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Research report

# A second tryptophan hydroxylase isoform, TPH-2 mRNA, is increased by ovarian steroids in the raphe region of macaques

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**Abstract**

Recently, a second gene that codes for the rate-limiting enzyme in serotonin synthesis was found in brain, named tryptophan hydroxylase-2 (TPH-2). We sequenced overlapping segments (251 and 510 bp) of 5′ monkey TPH-2 and questioned whether TPH-2 is regulated by estrogen (E) and progesterone (P) in serotonin neurons of macaques. Monkey TPH-2 was 97% homologous to human TPH-2 and 65% homologous to monkey TPH-1 in the coding region. Spayed monkeys were administered placebo, E-only, P-only, or E + P for 1 month via Silastic implants ($n$ = 4/treatment) and the midbrain was utilized for TPH-2 in situ hybridization (ISH). Additional monkeys ($n$ = 3/treatment) were used to determine the relative abundance of TPH-2 mRNA with quantitative (q) RT-PCR. In the ISH assay, all of the hormone treatments caused a significant and similar increase in TPH-2 mRNA optical density (fourfold; $P < 0.004$) and positive pixel area (twofold; $P < 0.002$) over spayed controls. Treatment with E or E + P for 1 month increased the relative abundance of TPH-2 mRNA over spayed controls in the qRT-PCR assay (ANOVA $P < 0.05$ and $P < 0.007$, respectively). In conclusion, ovarian steroids stimulate TPH-2 mRNA expression, which could in turn cause an increase in serotonin synthesis. This would impact many of the neural functions that are governed by serotonin.


*Theme:* Endocrine and autonomic regulation
*Topic:* Neuroendocrine regulation: other

*Keywords:* Serotonin; Macaques; Tryptophan hydroxylase genes; Estrogen; Progesterone; In situ hybridization; Quantitative PCR

## 1. Introduction

Serotonin modulates an ever-growing number of autonomic functions [8], cognitive domains [19], affective, anxiety, and stress-related disorders [7,14,26,27] and even metabolic syndromes [20]. As such, the serotonin neural system is a target of pharmacotherapies, steroid hormones, cytokines, neuropeptides, and growth factors, all of which impact the generation and efficacy of serotonin neurotransmission.

Hormone therapy has, until recently, been considered beneficial in menopausal women for neural functions underlying mood and cognition [24,25]. The Women's Health Initiative Memory Study trial (WHIMS) has shaken that premise with several negative outcomes in elderly postmenopausal women given equine estrogens and a synthetic progestin [9]. Animal models using natural hormones have reached quite different conclusions [5,10,17], but further study is needed.

This laboratory has devoted effort toward understanding the actions of ovarian hormones in serotonin neurons and their terminal fields with a macaque model of surgical menopause [2]. We found that estrogen with or without supplemental progesterone regulates the expression of

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pivotal serotonin-related genes and proteins in a pattern suggestive of increased serotonin production, increased neural firing, and decreased degradation [3,4,15,16], which should be beneficial to mood or integrative cognition.

Tryptophan hydroxylase or TPH is the rate-limiting enzyme for the synthesis of serotonin and TPH protein is translated from two mRNA transcripts, TPH-1, and TPH-2, with a large degree of homology in the coding regions, but divergence in the 5′ untranslated regions [30]. TPH-1 appears to be a relatively rare transcript in the brain and it is used predominantly in peripheral cell types that make serotonin [31]. In contrast, TPH-2 is the more prevalent transcript used in the brain.

In earlier work, it appeared that TPH-1 was a very rare transcript in brain and detection required low stringency conditions, but estrogen, with or without supplemental progesterone, increased TPH-1 mRNA levels in the dorsal raphe of macaques [23] and mice [12]. Today, we cannot rule out the possibility that the TPH-1 riboprobes may have poorly hybridized to TPH-2. Nonetheless, Western blots indicated that ovarian hormone replacement also increased total TPH protein [3]. Indeed, TPH protein levels were robust. Prior to the discovery of TPH-2, the large amount of protein that appeared to follow the rare transcript expression was interpreted as highly efficient translation. However, the new discovery suggests that in the monkey dorsal raphe (1) TPH-2 mRNA could be the more prevalent TPH transcript, (2) TPH-2 could be the transcript that largely generates TPH protein, and (3) TPH-2 could be regulated by ovarian hormones.

To further these hypotheses, a monkey-specific TPH-2 cDNA was constructed that spanned 5′ untranslated region and 5′ coding region from bp 56 to 306, which is contiguous only in TPH-2 mRNA. This cDNA was used for the production of riboprobe and for the detection of TPH-2 mRNA in the dorsal raphe of monkeys. In situ hybridization was applied to midbrain sections containing the dorsal raphe from spayed monkeys treated subcutaneously with placebo, estrogen, progesterone, or estrogen plus progesterone. In addition, to examine the relative expression of TPH-2, quantitative (q) RT-PCR was conducted on RNA extracted from the dorsal raphe region of spayed animals treated with placebo, estrogen, or estrogen plus progesterone.

## 2. Materials and methods

This study was approved by the Oregon National Primate Research Center (ONPRC) Institutional Animal Care and Use Committee.

### 2.1. Animals for in situ hybridization

Sixteen adult female rhesus monkeys (*Macaca mulatta*) were ovariectomized and hysterectomized (spayed) by the surgical personnel of ONPRC according to accepted veterinary protocol, approximately 3–6 months before assignment to this project. All animals were born in Oregon, were aged between 7 and 14 years, weighed between 4 and 8 kg, and were in good health.

During the menstrual cycle, the developing follicles produce estrogens for the first 14 days (follicular phase); upon ovulation of the dominant follicle, a corpus luteum develops and secretes progesterone for the remaining 14 days. Estrogen production also continues during the luteal phase. Based upon this profile, animals were either treated with placebo (spay-control group; $n = 4$), treated with estrogen (E) for 28 days (E group; $n = 4$), treated with placebo for 14 days and then treated with progesterone (P) for 14 days (P group; $n = 4$), or treated with E for 28 days and supplemented with P for the final 14 of the 28 days (E + P group; $n = 4$). The placebo treatment of the spay-control monkeys consisted of implantation with empty Silastic capsules (s.c.). The E-treated monkeys were implanted (s.c.) with one 4.5-cm E-filled Silastic capsule (i.d. 0.132 in.; o.d. 0.183 in.; Dow Corning, Midland, MI). The capsule was filled with crystalline estradiol (1,3,5(10)-estratrien-3,17-*b*-diol; Steraloids, Wilton, NH). The E + P-treated 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 anesthesia (ketamine–HCl, 10 mg/kg, s.c.; Fort Dodge Laboratories, Fort Dodge, IA).

The animals were processed in matched sets containing one animal from each treatment group. Each set was treated with hormones and euthanized at the same time. A total of four sets of animals were used yielding a final number of four animals in each of the four treatment groups. Serum E and P concentrations were measured with radioimmunoassay in the blood sample obtained at necropsy by the ONPRC Endocrine Services Laboratory to verify the efficacy of the implants.

### 2.2. Animals for quantitative (q) RT-PCR

An additional nine adult spayed female macaques were assigned for RNA extraction from the dorsal raphe region. These animals were born in China. Their approximate ages between 7 and 14 years were established by dental examination. They weighed between 4 and 8 kg and were in good health. These animals were also spayed between 3 and 6 months prior to assignment to this project. The animals were either treated with empty Silastic capsules (spay-control group; $n = 3$), treated with an E-filled capsule for 28 days (E group; $n = 3$), or treated with an E-filled capsule for 28 days and then supplemented with a P-filled capsule for the final 14 of the 28 days (E + P group; $n = 3$) as described above. Serum E and P concentrations were measured with radioimmunoassay in the blood sample obtained at necropsy by the ONPRC Endocrine Services Laboratory.