Abstract  Rationale: The serotonin neural system plays a pivotal role in mood, affective regulation and integrative cognition, as well as numerous autonomic functions. We have shown that ovarian steroids alter the expression of several genes in the dorsal raphe of macaques, which may increase serotonin synthesis and decrease serotonin autoinhibition. Another control point in aminergic neurotransmission involves degradation by MAO. This enzyme occurs in two isoforms, A and B, which have different substrate preferences.  Objectives: We questioned the effect of ovarian steroid hormones on MAO-A and MAO-B mRNA expression in the dorsal raphe nucleus and hypothalamus using in situ hybridization in non-human primates.  Methods: Rhesus monkeys (Macaca mulatta; n=5/group) were spayed and either placebo treated (controls), estrogen (E) treated (28 days), progesterone (P) treated (14 days placebo+14 days P), or E+P treated (14 days E+14 days E+P). Perfusion-fixed sections (25 µm) were hybridized with a 233 bp MAO-A, or a 373 bp MAO-B, radiolabeled-antisense monkey specific probes. Autoradiographic films were analyzed by densitometry, which was performed with NIH Image Software.  Results: MAO-A and -B mRNAs were detected in the dorsal raphe nucleus (DRN) and in the hypothalamic suprachiasmatic nucleus (SCN), preoptic area (POA), paraventricular nucleus (PVN), supraventricular nucleus (SON), lateral hypothalamus (LH) and ventromedial nucleus (VMN). MAO-A mRNA optical density was significantly decreased by E, P, and E+P in the DRN and in the hypothalamic PVN, LH and VMN. Ovarian hormones had no effect on MAO-B mRNA expression in the DRN. However, there was a significant decrease in MAO-B optical density in the hypothalamic POA, LH and VMN with E, P or E+P treatment. Pixel area generally reflected optical density.  Conclusions: Ovarian steroids decreased MAO-A, but not B, in the raphe nucleus. However, both MAO-A and B were decreased in discrete hypothalamic nuclei by hormone replacement. These data suggest that the transcriptional regulation of MAO by ovarian steroids may play a role in serotonin or catecholamine neurotransmission and hence, mood, affect or cognition in humans.

Keywords  Estrogen · Progesterone · Serotonin · Primate · Depression · Mood disorder · Hormone replacement therapy

Introduction  Monoamine oxidase A and B (MAO-A and MAO-B) are the central enzymes that catalyze oxidative deamination of biogenic amines in the central nervous system and peripheral tissues (Von Korff 1979). Inhibitors of MAO were among the first pharmacotherapies successfully used for the treatment of depression. It is generally thought that serotonin neurotransmission is dysfunctional in depression. Thus, the ability of MAO inhibitors to relieve depression suggests that MAO plays a functionally significant role in serotonin metabolism. Moreover, there is a delay in the onset of therapeutic efficacy which occurs approximately 2 weeks after daily treatment with the MAO-A inhibitor, clorgyline, correlating with desensitization of serotonin autoreceptors (Blier and de Montigny 1985).

The substrate specificity of MAO-A and B varies slightly between species. In human, monkey and rat,
MAO-A selectively degrades serotonin and norepinephrine whereas MAO-B predominantly metabolizes dopamine (Tipton et al. 1982; Youdim and Finberg 1991). Serotonin degradation by MAO-B has also been reported in rat, bovine, and pig brain (Ekstedt and Oreland 1976; Achee and Gabay 1977; Mitra and Guha 1980; Luine and Paden 1982; Tipton et al. 1982). In mice, dopamine is preferentially oxidized by MAO-A (Steyn et al. 2001). Anatomically, MAO-A has been localized largely to catecholaminergic neurons and MAO-B has been largely localized to serotonin neurons. However, MAO-A mRNA has also been detected in the monkey dorsal raphe nucleus, which contains a large population of serotonin neurons (Saura et al. 1982, 1996; Westlund et al. 1985, 1988; Richards et al. 1992; Luque et al. 1996). Among the species, MAO isoform mRNA expression was consistent with protein localization (Richards et al. 1992; Luque et al. 1996).

A body of data suggests that estradiol (E) has the potential to improve mood and cognition (McEwen 1999), although much remains unknown regarding the sites and mechanisms of action of E in the central nervous system (CNS). We have found that E changes the expression of pivotal genes in serotonin neurons in a manner that could increase serotonin synthesis and decrease serotonin autoinhibition (Pecins-Thompson et al. 1996, 1998; Pecins-Thompson and Bethea 1998). In addition, our laboratory has shown that progesterone (P) administered after E priming significantly increased the 5-HT/5-HIAA ratio in CSF of monkeys, due to a significant decrease in 5-HIAA (Bethea et al. 1999). These data led to the hypothesis that overall serotonin neurotransmission may be increased by actions of E on tryptophan hydroxylase (TPH), serotonin reuptake transporter (SERT), and 5HT1A autoreceptor gene expression, and then further facilitated by adding P to the E regimen to act on the expression of MAO-A or B in a fashion that would decrease serotonin degradation. MAO-A and MAO-B are encoded by separate genes (Bach et al. 1988), which also raises the possibility of differential regulation by ovarian steroids.

Previous studies in rats indicated that hypothalamic MAO-A activity decreased with acute E, and the addition of P to E-primed rats restored MAO-A activity (Luine and Rhodes 1983; Ortega-Corona et al. 1994). MAO-B activity in the rat hypothalamus was decreased with E and E+P (Ortega-Corona et al. 1994). However, in the locus coeruleus and cerebellum of the rat, MAO-A activity decreased and MAO-B activity increased with acute and chronic E (Chevillard et al. 1981). The actions of E and P in the serotonin system differ in various respects between rodents and primates. For example, the effect of E on tryptophan hydroxylase mRNA expression and on SERT mRNA expression in the raphé nuclei are different in monkey and rat (Pecins-Thompson et al. 1996, 1998; Alves et al. 1997; McQueen et al. 1997). Therefore, we questioned whether ovarian steroids would decrease MAO-A and MAO-B mRNA in the monkey dorsal raphe and hypothalamic nuclei, areas of the brain that contain significant populations of nuclear receptors for E and P. We also sought evidence that P may amplify the effect of E on the transcription of MAO-A or MAO-B.

Materials and methods

Animals and experimental groups

This study was approved by the Oregon Regional Primate Research Center (ORPRC) Animal Care and Use Committee. Adult female rhesus monkeys (Macaca mulatta) were ovariotomized and hysterectomized (spayed) by the surgical personnel of ORPRC between 3 and 6 months before assignment to this project according to accepted veterinary surgical protocol. All animals were born in Oregon, weighed between 4 and 8 kg, and were in good health.

For examination of the regulation of MAO-A and MAO-B mRNA, 20 spayed rhesus macaques were obtained and either treated with placebo (empty Silastic capsules; control group), or treated with E for 28 days (E group), or treated with placebo for 14 days and then treated with P for 14 days (P group), or treated with E for 28 days and then supplemented with P for the final 14 of the 28 days (E+P group). The animals were processed in matched sets containing one animal from each treatment group. Each set was treated with hormones and killed at the same time. A total of five sets of animals were used yielding a final number of five animals in each of the four treatment groups. The administration of E for 28 days supplemented with P for the last 14 days of the 28-day treatment period has been shown to cause differentiation of the uterine endometrium in a manner similar to the normal 28-day menstrual cycle (Brenner and Maslar 1988).

Surgery and treatments

All animals were spayed. The control monkeys were implanted (SC) with empty Silastic capsules. The E-treated monkeys were implanted (SC) with one 4.5-cm E-filled Silastic capsule (i.d. 0.132 in.; o.d. 0.183 in.; Dow Corning, Midland, Mich., USA). The capsule was filled with crystalline estradiol (1,3,5(10)-estratrien-3,17-b-diol; Steraloids, Wilton, N.H., USA). The E+P group received an E-filled capsule, and 14 days later, received one 6-cm capsule filled with crystalline P (4-pregnen-3,20-dione; Steraloids). The P-treated group received an empty Silastic capsule, and 14 days later received a P-filled capsule. All capsules were placed in the periscapular area under ketamine anaesthesia (ketamine HCl, 10 mg/kg, SC; Fort Dodge Laboratories, Fort Dodge, Iowa, USA).

Tissue preparation

The monkeys were killed at the end of the treatment period according to procedures recommended by the Panel on Euthanasia of the American Veterinary Association. Each animal was sedated with ketamine, given an overdose of pentobarbital (25 mg/kg, IV), and exsanguinated by severance of the descending aorta. The left ventricle of the heart was cannulated and the head of each animal was perfused with 1 l of saline followed by 7 l of 4% paraformaldehyde in 3.8% borate, pH 9.5 [both solutions made with DEPC treated water (0.1% diethyl pyrocarbonate) to minimize RNase contamination]. The brain was removed and dissected. Tissue blocks were postfixed in 4% paraformaldehyde for 3 h, then transferred to 0.02 M potassium phosphate buffered saline (KPBS) containing 10%, followed by 20% glycerol and 2% dimethyl sulfoxide (DMSO) at 4°C for 3 days to cryoprotect the tissue. After infiltration, the block was frozen in isopentene cooled to –55°C, and stored at –80°C until sectioning which occurred within 6 months of storage. Sections (25 µm) were cut on a sliding microtome, mounted at–80°C until sectioning which occurred within 6 months of storage, the block was frozen in isopentene cooled to –55°C, and stored in 30%, followed by 20% glycerol and 2% dimethyl sulfoxide (DMSO) at 4°C for 3 days to cryoprotect the tissue. After infiltration, the block was frozen in isopentene cooled to –55°C, and stored at –80°C until sectioning which occurred within 6 months of storage. Sections (25 µm) were cut on a sliding microtome, mounted.