpful in clearly defining steroid-sensitive afferent input to the serotonin system.

### *Afferents to Median Raphe*

The serotonin neurons of the primate median raphe nucleus appear to contain E-inducible PR (Bethea, 1994). ERβ mRNA, but not ERα mRNA, was detected in the rat median raphe (Simerly et al., 1996; Shugrue et al., 1997a). The median raphe also receives afferent input from the telencephalon, diencephalon, and brainstem, some of which may be responsive to E and/or P (Behzadi et al., 1990). The few projections from the lateral and dorsal hypothalamus and the hypothalamic preoptic area, all of which contain dense populations of ERα, ERβ, and PR, are potential candidates for mediating steroid-sensitive information to the median raphe. The lateral habenula of the thalamus contains a dense population of ERα mRNA-positive cells in the rat, and it projects to both the medial and dorsal raphe. The median raphe also receives input from the medial prefrontal cortex, thought to play a role in emotion, novelty-

seeking, and addiction (Tulving et al., 1994; Flores et al., 1996; Reiman et al., 1997). Although this area is not known to express ERα or PR in adults (Simerly, 1993), it was recently shown to contain significant amounts of ERβ mRNA (Shughrue et al., 1997a).

### *Afferents to Medullary Nuclei*

The serotonergic cells of the caudal pons and medulla can be largely grouped into the raphe magnus and the raphe obscurus. The 5HT cells in these nuclei project to the spinal cord and are involved in a number of autonomic functions. The raphe magnus has received the greatest attention, because it is heavily involved in pain perception, but it should be noted that 5HT neurons are a small percentage of the total population of the raphe magnus (Mason and Leung, 1996; Skinner et al., 1997; Mason, 1997). Stimulation of this area produces analgesia (Vasko et al., 1984). Estrogen appears to lower pain thresholds and heighten sensitivity to pain (Martinez-Gomez et al., 1994; Gordon and Soliman, 1996), whereas the analgesia of pregnancy depends largely on progestins (Medina et al., 1993). ERβ mRNA was detected in the raphe magnus, but neither ERα nor PR has been detected in the medullary nuclei. Thus, E may have a direct action on neurons involved in pain via ERβ. It is also reasonable to ask whether E or P act on neurons that project to the raphe magnus. In the rodent, there is a significant innervation of the raphe magnus from the periaqueductal gray, which contains ERα and ERβ mRNA-positive cells. In addition, the raphe magnus receives serotonergic innervation from the dorsal raphe (Beitz, 1982, 1990). The 5HT cells in the dorsal raphe contain PR in the primate (Bethea, 1994), whereas smaller interneurons of the dorsal raphe contain ERα and PR in the rat (Alves et al., 1996). Moreover, a dense amount of ERβ was detected in the rat dorsal raphe (Shughrue et al., 1997a). Therefore, there is significant potential for E and P to modulate information going to the raphe magnus, thereby impacting the role of serotonin in pain or analgesia.

### *Serotonergic Terminal Fields*

E and P nuclear receptors are densely localized in several areas of the brain that receive serotonergic innervation. If a neuron contains ER and/or PR and is also innervated by serotonin neurons, then E and/or P may affect gene expression for serotonin receptors or coupling proteins within the target neuron. There are seven major types of 5HT receptors, each of which exhibit subtypes or isoforms (Uphouse, 1997). Thus, ovarian steroid hormones could alter the sensitivity or responsivity of target neurons in serotonin terminal fields.

Although information is limited on the serotonergic innervation of ER- and PR- containing neurons, a few pivotal populations have been identified with double-electron immunocytochemistry. In E-treated guinea pigs, symmetric serotonin synaptic contacts were detected on PR-positive neurons in the lateral aspects of the medial basal hypothalamus, but not in the preoptic area (Brown et al., 1990). In the green monkey, boutons immunoreactive for serotonin were also detected on hypothalamic neurons containing PR (Leranth et al., 1992). The majority of the PR-positive neurons in both of these studies were of a GABAergic phenotype. Together these observations indicate that serotonin neurons innervate hypothalamic GABA neurons, which contain PR. GABA neurons are generally inhibitory, so P could act to alter serotonin receptor expression in the GABA neurons, thereby modulating the response of this inhibitory system to serotonin. Double *in situ* hybridization studies to determine the subtype of serotonin receptor expressed by GABA neurons are now feasible and anticipated. This information will reveal whether serotonin inhibits or stimulates GABA neurons in specific brain regions.

In a more general manner, it is informative to compare maps of steroid receptors with the projection areas of serotonin neurons. The ascending serotonin neurons from the dorsal and median raphe travel through the median forebrain bundle in the diencephalon and project to nearly every area of the brain. Informa-

tion on serotonin terminal fields can be synthesized with knowledge of steroid receptor localization in the rat brain from several excellent reviews (Simerly et al., 1990; Simerly, 1993; Halliday et al., 1995; Shughrue et al., 1997a).

There is a prominent 5HT innervation to most of the cytoarchitectural areas of the cortex from both the dorsal raphe and the median raphe in a topographical manner. The adult rat isocortex does not express mRNA for ERα, but there is a significant level of ERβ in cortex (Shughrue et al., 1997a). Extrapolating from this observation, it is possible that E could alter serotonin receptor expression in the rat cortex through a genomic mechanism employing ERβ. The olfactory bulb receives a significant serotonin projection and expresses a minor amount of ERα mRNA in the mitral layer, accessory olfactory bulb, the anterior olfactory nucleus, the islands of Calleja, and in layer III of the piriform cortex (Simerly et al., 1990). However, dense populations of cells expressing ERβ were found in the anterior olfactory nucleus and the islands of Calleja (Shughrue et al., 1997a), indicating this may be one mechanism by which E alters serotonin receptors in the rodent olfactory system.

The hippocampus plays an important role in learning and memory, and evidence has accumulated that clearly indicates that ovarian steroids regulate the number of synapses in the rat hippocampal CA1 region (Desmond and Levy, 1997). The hippocampus is heavily innervated with contributions from both the dorsal and median raphe nuclei, with CA1 and CA2 regions receiving the densest innervation (Halliday et al., 1995). The entorhinal area, the presubiculum, and the pyramidal layer cells of the subiculum (particularly CA1 and CA2) express a minor amount of ERα mRNA (Simerly et al., 1990). However, ERβ is densely expressed in CA1–CA3 and the subiculum, further suggesting that E may alter gene expression of serotonin receptors in the hippocampus through this novel form of ER. Moderate PR mRNA labeling is found over the pyramidal cells in region CA3 (Simerly, 1993). However, the integrated data suggest that the area where E is most likely to regulate serotonin receptors is CA1. Curiously, the androgen receptor (AR) is densely expressed in every layer of the hippocampal formation in the female rat (Simerly et al., 1990).

Although the function of the septal area is poorly understood, lesions of the septal nuclei result in a syndrome of hyperreactivity, amnesia, and hypersexuality (Cavazos et al., 1997). The septal area is also heavily innervated by serotonin axons (Halliday et al., 1995), and ERα mRNA is densely expressed in the ventral division (Simerly et al., 1990), whereas ERβ is more prevalent in the medial division (Shughrue et al., 1997a). ERα and ERβ mRNA expression is prominent in several subdivisions of the bed nuclei of the stria terminalis, particularly in the posterior division, which receives axons from the dorsal raphe (Halliday et al., 1995; Shughrue et al., 1997a). The bed nucleus of the stria terminalis is consistently activated by copulatory behavior in male rats, and it contains a high concentration of aromatase-containing neurons (Bialy and Kazzmarek, 1996). This nucleus is also a major target for the central effects of oxytocin (Wakerley et al., 1995). Therefore, the potential for E to alter serotonin receptor expression and, thereby, serotonin sensitivity in this critical area is significant.

The subfornical organ which plays a role in fluid homeostasis (Bourque et al., 1994; McKinley et al., 1996; Nomura et al., 1997), contains a dense concentration of ERα mRNA (Simerly et al., 1990), but not ERβ mRNA (Shughrue et al., 1997a), and it as well as other circumventricular organs, are densely innervated by 5HT fibers. (Halliday et al., 1995).

The dorsal raphe has a heavy projection to the midline and intralaminar thalamic nuclei (Halliday et al., 1995), which plays a role in the regulation of the state of arousal (Pare and Llinas, 1995; Steriade, 1996). In addition, these thalamic nuclei have been implicated in the regulation of body weight and temperature (Travis et al., 1995; Purvis and Duncan, 1997), and they also contain moderate levels of ERα and ERβ mRNA (Simerly et al., 1990; Shughrue

et al., 1997a). Serotonin cells in the medial lemniscus also project to the midline thalamic nuclear group (Holliday et al., 1995), which expresses a significant amount of ERα mRNA (Simerly et al., 1996). Finally, the median raphe projects to the lateral habenula (Halliday et al., 1995), which contains a very dense population of ERα mRNA-positive cells (Simerly et al., 1990). Therefore, the potential for E regulation of serotonin receptor expression or coupling mechanisms in these thalamic areas is great. In contrast, the basal ganglia, which are involved in higher-order motor control (Houk, 1997) receive a dense 5HT innervation from the dorsal raphe, but contain very little ERα mRNA (Simerly et al., 1996). Many of the 5HT neurons in this projection also send collaterals to the substantia nigra, also involved in motor control (Kawaguchi, 1997), which is completely devoid of ERα mRNA (Simerly et al., 1990). Nonetheless, the pars compacta zone of the substantia nigra (motor control) exhibits a minor amount of ERβ mRNA (Shughrue et al., 1997a).

The hypothalamus, a complex region controlling neuroendocrine and autonomic functions, contains dense populations of ERα, ERβ, and PR-positive neurons, and receives heavy projections from the midbrain dorsal and median raphe, which deserve closer inspection. The suprachiasmatic nucleus, origin of endogenous circadian rhythms, receives one of the densest 5HT innervations in the forebrain and contains ERα mRNA in the rat (Simerly et al., 1990), but neither ERα nor PR protein or mRNA is present in the suprachiasmatic nucleus of rhesus monkey (Bethea et al., 1996). In ovariectomized rats, E alters the diurnal rhythm of 5HT turnover in the suprachiasmatic nucleus. However, the effect of E on 5HT turnover in suprachiasmatic nucleus is difficult to attribute to the ER in this region, since the 5HT is derived from the raphe nuclei. The lateral part of the medial preoptic area is also heavily innervated by 5HT axons, which have a high probability of contacting ERα, ERβ, and PR-expressing cells in this densely steroid receptor-positive area. The ventromedial nucleus receives a serotonergic innervation, which influences lordosis behavior in rats and contains a pivotal population of ERα and PR-positive neurons. The interaction of ERα, ERβ, and PR with 5HT in the ventromedial nucleus needs further clarification, but recent experiments have elicited lordosis behavior with an intracerebroventricular injection of serotonin in PR knockout mice (Mani etal., 1996). In contrast, the mamillary nuclei are nearly devoid of ERα and ERβ mRNA, but receive a dense serotonergic innervation as well. In summary, it is reasonable to expect that E and/or P modifies 5HT receptor expression in certain hypothalamic nuclei/5HT terminal fields. Experiments in this lab are currently under way to determine the effect of E and P on postsynaptic serotonin 1A, 2A, and 2C receptor mRNA in the hypothalamus of nonhuman primates and to characterize the phenotype of the neurons that express these receptors.

The amygdala, an area thought to regulate emotional responses and aggression, receives a significant serotonergic innervation predominantly to its central, lateral, and basolateral nuclei from the dorsal and median raphe nuclei (Halliday et al., 1995). Several of the amygdalar nuclei contain the densest populations of ERα outside of the hypothalamus, but of the nuclei that receive heavy serotonergic innervation, only the central nucleus contains a high density of ERα mRNA-positive cells (Simerly et al., 1990). Alternatively, the rest of the amygdaloid complex, including the amygdalohippocampal nucleus, the cortical nuclei, and the medial nuclei, contain extremely high levels of ERβ (Shughrue et al., 1997a). Thus, there are two intracellular signaling pathways for E that could alter serotonin receptor levels in this pivotal limbic area.

## Effect of E and P on Serotonin Neurons

### *Serotonin Synthesis and Serotonin Levels*

Female rats have an overall higher level of 5HT in the CNS than males (Renner et al., 1985;

Carlsson and Carlsson, 1988). A review of older data on ovarian steroid action in rats suggested that the treatment of ovariectomized rats with E alone, E + P, or even P alone increases serotonin neuronal function as deduced from measurement of 5HT content in either the dorsal raphe or in a number of terminal fields, particularly the hypothalamus (Wirz-Justice et al., 1994). In the dorsal raphe, E has been observed to increase the level of 5HT (Ladisich, 1974; Cone et al., 1981; Di Paolo et al., 1983) and 5-hydroxyindole acetic acid (5HIAA), the major metabolite of 5HT, usually cited as an indication of overall 5HT turnover (Renner et al., 1986). Several authors report an increase in 5HT levels, 5HT uptake, or 5HIAA after E or E + P in various hypothalamic nuclei (Wirz-Justice et al., 1974; Johnson and Crowley, 1983; King et al., 1986; Renner and Luine, 1986; Morissette et al., 1990). In contrast, other studies report decreases in 5HT or turnover in discrete hypothalamic nuclei with E or E+P treatment, particularly in the suprachiasmatic nucleus and ventromedial nucleus, associated with lordosis (Renner et al., 1987; Cohen and Wisch, 1988; James et al., 1989; Bitar et al., 1991; Gereau et al., 1993). These differences are difficult to reconcile. We found that in ovariectomized guinea pigs treated with E for 28 d or with E supplemented with P for the last 14 of 28 d, there was a significant increase in the serotonin content of the dorsal raphe (Bethea et al., 1995) and in the hypothalamus only with E + P treatment (Fig. 2). However, this chronic hormone treatment differs significantly from the more acute hormone treatments required for manifestation of female sexual behavior.

Part of the inconsistencies between studies may be owing to the interpretation of the meaning of changes in the levels of 5HIAA. The level of 5HIAA can be interpreted in several, nondefinitive ways. Also, turnover or metabolism, usually expressed as the ratio of 5HIAA/5HT, can also be confounding. Decreases in 5HIAA/5HT are frequently interpreted as a decrease in turnover. Therefore, a decrease in serotonergic activity, but mathematically a decrease in 5HIAA/5HT is the same as an increase in the inverse ratio, 5HT/5HIAA. This would indicate a greater relative level of transmitter to metabolite and, thus, more potential serotonergic action. A

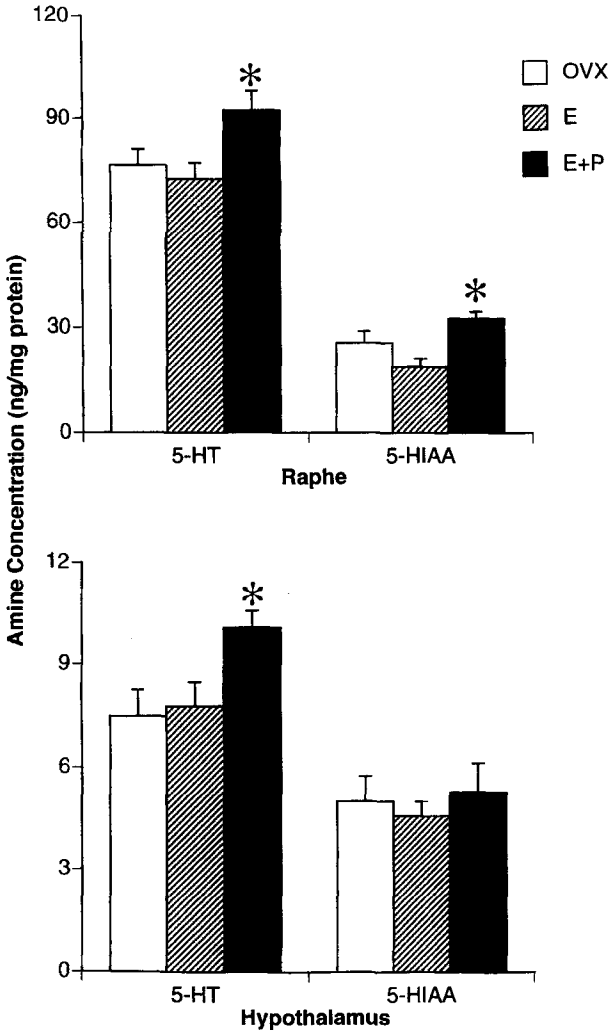


Fig. 2. The serotonin (5HT) and 5HIAA content of the dorsal raphe region and the medial basal hypothalamus in steroid treated guinea pigs. As shown on the ordinates, there is a much higher concentration of 5HT and 5HIAA in the dorsal raphe than in the hypothalamus. There was a significant increase in 5HT content with E + P treatment in the dorsal raphe (cell bodies) and hypothalamic terminal field ($n$=5 animals group, significantly different from ovx control at $p < 0.05$, ANOVA, and Mann-Whitney posthoc comparison). Bottom panel—previously unpublished; top panel—redrawn from Bethea et al. (1995).

decrease in 5HIAA/5HT has also been called a decrease in metabolism. Unfortunately, many studies only measure 5HIAA and then attempt interpretations, which are not informative without knowledge of 5HT levels. In summary, changes in 5HIAA or the ratio of 5HIAA to 5HT cannot be interpreted unambiguously.

Pharmacological manipulations of 5HT with steroid treatment can clarify the effect of E and P on the 5HT system. For example, pargyline blocks monoamine oxidase (MAO) activity, which allows 5HT to accumulate. A greater accumulation of 5HT after pargyline occurred with P treatment in E-primed vs ovariectomized rats, indicating that P caused an increase in serotonin synthesis (Walker and Wilson, 1983). In addition, P partially restored hypothalamic levels of 5HT in rats treated with the synthesis inhibitor, parachlorophenolalanine (pCPA), and administration of the P synthesis inhibitor, WIN 32729, apparently reduced 5HT utilization as indicated by a decrease in 5HIAA (Walker and Wilson, 1983). The concept that P amplifies serotonergic signals regulating the luteinizing hormone (LH) surge (Hery et al., 1976) is supported by the observation that lesions of serotonergic cells with 5, 7- dihydroxytryptamine (DHT) completely disrupt the estrous cyclicity (Meyer et al., 1983).

Studies in which 5HT levels are measured in discrete brain areas with microdialysis are more straightforward to interpret. In ewes, E treatment increased 5HT levels in microdialysate samples of the mediobasal hypothalamus (Fabre-Nys et al., 1994). In microdialysate samples from the nucleus accumbens, the ratio of 5HIAA/5HT decreased at proestrous and with E treatment of ovariectomized rats (Shimizu and Bray, 1993). The authors interpreted this as a steroid-induced decrease in 5HT metabolism (therefore, breakdown). Thus, the data are consistent with an increase in serotonergic activity in the presence of E. However, in the hypothalamus of rats, baseline 5HT levels were significantly lower in estrous than in male rats, but no significant difference was observed between estrous and diestrous levels (Gundlah et al., 1998). Similarly, injection of E-primed rats with P produced a decrease in extracellular 5HT in the midbrain central gray after 20 min and produced a decrease in 5HT in the ventromedial nucleus after 60 min (Farmer et al., 1996). The rapid effect of P on 5HT levels in the central gray suggests a nongenomic mechanism may be involved.

Further studies in rats using microdialysis also indicate that ovarian steroids may cause a decrease in 5HT release. Changes in extracellular 5HT after maximally blocking reuptake reflect changes in 5HT release. The increase in extracellular levels of 5HT induced by a maximally effective dose of the reuptake inhibitor, paroxetine, was blunted in estrous female rats compared to diestrous females or male rats (Gundlah et al., 1998). This suggests that there is a decrease in 5HT release in the ventromedial nucleus during estrous, a time when ovarian hormones would be in effect. In addition, the fenfluramine-induced increase in 5HT observed in male rats was blunted in females. This gender difference was observed only at a high dose of fenfluramine, further suggesting that males have a larger releasable pool of 5HT in the hypothalamus than females (Gundlah et al., 1998).

In summary, more recent studies in rats with microdialysis probes indicate that ovarian hormones decrease 5HT release in the ventromedial nucleus and that females have a smaller releasable pool of 5HT in the hypothalamus than males. This information is not completely consistent with the earlier studies in which large areas of the brain (rat or guinea pig) were homogenized for serotonin measurement after E and P treatment (Wirz-Justice et al., 1974; Johnson and Crowley 1983; King et al., 1986; Renner and Luine, 1986; Morissette et al., 1990; Bethea et al., 1995). However, the decrease in 5HT observed in the microdialysis studies does temporally coincide with an increase in lordosis and proceptive behavior that occurs in rats following treatment with ovarian steroids. (Glaser et al., 1983; Farmer et al., 1996). In addition, lordosis behavior was facilitated in rats after a 90% depletion of 5HT in the ventrome-

dial nucleus owing to local injection of the neurotoxin, 5, 7-DHT (Frankfurt et al., 1985). Together these studies indicate that the method and time of steroid treatment, the type of measurement applied, the area of the brain examined, the species and the gender can all influence the level of 5HT observed.

The types of studies described above are not possible with human subjects. Therefore, hormone secretion, particularly prolactin secretion, is frequently used as an indication of serotonin neural function in humans (Van de Kar, 1991). Furthermore, to gain insight into the synthetic activity of the serotonin system, tryptophan loading or tryptophan depletion followed by hormone measurement or mood assessment has been used in humans as an indication of serotonin synthesis. Normal healthy women experienced a marked decrease in several parameters of mood following tryptophan depletion, whereas men did not, (Ellenbogen et al., 1996), indicating that the serotonin system is more vulnerable to alterations in precursor availability in women than men. In women infused with tryptophan either before menstruation (late-luteal phase—high E and P) or several days after menstruation (follicular phase—low E and no P), there was a greater increase in prolactin secretion after tryptophan loading in the late-luteal phase (Bancroftt et al., 1991). This further indicates that the ovarian steroids increased the synthetic capacity of the serotonin system. Prolactin release after administration of the serotonin-releasing agent, *d*-fenfluramine, is also used as an indication of serotonin stores (O'Keane and Dinan 1991). The prolactin response to *d*-fenfluramine was maximal at midcycle, corresponding to the peak of follicular estradiol (O'Keane et al., 1991), further indicating that E increases the synthetic capacity of serotonin neurons in humans.

Another common approach to deduce the effect of E and P on serotonergic activity in humans involves measurement of cerebrospinal fluid (CSF) levels of 5HT and 5HIAA with lumbar puncture. This approach is fraught with caveats, including whether the levels of these amines in CSF reflect the levels found in brain tissue and whether a lumbar puncture accurately reflects the level of the amines that might be found in the cerebral ventricles. Serotonin is frequently undetectable in CSF, so the concept of using only 5HIAA levels to indicate serotonin metabolism is again called into question. Nonetheless, some interesting data have emerged. When CSF monoamines were examined across the menstrual cycle of healthy women, there was no marked change between the follicular phase (E-dominated) and luteal phase (P-dominated). However, overall, there was a significant correlation between 5HIAA and homovanillic acid (HVA), metabolites of 5HT and dopamine (DA), respectively, which are generated by MAO. In addition, there was a significant negative correlation between the levels of ovarian steroids and the ratio of HVA/5HIAA (Eriksson et al., 1994). That is, the higher the level of ovarian steroids, the lower the level of HVA/5HIAA. This could be interpreted in several ways. One possibility is that ovarian steroids increase the turnover of 5HT, thereby increasing 5HIAA. We recently measured CSF levels of the biogenic amines in ovariectomized/hysterectomized monkeys treated with E (28 d) or E + P (14 d E + 14 d E + P) employing puncture of the cisterna magna. Although there was little change in the concentration of 5HT, there was a significant decrease in the concentration of 5HIAA with E + P treatment producing a marked decrease in the 5HIAA/5HT ratio (Schutzer et al., 1997). These data further indicate that P may decrease the degradation of 5HT, but the results in monkeys are not consistent with the human studies described above. We also found that in monkeys, there was a decline in the HVA/DA ratio, further suggesting that in E-primed animals, P may downregulate MAO (Fig. 3).

Another recent approach to the measurement of 5HT function in humans is with in vivo PET scan studies of 5HT synthesis. Lower 5HT synthesis was reported in human females (Nishizawa et al., 1997) which is consistent

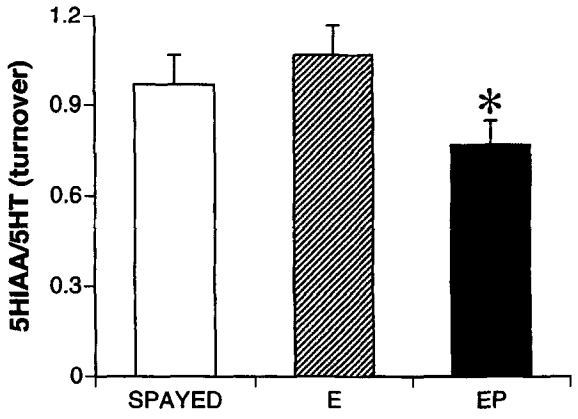


Fig. 3. The ratio of 5HIAA to 5HT in the CSF of pigtail macaques, which were ovariectomized and hysterectomized (spayed) or treated with E for 28 d (E) or treated with E for 28 d with addition of P for the last 14 of the 28-d period. The monkeys were anesthetized with Ketamine, and a sample of CSF was obtained immediately from the cisterna magna using a 6-in. spinal needle. There was a significant reduction in the ratio of 5HIAA/5HT in the animals treated with E + P ($*p < 0.05$, ANOVA with Student Newman Keuls posthoc comparison). This could be interpreted as a relative increase in the amount of transmitter to metabolite, and it further suggests that P may decrease the degradative activity of MAO (Schutzer et al., 1997).

with the results from typtophan depletion studies. However, another group obtained the opposite result employing the same methods (Chuganl et al., 1998). Future studies may be in order in which variables, such as the phase of the menstrual cycle, are carefully controlled.

Serotonin levels in plasma are higher in the follicular phase of cycling women than in postmenopausal women (Blum et al., 1996), and E-replacement therapy (ERT) in postmenopausal women caused a modest increase in plasma 5HT. However, a review of various dependent variables across the menstrual cycle suggested that plasma 5HT was lowest at the time of highest E during the periovulatory phase (Leibenluft et al., 1994). E treatment, orally or transdermally, increased urinary excretion of 5HIAA, and this was interpreted as an enhancement of serotonin turnover (Lippert et al., 1996). It follows that if E increases plasma 5HT (Blum et al., 1996) then peripheral oxidation (by MAO) would lead to increased excretion of 5HIAA.

In summary, the most recent data from rats, primates, and humans suggest that there may be a species difference in the effect of ovarian steroids on serotonin neural function. E causes a decrease in hypothalamic serotonin in rats, but data from nonhuman primates suggest that E + P increases 5HT. Limited experimental information from humans is consistent with a stimulatory effect of E on serotonin synthesis.

## *Tryptophan Hydroxylase*

TPH is the rate-limiting enzyme in the overall synthesis of 5HT, and it converts the amino acid, tryptophan, to 5-hydroxytryptophan (5HTP), which is then converted to 5HT. Therefore, the level of activity of this pivotal enzyme probably plays a role in the level of 5HT achieved in either cell bodies or axon terminals. Information regarding the effect of E and P on TPH expression or activity is meager. In nonhuman primates, the observation that PR colocalize in 5HT neurons and, in addition, that PR in the dorsal raphe is markedly induced by E treatment indicates that primate serotonin neurons contain ER and PR (Bethea, 1994). Since these receptors are nuclear transcription factors, it follows that in the presence of E or P, the steroid receptors will act on gene transcription in some manner. In rats, however, ER and PR do not colocalize in 5HT neurons. Rather, the nuclear receptors appear to be in smaller interneurons of the dorsal raphe (Alves et al., 1996). Therefore, one may not necessarily expect E or P to alter gene transcription via a direct mechanism in the rat serotonergic system.

We questioned whether E and/or E + P increased TPH gene expression in nonhuman primates. The expression of mRNA for TPH was examined in ovariectomized plus hysterectomized controls (spayed), E-treated (28 d) and E + P-treated monkeys (14 d E + 14 d E

+ P) using *in situ* hybridization and a 249-bp TPH cRNA monkey specific probe ($n$ = 5 animals/group). Densitometric analysis of film autoradiographs revealed a ninefold increase in TPH mRNA in E-treated macaques compared to spayed animals ($P < 0.05$). With supplemental P treatment, TPH mRNA signal was increased fivefold over spayed animals ($P < 0.05$), but was not significantly different compared to E-treated animals. These results were verified by grain counts from photographic emulsion-coated slides as illustrated in Fig. 4. There were significantly higher single-cell levels of TPH mRNA in serotonergic neurons of the dorsal raphe in E- and E + P-treated groups ($p < 0.05$). These data indicate that E induces TPH gene expression in nonhuman primates and the addition of P has little additive effect on TPH gene expression (Pecins-Thompson et al., 1996). Recent examination of the regulation of TPH mRNA levels found no effect of E or P replacement in ovariectomized rats (Alves et al., 1997). This is quite different from the regulation observed in primates, but it is consistent with the absence of ER and PR in rat serotonergic neurons.

Our previous observations in guinea pigs indicated that E had little effect on prolactin secretion (similar to primate) and that E did not significantly increase the serotonin content of the dorsal raphe (Bethea et al., 1995) or hypothalamus (Fig. 2). Rather, addition of P to the E regimen increased the serotonin content of the dorsal raphe and hypothalamus, which correlated with an increase in prolactin secretion. Thus, E increased TPH mRNA in monkey, and P had no further effect, but E + P and not E alone increased the level of serotonin, the final transmitter product in the guinea pig model. Previous studies in rat suggested that the translational efficiency of TPH mRNA varied between the pineal gland and the dorsal raphe (Dumas et al., 1989). We questioned whether translational efficiency could be modified by steroid hormones, and examined the levels of TPH protein in the dorsal raphe of ovariectomized, E-treated, and E + P-treated guinea pigs with Western blotting and densitometric

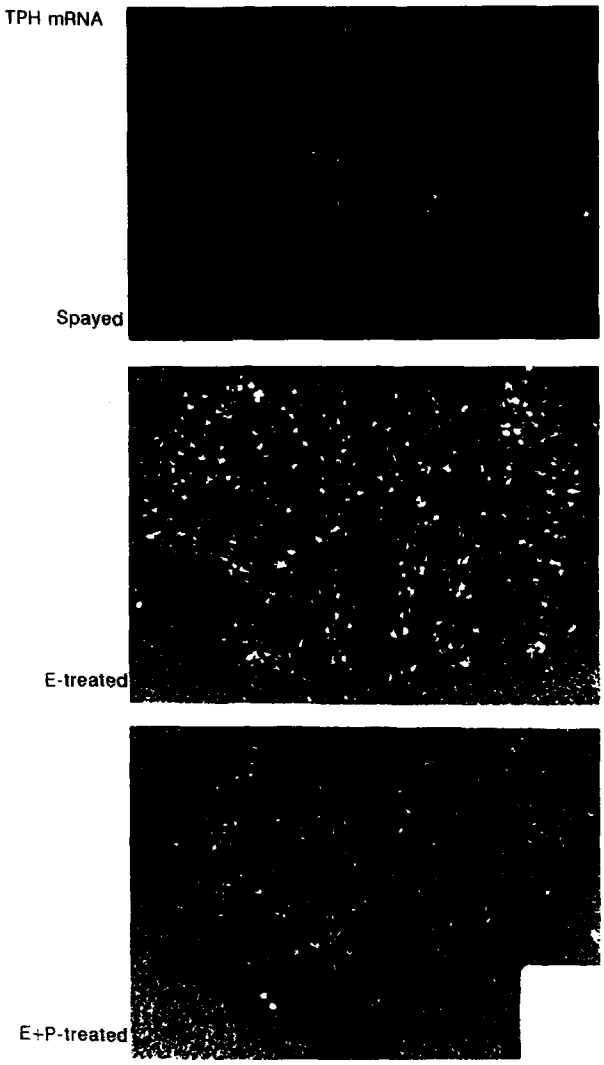


Fig. 4. Dark-field photomicrographs of cells in macaque dorsal raphe, which were labeled for TPH mRNA by ISH followed by emulsion development of silver grains. Representative raphes from a spayed (top), an E-treated (middle) and an E + P-treated monkey (bottom) are shown. Magnification equals 12.5X through the microscope lens. (Reprinted from Pecins-Thompson et al. [1996])

analysis. E and E + P treatment caused a similar and significant increase in the level of TPH protein compared to ovariectomized controls (Fig. 5). Therefore, TPH protein levels in steroid-treated guinea pigs were regulated in the same

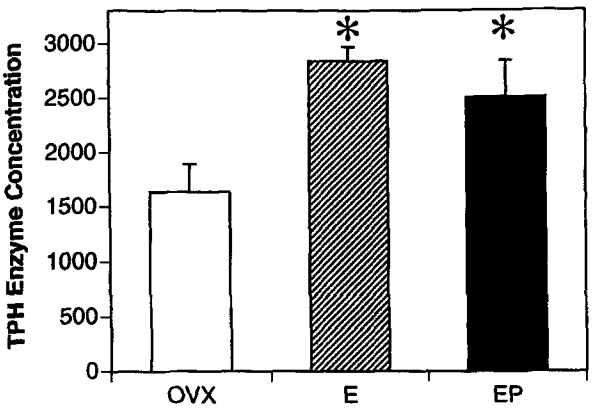


Fig. 5. TPH enzyme concentrations in the dorsal raphe of steroid treated guinea pigs as determined with Western blotting and densitometric analysis of protein bands on chemilumenescent sensitive film. The guinea pigs were ovariectomized controls (OVX), ovx and treated with E for 28 d, or treated with E for 28 d supplemented with P for the last 14 of the 28-d period. Serum E concentrations (pg/mL) equaled 36 ± 2.6 in the controls and 141.8 ± 36.7 in the E-treated groups. Serum P concentrations (ng/mL) equaled 0.07 ± 0.06 in the control and E-treated groups and 4.1 ± 0.4 in the E + P-treated group. There was a significant increase in TPH protein concentration in the dorsal raphe of the E and E + P-treated guinea pigs relative to ovx controls. *Significantly different at $p < 0.05$, ANOVA followed by Student Newman Keuls posthoc comparison.

manner as TPH mRNA levels in primates. Moreover, the E and E + P-induced increase in TPH protein levels occurred in the same animals that only demonstrated an increase in hypothalamic serotonin levels with E + P treatment. In summary, there remains a discrepancy in the steroid regulation of TPH protein levels, TPH mRNA levels, and the final transmitter product in E and E + P-treated guinea pigs or monkeys. This observation, together with the measurement of a lower 5HIAA/5HT ratio in the CSF of E + P-treated monkeys has furthered the hypothesis that P may decrease the degradation of 5HT, possibly by regulation of MAO.

One salient question that arises from this data pertains to the postmenopausal state. Extrapolating from the monkey data, one would speculate that postmenopausal women have a lower level of serotonin synthesis owing to lower levels of TPH mRNA. However, not all postmenopausal women are depressed (Avis et al., 1994; Weissman and Olfson, 1995; Pearl Stein, 1995). In addition, it seems that surgical menopause is more indicative of onset of negative affect (Dennerstein, 1996; Sherwin, 1996) than natural menopause and, furthermore, that women who experience postmenopausal depression are more likely to have experienced depressive episodes prior to menopause (Stewart and Boydell, 1993; Avis et al., 1994; Pearlstein 1995). Based on the observation that TPH mRNA does exhibit variations in translational efficiency in rat dorsal raphe and pineal gland (Dumas et al., 1989), we questioned whether there could be an adjustment of translational efficiency in the dorsal raphe with time after ovariectomy and hysterectomy (spayed). The dorsal raphe was obtained from one monkey each at 2, 6, 22, and 40 min and subjected to Western blot analysis for TPH protein. The same amount of total soluble protein was loaded on the gel, so that the TPH signal could be compared on a relative basis using densitometric analysis. There was a significant increase in TPH protein with time after ovariectomy (Fig. 6). The highest concentration of protein was observed in the monkey that had been spayed for 40 min. Examination of the level of TPH mRNA with time after ovariectomy is needed, but these preliminary data suggest that after the loss of ovarian function, there may be an adjustment in the translational efficiency, so that TPH protein levels recover in many women. This also suggests that this mechanism is a potential point of vulnerability in women who experience depressive episodes both pre- and postmenstrually.

### *Serotonin Reuptake Transporter (SERT)*

The primary means by which serotonergic neurons control extracellular 5HT levels is via the reuptake transporter (SERT), which acts to reduce the concentration of serotonin in the synaptic cleft (Blakely et al., 1994). Dysfunction

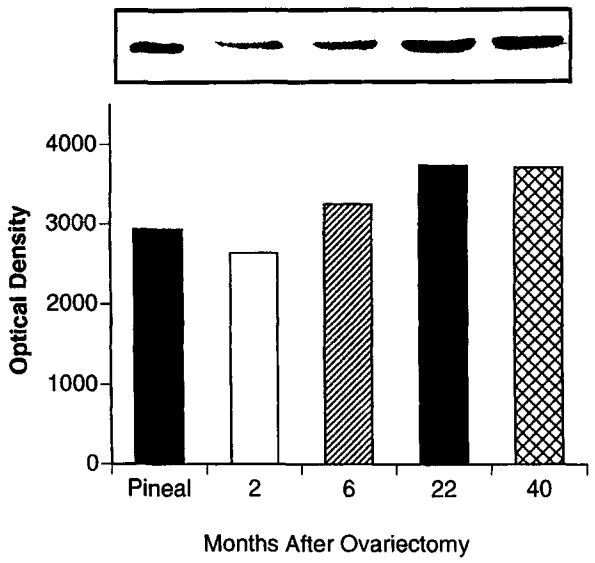


Fig. 6. Western blot of dorsal raphe extracts (300 µg soluble protein) from ovariectomized rhesus monkeys and densitometric analysis of band density. One monkey each at 2-, 6-, 22-, and 40-min postovariectomy were analyzed, which does not enable statistical analysis. Nonetheless, there appears to be a gradual increase in TPH enzyme concentration in the dorsal raphe with time after ovariectomy.

of SERT-mediated uptake of serotonin has been implicated in depression and anxiety disorders (Siever et al., 1991). Moreover, SERT is the site of action of widely used antidepressants known as selective serotonin reuptake inhibitors (Barker and Blakely, 1995). Thus, by altering the expression or activity of SERT, ovarian steroids could alter serotonergic neurotransmission.

Determination of the effect of E and P on SERT has involved studies of the level of specific binding of radiolabeled ligands, examination of the uptake of radiolabeled serotonin into slices of brain, and most recently, measurement of SERT mRNA levels with *in situ* hybridization. The usefulness of data obtained with radiolabeled ligands that bind to SERT is limited by the specificity of the ligand. Those compounds, which also bind to the norepinephrine (NET) or dopamine (DAT) transporters, confound the ability to obtain information specifically about SERT.

This laboratory has examined the effect of E alone and E + P treatment on SERT mRNA levels in nonhuman primates using *in situ* hybridization (Pecins-Thompson et al., 1998a). The expression of mRNA for SERT was examined in ovariectomized plus hysterectomized controls (spayed), E-treated (28 d) and E + P-treated monkeys (14 d E + 14 d E + P) using *in situ* hybridization and a 253-bp human SERT cRNA subclone constructed from the full-length human SERT cDNA ($n = 5$ animals/group). Densitometric analysis of autoradiographs with gray-level thresholding was performed at five levels of the dorsal and median raphe. The number of pixels exceeding background in defined areas was obtained (pixel number). The average pixel number for spayed, E- and E + P-treated groups was 22,280 ± 3517; 15,227 ± 1714, and 14,827 ± 2042, respectively, in the combined dorsal and median raphe. In the E- and E + P-treated groups compared to the control group, there was a 32% and 33% decrease in SERT mRNA signal represented by pixel number (ANOVA, $p < 0.05$). Therefore, the SERT mRNA signal in E- and E+P-treated groups was significantly less than the spayed control group, but there was no difference in the SERT mRNA signal between the E- and E + P-treated groups. Also, there were significantly fewer SERT mRNA-positive cells in the dorsal raphe of E- and E + P-treated groups (ANOVA, $p < 0.001$). Therefore, E, with or without P, reduces SERT mRNA expression (Fig. 7). These data suggest that one action of E replacement therapy in postmenopausal women may be to decrease expression of the SERT gene. If the E-induced decrease in SERT mRNA is manifested by a decrease in SERT protein, then 5HT may remain in the extracellular space for a longer period of time and continue neurotransmission when E is present. Alternative interpretations are also feasible depending on autoreceptor involvement.

These results are consistent with some studies in rats and humans, but not others. Earlier


Fig. 7. Representative dark-field photomicrographs of cells of the dorsal raphe that were labeled for SERT mRNA by *in situ* hybridization followed by emulsion development of silver grains. There was a significant reduction of SERT mRNA signal (number of cells and grains per cell) in the dorsal raphe of monkeys treated with E and E + P (Reprinted from Pecins-Thompson et al. [1998a]).

studies examined the effect of E on imipramine binding sites in the rodent brain and on platelets. Imipramine is thought to bind to the reuptake transporters for serotonin and norepinephrine (Paul et al., 1984). Both [$^3$H]imipramine and [$^3$H]serotonin uptake increased by 20–30% in the frontal cortex and hypothalamus of ovariectomized rats treated with E for 12 d (Rehavi et al., 1987; Attali et al., 1997). In addition, a significant increase in imipramine binding sites on platelets was reported after E treatment in surgically menopausal women that correlated with an improvement in their depression scores (Sherwin and Suranyi-Cadotte, 1990). Furthermore, imipramine binding to platelets was lower in women experiencing late-luteal phase dysporhia (Rojansky et al., 1991). The increase in imipramine binding in E-treated rats and humans is thought to represent an increased density of serotonin reuptake sites, which could act to decrease serotonin in the extracellular space. These observations are difficult to reconcile with an improvement in mood or the E-induced decrease in SERT mRNA observed in primates in this study. In a series of autoradiographic studies on postmortem human brain, gender differences were not discerned with [$^3$H]imipramine binding (Gross-Isseroff and Biegon, 1988; Gross-Isseroff et al., 1988, 1989), nor were gender differences reported in platelet binding between normal men and women (Haibreichet et al., 1991). However, imipramine is not a selective ligand for the serotonin reuptake transporter.

It was recently reported that E increased SERT binding sites in the amygdala, lateral septum, and hypothalamus, and also increased SERT mRNA expression in the dorsal, but not the median raphe nuclei in rats (McQueen et al., 1997). However, the duration of E treatment was vastly different from the monkey paradigm; that is, the rats were ovariectomized, immediately injected with E, and killed the following day. Therefore, the difference in the length of the steroid treatment may account for the different results. Moreover, there was no increase in the SERT mRNA grains per cell, only in the number of cells detected. Also, the hybridization assay was conducted on brain tissue that was not fixed. Thus, there are significant differences between our study in primates and the study with rats (McQueen et al., 1997), both in the paradigm as well as in the technique. In contrast, [$^3$H]paroxetine binding, a selective indicator of serotonin reuptake sites, decreased in the hippocampus of E-treated rats (Mendelson

et al., 1993). These data are consistent with the E-induced decrease in SERT mRNA observed in the primate study and with the beneficial effect of E on mood and cognition.

Serotonin reuptake inhibitors are being used effectively in the treatment of premenstrual or late luteal phase dysphoric disorder (LLPDD) (Sundblad et al., 1993; Eriksson et al., 1995; Steiner et al., 1995; Yonkers et al., 1996a,b; Steiner, 1996). Comparison of the ovarian steroid regulation of SERT mRNA in primates with women who experience late-luteal phase dysphoria indicates a dichotomy. That is, women with LLPDD become symptomatic 5–14 d before menstruation when E and P levels are relatively high. Based on our observations in monkeys, one would expect that the ovarian production of E and P during the luteal phase would increase TPH mRNA and decrease SERT mRNA, thus producing conditions optimal for mood. However, the inverse is true. Women with LLPDD show an improvement in mood when ovarian steroid hormone production is halted pharmacologically or surgically (Rubinow and Schmidt, 1995). These observations have led us to speculate that the regulation of serotonin gene expression by E and P in monkeys more closely resembles that of normal women, and that women with LLPDD exhibit a pathology that differs markedly from normal monkeys.

### 5HT Autoreceptors

The most widely studied of the 5HT autoreceptors is the 5HT1A receptor. The 5HT1A autoreceptor subtype is of significant importance, because it inhibits serotonin neural firing (Sprouse and Aghajonian, 1986), which leads to a suppression of serotonin synthesis, serotonin turnover and the release of serotonin in diverse projection areas (Bohmaker et al., 1993; Singh and Lucki, 1993; Sharp et al., 1993). However, this receptor also occurs postsynaptically. The 5HT1A receptor is particularly hard to study with radiolabeled ligands, because there are currently no ligands that distinguish between the pre- and postsynaptic receptor. However, it is obvious that the mRNA for the autoreceptor will only be located within the serotonin neurons *per se*. Therefore, this laboratory examined the effect of E and P on the mRNA levels for the 5HT1A autoreceptor in the dorsal and median raphe of rhesus macaques (Pecins-Thompson and Bethea, 1998b) using *in situ* hybridization and a 432-bp 5HT1A probe generated with PCR. 5HT1A mRNA levels were examined in monkeys that were ovariectomized-hysterectomized controls (spayed), E-treated (28 d), and E + P-treated (14 d E + 14 d E + P). Densitometric analysis of autoradiographs with gray level thresholding was performed at five levels of the dorsal raphe. The number of pixels exceeding background in defined areas was obtained (pixel number) as well as the mean optical density. In the E- and the E + P-treated groups compared to the control group, there was a 38% and 43% decrease in 5HT1A mRNA signal, respectively, represented by pixel number ($p < 0.05$). Mean optical density for 5HT1A was significantly decreased by E treatment (21%; $p < 0.05$) and then further decreased with the addition of P treatment (45%; $p < 0.01$). Also, the number of positive cells and the grains/cell were counted. There were significantly fewer 5HT1A mRNA-positive cells in the serotonergic neurons of the dorsal raphe in E- and E + P-treated groups than controls ($p < 0.001$). There were significantly lower single-cell levels of 5HT1A mRNA in serotonergic neurons of the dorsal raphe only in the E + P-treated group ($p < 0.05$). These results suggest that E reduces raphe 5HT1A gene expression and that the addition of P further reduces 5HT1A gene expression in nonhuman primates (*see* Fig. 8). Together these actions of E and P could increase serotonin neurotransmission, thereby elevating mood and/or altering neuroendocrine functions.

Another characteristic of compounds with medium to high efficacy for blocking 5HT1A receptors is an antidepressant-like activity when combined with selective serotonin reuptake inhibitors (Singh and Lucki, 1993). Serotonin reuptake inhibitors block serotonin reuptake via SERT. However, the therapeutic efficacy of serotonin reuptake inhibitors is typ-

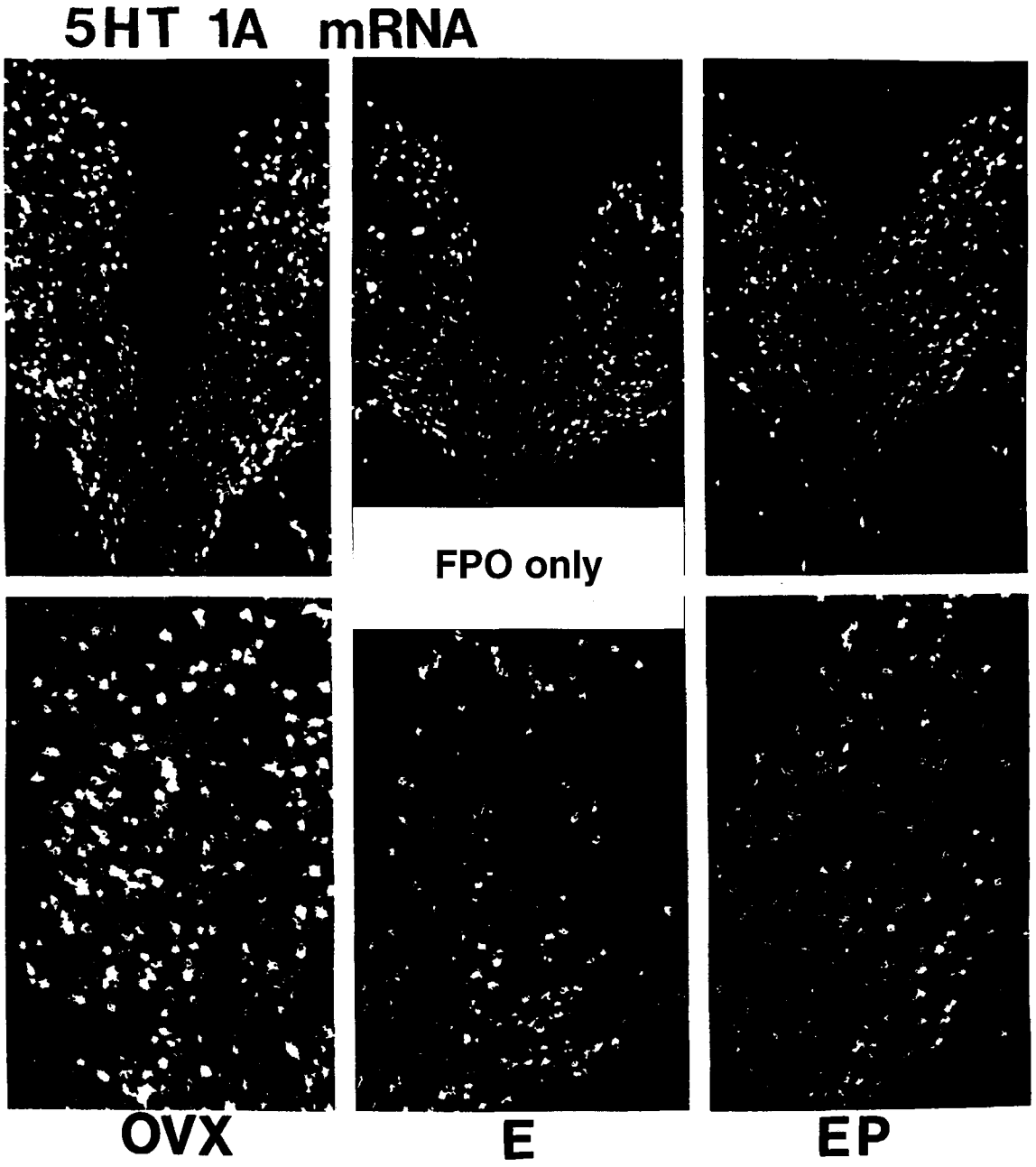


Fig. 8. Dark-field photomicrographs of cells in the dorsal raphe that were labeled for 5HT1A mRNA by *in situ* hybridization followed by emulsion development of silver grains. Representative raphes from an ovx, E-treated, and an E + P-treated monkey are shown. There was a significant decrease in the 5HT1A mRNA signal with E treatment and a further significant decrease in the signal with E + P treatment as determined from densitometric analysis of film autoradiographs as well as cell and grain counting on the emulsion developed sections ($p < 0.05$, ANOVA followed by Student Newman Keuls posthoc pairwise comparison) Magnification: top 12.5×, bottom 25×. (Reprinted from Pecins-Thompson and Bethea, 1998b.)

ically delayed for several weeks. Hypothetically, this delay is owing at least in part to acute increases in serotonin levels in the raphe that inhibit neuronal firing via the 5HT1A autoreceptor (Goodwin, 1996). Therefore, blockade of the autoreceptor may prevent the delay in therapeutic efficacy of the serotonin reuptake inhibitors, increasing their effectiveness. Recently, a 5HT1A antagonist was found to potentiate the antidepressant effects of a serotonin reuptake inhibitor in depressed patients (Artigas et al., 1994). This finding highlights the importance of E and P decreasing expression of the 5HT1A autoreceptor in order to facilitate serotonin neurotransmission.

Previous studies in rats are consistent with the ability of E and E + P to reduce 5HT1A autoreceptor mRNA. It has been shown that E reduces the ability of the 5HT1A agonist 8-hydroxy-2-(*di-n*-propylamino)tetralin (8-OH-DPAT) to decrease firing of dorsal raphe neurons in rats, indirectly indicating that E has reduced the 5HT1A/8-OH-DPAT binding sites (Lakoski, 1988). Another study has shown gender and estrous cycle effects of 8-OH-DPAT on hypothalamic levels of serotonin in rats (Maswood et al., 1995). In general, females had an overall greater decrease than males in the 5HIAA/5HT ratio in response to 8-OH-DPAT. One interpretation of these data could be that more serotonin was released in females. However, as previously discussed, it is difficult to interpret unambiguously the 5HIAA/5HT ratio. Additionally, there is a sex difference in the response of 5HT1 receptors to gonadal steroids (Fischette et al., 1983). Castration in male rats reduced 5HT1 binding in the midbrain, and E treatment reversed this effect. In postmortem human brains, there was a decrease in receptor labeling with [$^3$H]-8-OH-DPAT owing to age, but there was no effect observed with gender (Dillon et al., 1991). However, aged women lack significant levels of circulating E or P. Different results might be obtained if young premenopausal women were compared to men.

In summary, in both rats and primates, the majority of evidence suggests that E downregulates the 5HT1A autoreceptor activity or expression. This follows nicely the evidence that serotonin neurons in primates have ovarian steroid receptors (Bethea, 1993, 1994). However, because of the evidence that serotonin neurons in rats lack ER and PR (Alves et al., 1996), it is more difficult to propose a mechanism for the action of E on the 5HT1A autoreceptors in rats. Recent information suggests that ER-positive and PR raphe neurons in rat may express excitatory amino acids (S. E. Alves, 1998). Thus, one possible mechanism would involve a stimulatory effect of E or P on the excitatory interneurons, which in turn, would stimulate serotonin neurons leading eventually to a downregulation of the 5HT1A autoreceptor.

## Effect of E and P on Postsynaptic Receptors for 5HT

The number of identified postsynaptic receptors for 5HT has multiplied rapidly in recent years. Currently, there are seven major types of 5HT receptors, each of which may have more than one subtype. The effect of E and P on a few of these receptors has been examined with radiolabeled ligand binding, with pharmacological approaches, or with examination of mRNA levels.

The 5HT1 family includes a number of subtypes, 5HT1A, 5HT1B, 5HT1D, 5HT1E, and 5HT1F, each of which may exhibit isoforms (Hoyer and Martin, 1997). These receptors contain seven membrane-spanning domains and inhibit the adenylate cyclase system through G-proteins (Peroutka, 1994). Several studies suggest that antidepressants produce changes in the sensitivity of both 5HT1A autoreceptors and postsynaptic 5HT1A receptors (Biegon and McEwen, 1982; de Montigny et al., 1984; Goodwin et al., 1987; Singh and Lucki, 1993; Goodwin, 1996). The highest concentration of 5HT1A postsynaptic receptor protein or mRNA is found in limbic areas, such as the hippocampus, entorhinal cortex, and amygdala, as well as layers I and II of the cerebral cortex as deter-

mined with radioligand binding studies or *in situ* hybridization (Hoyer et al., 1986; Pazos et al., 1987; Chalmers and Watson, 1991). These areas also contain dense populations of cells that express ER and PR (Simerly et al., 1990; Simerly, 1993). Therefore, it is reasonable to question whether E and P regulate postsynaptic 5HT1A receptor mRNA and protein.

Reports of the action of ovarian steroids on 5HT1A postsynaptic receptors differ depending on the region examined. In one study, treatment with E significantly increased the density of 5HT1 receptors in the preoptic area, anterior hypothalamus, lateral septum, and arcuate-median eminence of ovariectomized rats (Biegon et al., 1982). However, a 14 d treatment with E or P induced a downregulation in 5HT1 receptors (and an increase in 5HT2 receptors) in the female rat cortex (Biegon et al., 1983). P given to E-treated rats had no effect on the E-induced reduction in 5HT1 receptors (Biegon et al., 1983). Moreover, a biphasic effect of E on the density of 5HT1 receptors has been shown in female rats (Biegon and McEwen, 1982). There was an initial acute reduction in serotonin receptor density throughout the brain, which was followed 48 to 72 h later by a selective increase in 5HT1A receptors in ER-containing brain regions. It was recently shown that E increases 5HT1A receptor mRNA in the mediobasal hypothalamus of female rats (Sinclair-Worley et al., 1997). The hippocampus contains little ERα, but dense ERβ (Simerly et al., 1990; Shughrue, 1997a). One laboratory reported that estrogen enhances 5HT1A sensitivity in the hippocampus by an action on postreceptor mechanisms (Clarke and Goldfarb, 1989; Clarke and Maayani, 1990). However, another group reported that there was little effect of long-term treatment with E on the active or passive membrane properties of the CA1 cells (Dijcks et al., 1994).

5HT1A receptors are decreased in a region-specific manner with aging in humans. [$^3$H]8-OH-DPAT labeling was reduced with age in several cortical and hippocampal regions and in the raphe of postmortem human brains (Dillon et al., 1991) suggesting that the decline in gonadal steroids may be accompanied by a decline in 5HT1A receptor expression. Gender had no effect on [$^3$H]8-OH-DPAT binding in aged humans, but circulating levels of ovarian steroids are probably absent in the aged women.

In women with LLPDD, there is a blunting of growth hormone (GH) release in response to L-tryptophan during the luteal phase compared to normal controls. The GH response is thought to be mediated by 5HT1A receptors, further suggesting that 5HT1A receptors may be downregulated in women with LLPDD (Bancroft et al., 1991). This suggestion is supported by a study showing that the prolactin response to challenge with the 5HT1A receptor agonist, buspirone, is significantly blunted during the follicular phase of women with LLPDD (Yatham, 1993). However, the latter data are ambiguous owing to the action of buspirone at dopamine D2 receptors, which inhibit prolactin secretion at the pituitary level. Nonetheless, the feedback regulation of 5HT1A receptor expression by E and P may be dysfunctional in women with LLPDD.

Studies are currently in progress in this laboratory to determine the effect of E and E + P on hypothalamic 5HT1A postsynaptic receptor mRNA in macaques. The phenotype of the neuron(s) expressing 5HT1A in the hypothalamus is also unknown at this time. Additional studies are anticipated in which double *in situ* hybridization will be applied to this question. In summary, the majority of studies in rats suggest that there is an increase in 5HT1A receptor density, binding, and mRNA with E treatment in the hypothalamus. This type of regulation may be missing in women with LLPDD and definitive studies in nonhuman primates are lacking.

The 5HT2 receptor family currently recognizes three subtypes termed 5HT2A, 2B, and 2C. These receptors are single G-protein-linked protein molecules, and they exhibit the classic features of G-protein-coupled receptors, that is, seven-transmembrane domains with an extracellular amino-terminus and an intracellular carboxyl-terminus. On agonist activation,

these receptors stimulate phosphoinositide metabolism, and they are considered Gq-protein-coupled receptors. All of these receptors have been cloned and exhibit similar size (458–471 amino acids) as well as close nucleotide homology. Subtypes have also been distinguished by their kinetics with various pharmacological agents (Hoyer et al., 1994). The 5HT2B receptor is largely located in peripheral tissues and will not be considered further here (Kursar et al., 1992; Loric et al., 1992) 5HT2A receptors are also diffusely distributed throughout the periphery (Bradley et al., 1986).

5HT2A receptors are found in the CNS, and the 5HT2A receptor has been implicated in suicide and depression (Fifa and Fillion, 1992). In the human, 5HT2A receptor mRNA was localized with *in situ* hybridization to cortex and hippocampus, but not in the raphe, cerebellum, substantia nigra, or striatum (Burnet et al., 1995). In rat brain, 5HT2A receptor mRNA was primarily localized to the olfactory bulb, anterior hippocampal rudiment, cortex, and claustrum (Wright et al., 1995). In contrast, radiolabeled ligand autoradiography studies localized 5HT2A receptor protein in the dorsal raphe of rats (Sumner and Fink, 1993). Recently, our lab has utilized *in situ* hybridization to map 5HT2A receptor mRNA in the primate hypothalamus. We have found that 5HT2A receptor mRNA is densely expressed in the periventricular nuclei, supraoptic nucleus, the mamillary bodies, and in the subthalamic capsule, and moderately expressed in the thalamus (Bethea et al., 1998). None of these areas contain dense populations of ERα or PR. However, ERβ has recently been colocalized to the vasopressin and oxytocin neurons of the periventricular nuclei and supraoptic nucleus (Shughrue et al., 1996), raising the possibility that 5HT2A receptors may be regulated by E in these areas via this novel form of the ER.

The 5HT2C receptor is the most prominent subtype in rat brain (Molineaux et al., 1989). The mRNA and protein is found in discrete regions of the rat brain, such as the choroid plexus, olfactory bulb, nucleus accumbens, amygdala, substantia nigra, and hypothalamus (Hoffman and Mezey, 1989; Wright et al., 1995; Wolf and Schutz, 1997). In primate hypothalamus, we have used *in situ* hybridization to localize 5HT2C receptor mRNA. Dense populations of 5HT2C-labeled cells are found in the anterior hypothalamus, periventricular nuclei, ventromedial nuclei, dorsal hypothalamic area, lateral hypothalamus, arcuate nucleus, and the infundibular nucleus (Bethea et al., 1998). Several of these areas also contain dense populations of ER- and PR-positive neurons, so there is significant potential for regulation of 5HT2C receptors by E and P.

A very few studies using radiolabeled ligand autoradiography have examined the effect of E and P on 5HT2A receptor protein in rats, but no information is available on ovarian steroid regulation of 5HT2C receptors in any species. A single injection of exogenous E given to ovariectomized rats induced a significant increase in 5HT2A receptor labeling in the dorsal raphe nucleus, anterior frontal, anterior cingulate, and primary olfactory cortex as well as the nucleus accumbens and the amygdala (Sumner and Fink, 1993, 1995; Fink and Sumner, 1996). Moreover, at the time of the spontaneous E-induced LH surge, 5HT2A receptor densities increase compared to diestrous females or males in the frontal and cingulate cortex, olfactory tubercle, and nucleus accumbens (Sumner and Fink, 1997). Finally, ovariectomy reduced 5HT2A receptor mRNA and protein, and E-replacement reversed this effect (Cry et al., 1996). The nucleus accumbens receives major inputs from the amygdala, and projects to the cortex and hypothalamus (Zahm and Brog, 1992). These regions are essential for cognition, emotion, mental state, mood, and neuroendocrine control. Thus, the E-stimulated increase in 5HT2A receptor densities in the above-stated regions, including the accumbens and amygdala, may be related to control of pituitary hormone secretion, mating, and motor behavior as well as mood.

Pharmacological studies to clarify the effect of E and P on 5HT2A or 2C receptors have been limited by the lack of selective antagonists.

Unfortunately, ketanserin and other available pharmacological agents act at both 2A and 2C receptors, so studies of this nature do not discern which receptor subtype is involved. Nonetheless, ketanserin blocked the E-induced LH surge in cycling rats (Tanaka et al., 1993). In addition, there is a large body of literature indicating that either 5HT2A or 2C receptors mediate serotonergic stimulation of prolactin secretion (Jørgender et al., 1992, 1993; Rittenhouse et al., 1993; Albinsson et al., 1994; van de Kar et al., 1996; Coccaro et al., 1996; Lacau-Mengido et al., 1996) and prolactin secretion is significantly augmented by E and P (Bethea et al., 1996). The studies of Fink and colleagues encourage speculation that one of the mechanisms by which E and P increase prolactin secretion is via stimulation of expression of 5HT2A or 2C receptors. Several studies have implicated the paraventricular nucleus in mediating the 2A/2C agonist-induced increase in prolactin secretion (Rittenhouse et al., 1993; Bagdy and Makara, 1994, 1995; Bagdy, 1996). In the primate, the paraventricular nucleus contains a much heavier concentration of 5HT2A than 2C mRNA, suggesting that the effect of the mixed agonists in this nucleus on prolactin secretion may be via 5HT2A rather than 2C receptors. ERα and PR mRNA labeling is notably light in the paraventricular nucleus (Simerly et al., 1990), but ERβ mRNA labeling is very dense (Shughrue et al., 1997a).

The 5HT2A/2C receptors have also been shown to stimulate lordosis. Local infusion or systemic administration of 2A/2C antagonists decreases lordosis behavior. The decline is smaller during proestrous, suggesting that ovarian steroids shift the dose–response curve of 2A/2C antagonists to the right (Uphouse et al., 1996). In addition, a 2A/2C agonist infused locally into the ventromedial nucleus protected against the decrease in lordosis elicited by serotonin or 5HT1A agonists in proestrous rats (Maswood et al., 1996).

The mixed serotonin 1B/2C receptor agonist mCPP has been used in clinical research studies to examine the effect of E on the sensitivity of the serotonin system. In postmenopausal women without E treatment, prolactin secretion in response to mCPP was blunted compared to postmenopausal women who received 30 d of E via transdermal patch (Halbreich et al., 1995). Postmenopausal women also had a blunted prolactin response to mCPP compared to women of reproductive status (Halbreich et al., 1995). Women with PMS also showed a blunted cortisol response to mCPP during both the follicular and luteal phases of the menstrual cycle (Su et al., 1997). In contrast, normal men had significantly higher levels of 5HT2 receptor binding capacity than normal women in the frontal and cingulate cortices as determined with PET and the radiotracer, [$^{18}$F]-labeled altanserin (Biver et al., 1996).

In summary, ovarian steroids probably regulate the expression of 5HT2A and 2C receptors. There is direct evidence that E increases 5HT2A receptor densities in brain regions associated with both mood and reproductive status. Moreover, E-replacement therapy augments the prolactin response to challenge with a 1B/2C serotonin receptor agonist, mCPP, and there is evidence that the steroid milieu alters the lordosis response to 2A/2C agonists and antagonists.

The 5HT3 receptor is a ligand-gated ion channel. Like other ligand-gated ion channels, the 5HT3 receptor consists of four transmembrane segments and a large extracellular N-terminal region incorporating a cysteine–cysteine loop and potential *N*-glycosylation sites (Mircq et al., 1991). There have been no studies to examine the effect of ovarian steroids on the expression of the 5HT3 receptor. One study noted an increase in prolactin pulse frequency after 5HT3 receptor blockade with the antagonist ondansetron administered daily for the duration of a menstrual cycle (Ulrich et al., 1994). In rats, however, the systemic administration of neither 5HT3 agonists nor antagonists had any effect on lordosis (Tanco et al., 1993, 1994). There is currently no information available on the regulation of 5HT4, 5HT5, 5HT6 or 5HT7 receptors by E or P (Peroutka, 1994). Nonetheless, the localization of the 5HT7 receptor in the hypothalamus and its

potential involvement in circadian rhythms (Lovenberg et al., 1993) make it a likely candidate for steroid hormone regulation.

### Degradative Enzymes

MAO-A and B are the main enzymes that catalyze oxidative deamination of biogenic amines in the CNS and peripheral tissues (Von Korff, 1979). In the human brain, MAO-A selectively degrades 5HT and norepinephrine, but MAO-B predominantly metabolizes dopamine (Saura et al., 1982; Youdim and Finberg, 1991). A differential physiological function for these enzyme isoforms is supported by the suggestion that MAO-A and MAO-B are encoded by separate genes (Bach et al., 1988). Human, monkey, and rat MAO-A and MAO-B proteins and mRNAs have been localized largely to catecholaminergic and 5HT neurons, respectively (Saura et al., 1982; Westlund et al., 1985, 1988; Richards et al., 1992; Luque et al., 1996).

We reported that P added to an E treatment regimen decreased the ratio of 5HIAA/5HT or increased the ratio of 5HT/5HIAA in CSF of female monkeys (Schutzer et al., 1997). In addition, we found that treatment of male macaques with the progestin agonist, cyproterone acetate, decreased 5HIAA, which correlated with a decrease in self-mutilating behavior (Eaton et al., in press). The progestin-induced decreases in 5HIAA correlated with a concomitant decrease in HVA, the major metabolite of dopamine. These data have encouraged speculation that P may decrease the functional activity of the MAOs, either at the level of gene expression, protein expression, or phosphorylation.

A decrease in the activity of MAO-A or B would be reflected by a decrease in the metabolites, 5HIAA and HVA, which in turn may indicate a relative increase in the availability of active transmitter to inactive metabolite. This reasoning is supported by the fact that MAO inhibitors are effective antidepressants and MAO inhibitors act by decreasing degradation of 5HT and dopamine. This decrease in degradation is thought to increase transmitter availability in the extracellular space. In fact, early studies demonstrated that MAO inhibitors increase the concentration of serotonin and decrease the concentration of 5HIAA in rat brain and in human plasma (Juorio et al., 1986; McKenna et al., 1992; Celada et al., 1992).

Studies prior to about 1985 did not distinguish between MAO-A and B, so early data on the steroid regulation of either plasma MAO (which is predominantly MAO-B from platelets) or on the steroid regulation of brain MAO (which could be either MAO-A or B) will not be reviewed here (*see* Sherwin, 1996 for review). Reports on the regulation of specific isoforms of MAO by ovarian steroids in the rat brain have been conflicting. Hypothalamic MAO-A in the rat has been shown to increase after E + P treatment, whereas MAO-B decreased (Ortega-Corona et al., 1994). In contrast, MAO-A activity decreased and MAO-B activity increased in the locus ceruleus and cerebellum of the rat with acute and chronic E (Chevillard et al., 1981). Future studies in primates will determine if E or P alters the expression or activity of MAO-A, which would in turn alter overall serotonergic neurotransmission.

## Other Considerations

### Mechanism of Action of E and P in Serotonin Neurons

We have shown that E increases TPH mRNA and decreases SERT and 5HT1A mRNAs in serotonin neurons of macaques. The effects of E on the expression of the different mRNAs may be direct via nuclear ER, or it could be the result of enhanced afferent neurotransmission from another system that is responsive to E. Although ER have not been observed in serotonin neurons, we demonstrated that serotonin neurons contain PRs, which are markedly induced by E treatment (Bethea, 1994). The induction of PR by E is dependent on nuclear ERs in the hypothalamus and other reproduc-

tive tissues (Clarke 1990; Bethea et al., 1996). In the guinea pig hypothalamus, ER were colocalized in all neurons containing PR (Blaustein and Turcotte, 1989). Together this data suggest that primate serotonin neurons contain nuclear ER, either of the α or β type.

We currently speculate that the stimulatory effect of E on TPH mRNA and the inhibitory effect of E on SERT mRNA and 5HT1A mRNA expression in macaques are mediated directly in the serotonin neuron by nuclear ER. However, an E response element (ERE) has not been reported for the TPH, SERT, or 5HT1A genes, nor has an inhibitory ERE been identified. This introduces the question of how, at a molecular level, can E stimulate the TPH gene (Pecins-Thompson et al., 1996) and inhibit the SERT (Pecins-Thompson et al., 1998a) and 5HT1A (Pecins-Thompson et al., 1998b) genes in the same cell presumably using the same wild-type ER. E binds to the (ER) which then increases transcriptional activity by acting directly through EREs (Tsai and O'Malley, 1994) or via protein–protein interactions at AP-1 sites (Webb et al., 1995). It has been recently recognized that other proteins, called coactivators or corepressors, can interact with steroid receptors and the transcriptional apparatus (Katzenellenbogen et al., 1996). It has also been suggested that coactivators or corepressors may be specific for particular target genes (Yen, 1996). Thus, it is possible that the TPH and SERT or 5HT1A genes have different coproteins that interact with ligand-bound ER. Recently, several laboratories showed that ER sequesters nuclear factor-kappa B (NF-κB) leading to transcriptional repression of genes that have NF-κB sites in the promoter region (Greene et al., 1996; Mckay and Cidlowsky, 1998; Wissink et al., 1998). In one study, the repression occurred in the same cells where ERα acts to increase transcription of the PR gene. This type of mechanism may be operational in serotonin neurons. The SERT gene has NF-κB sites in the promoter region, which could constitutively drive transcription. With E treatment, ER may sequester NF-κB or another important nuclear transcription factor, and thereby reduce transcription of SERT while binding to potential EREs in the promoter region of the TPH gene and increasing TPH gene transcription.

In addition, the promoter region of the SERT gene contains two AP1 binding sites (Heils et al., 1995). Using the promoter region of the collagenase gene, Kushner and colleagues have shown that ERα enhances the interaction of *fos* and *jun* at AP1 sites via protein–protein interactions with the cAMP response element binding protein (CBP/P160). These data suggest that the AP1 binding site may be an important route by which ERα affects target tissues in vivo (Webb et al., 1995; Ribeiro et al., 1995). The effect of E on TPH, SERT, or 5HT1A mRNA stability is unknown.

### *Nongenomic Actions of Ovarian Steroids*

Evidence is also accumulating that steroids may have rapid, nongenomic actions at the level of the membrane (Crawley et al., 1986; McEwen, 1995; Majewska, 1992; Orchinik and McEwen, 1993) and serotonin neurotransmission may be modulated in this manner as well. There are several metabolites of P (neurosteroids) that act at GABA-A receptors, and may be anxiolytic, analgesic, sedative, hypnotic, or anesthetic (Schumacher et al., 1989; McEwen, 1991; Orchinik and McEwen, 1993). These actions are similar to the actions of the benzodiazapines. Some evidence is available to suggest that there is a lower level of the progestin metabolite, allopregnanolone, during the luteal phase in serum of women with LLPDD (Rapkin et al., 1997). In addition, plasma GABA levels were lower during the luteal phase in woman with LLPDD (Halbreich et al., 1996), and changes in GABA-A receptor binding have been reported during pregnancy in the rat (Majawska et al., 1989). Although the therapeutic efficacy of antidepressants or ovarian steroids to alleviate depression has a significant time delay, it would be premature to rule out a role of neurosteroids or nongenomic

actions of ovarian steroids in the regulation of mood. Nonetheless, the effect of P on prolactin secretion appears to require nuclear PR, since RU 486 blocks P-induced prolactin secretion (Pecins-Thompson and Bethea, 1997). In addition, prolactin secretion is elevated between 36 and 48 h after an acute injection of P. This time delay also suggests that transcriptional events may be involved.

### Synthetic Steroids

We have shown that E alone and E + P act to increase TPH mRNA and protein levels, to decrease SERT mRNA, and to decrease 5HT1A autoreceptor mRNA expression. In concert, these actions could lead to increased serotonin synthesis, decreased serotonin reuptake, and a release of autoreceptor inhibition of serotonin neural firing. It is reasonable to believe that together these actions of E and P to facilitate serotonin neurotransmission would act to improve mood. However, in clinical studies, the administration of the synthetic progestin, medroxyprogesterone (MPA), attenuated the beneficial effect of E replacement therapy on mood (Holst et al., 1989; Sherwin and Gelfand, 1989; Sherwin, 1991, 1996). The effect of medroxyprogesterone on mood in women is not consistent with the experimental results obtained in monkeys using natural P as described above. It should be noted that another study found that addition of natural P to an E-replacement regimen caused a pleasant tranquilizing effect (de Lignieres and Vincens, 1982). Together these data suggest that the molecular action of the synthetic progestin, medroxyprogesterone, differs significantly from the action of natural P in serotonin neurons. This speculation has received recent support from studies in cardiovascular physiology in which medroxyprogesterone abolishes the beneficial effect of E on various parameters of cardiovascular function, whereas natural P does not (Wagner et al., 1996; Clarkson et al., 1997; Miyagawa et al., 1997a,b).

### Clinical Considerations

The role of serotonin in depression is widely recognized (Owens and Nameroff, 1994). However, depression may be manifested in various subtypes, which could involve different contributions from various neurotransmitter systems. For example, stress-induced depression appears to involve the noradrenergic neurons of the locus ceruleus (Berridge et al., 1993; Valentino et al., 1993; Weiss J. M. et al., 1994; Weis G. K. et al., 1994). Mortola and colleagues found that depressive episodes occurring in the luteal phase of the menstrual cycle in women with LLPDD are different from endogenous depression as determined with cortisol parameters and psychological indices (Mortola et al., 1989). An increasing number of selective serotonin reuptake inhibitors have therapeutic efficacy as antidepressant drugs (Fuller and Wong, 1990), and at least two of these, fluoxetine (Rickels et al., 1990; Stone et al., 1990; Mortola, 1994; Steiner et al., 1995; Steiner, 1996) and sertraline (Yonker et al., 1997), are effective in treating PMS. It is currently not possible to identify patients who would respond best to selective serotonergic, noradrenergic, or dopaminergic drugs. The respective contribution of the different aminergic neural systems to depression related to reproductive function vs depressions of other etiologies is therefore of importance for appropriate and specific treatment.

Based on this rationale, we examined the expression of the mRNA for the NET in the locus ceruleus from the same monkeys that exhibited regulation of dorsal raphe TPH, SERT, and 5HT1A mRNAs by E and/or P. We could detect no difference in NET mRNA levels in the locus ceruleus of ovariectomized, E-treated, or E + P-treated monkeys, nor could we detect ERα or PR in the noradrenergic neurons of this nucleus (Schutzer and Bethea, 1997).

The combined roles of serotonin and ovarian steroids in mood disorders related to reproductive function in women has only recently received significant clinical research. In a mul-

ticenter geriatric depression trial, older women receiving steroid hormone-replacement therapy in any form showed a significantly better response to fluoxetine (Prozac, 20 mg/d) than women not on hormone-replacement therapy. In fact, fluoxetine did not significantly enhance mood compared to placebo in patients who were not on steroid hormone-replacement therapy (Schneider et al., 1997). However, there was a large placebo effect in this study, which requires cautious interpretation.

## Conclusions

Evidence is accumulating that the ovarian steroids, E and P, affect numerous functional properties of the serotonin neural system, including gene expression for TPH, the serotonin reuptake transporter, the 5HT1A autoreceptor, several of the postsynaptic receptors, and probably the degradative enzyme, MAO-A. Therefore, there is a significant biological basis by which ovarian steroid hormones can influence mood, hormone secretion, autonomic function, and cognition. Nonetheless, many questions remain to be answered.

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