Sex Differences in Serotonin 1 Receptor Binding in Rat Brain

Abstract. *Male and female rats exhibit sex differences in binding by serotonin 1 receptors in discrete areas of the brain, some of which have been implicated in the control of ovulation and of gonadotropin release. The sex-specific changes in binding, which occur in response to the same hormonal (estrogenic) stimulus, are due to changes in the number of binding sites. Castration alone also affects the number of binding sites in certain areas. The results lead to the conclusion that peripheral hormones modulate binding by serotonin 1 receptors. The status of the serotonin receptor system may affect the reproductive capacity of an organism and may be related to sex-linked emotional disturbances in humans.*

Steroid hormones act on their specific receptors in discrete brain regions (*1*) and influence neuronal chemistry and function (*2*). The modulation of neurotransmitter receptor binding by hormones (*3, 4*) may alter sensitivity to neurotransmitters, thus altering brain responsiveness to incoming stimuli.

Serotonin is among the putative neurotransmitters linked to steroid hormone action; it influences sexual behavior (*4, 5*) and the preovulatory surge of luteinizing hormone (*4, 6*). Binding to serotonin 1 receptors fluctuates over the estrous cycle (*7*), and administering estrogen increases the number of serotonin 1 receptors in several estrogen-sensitive brain regions of rats subjected to ovariectomy. Estrogen apparently alters the response of the serotonin 2 receptor system as well (*8*).

The effect of estrogen binding by serotonin 1 receptors in females subjected to ovariectomy occurs in specific nuclei (*3*) implicated in the control of gonadotropin release (*9*). We have investigated the role of sex steroids on these and other areas in male and female rats to examine possible mechanisms of gonadotropin secretion characteristic of male and female mammals. We now report that male and female rats subjected to gonadectomy exhibit differences in binding to serotonin 1 receptors in specific nuclei in response to the same estrogenic stimulus. In addition, castration of male rats alters the number of serotonin 1 receptor sites in discrete areas of the brain, and estrogen can reverse the effects of castration only in the midbrain.

Adult Sprague-Dawley rats of both sexes (minimum age of males, 8 weeks) were matched by weight and maintained on a 14:10 light:dark cycle (lights were on from 0500 to 1900 hours) under standard laboratory conditions. Gonads were removed at least 1 week before subcutaneous hormone injection, which consisted of 10 μg of estradiol benzoate (EB) or sesame oil at 0 and 24 hours. Animals were decapitated 48 hours later; all injections and decapitations occurred between 1100 and 1300 hours. Some brains were frozen onto cryostat chucks and their nuclei dissected according to the method of Palkovits (*10*); others were grossly dissected and frozen on dry ice as were pituitaries.

Nuclei studied were those which had previously demonstrated increases in serotonin 1 receptor binding in response to estradiol treatment in rats subjected to ovariectomy (*3*). Other limbic-mesencephalic areas containing high to moderate amounts of estrogen-concentrating cells (*11*), high to moderate densities of serotonin 1 receptors (*12*), or both, were also studied because of their involvement in steroid and gonadotropin secretion (ventral subiculum) (*13*), sex-related electrophysiological responses (central gray) (*14*), and steroid-induced changes in neurotransmitter content (dorsal raphe nucleus) (*15*).

The assay for binding of tritium-labeled serotonin was performed on homogenates obtained from microdissected nuclei, and saturation analysis was performed on washed membrane preparations [tritiated-serotonin (26 to 30 Ci/mmole, 0.5 to 4.0 n*M*) in the presence or absence of 6 μ*M* unlabeled serotonin] (*3*). Baseline serotonin 1 receptor binding in homogenates obtained from microdissected nuclei in male and female rats from which gonads were removed is shown in Table 1. In females moderate binding was observed in preoptic-hypothalamic nuclei, compared with other brain areas (*3, 12*). Extensive binding was observed in the lateral septum and the dorsal raphe nucleus. Males exhibited more serotonin 1 receptor binding than females did in the gonadectomized state in both medial (35 ± 9 percent) and lateral (39 ± 19 percent) preoptic nuclei; however, binding in this region is not homogeneous (*16*). No other differences in baseline binding between males and females subjected to gonadectomy were found.

Binding by serotonin 1 receptors in microdissected nuclei in response to treatment with EB is shown in Fig. 1. Females exhibited increases in binding in the lateral septum, lateral preoptic area, anterior hypothalamic nucleus, and the arcuate nucleus–median eminence, as previously reported (*3*). In the anterior hypothalamic nucleus, estrogen treatment increased binding equally in male and female rats. However, different patterns in males and females were seen in the preoptic area (both medial and lateral), lateral septum, arcuate nucleus–median eminence, central gray, and the dorsal raphe nucleus. Other brain nuclei did not respond significantly to estrogen administration in either male or female rats, but contained high-to-moderate amounts of estrogen-concentrating cells.

To determine whether the effects of

Table 1. Specific binding of tritiated serotonin to serotonin 1 receptors in microdissected nuclei obtained from male and female rats subjected to gonadectomy. Results are means ± standard error of the mean of *N* experiments, each representing three pooled animals for each treatment group assayed in duplicate. Assay conditions were similar to those previously described (*3*). In brief, homogenates were incubated for 10 minutes at 37°C, to allow endogenous monoamine oxidase to catabolize endogenous serotonin, and then incubated for 10 minutes at 37°C with 2.0 n*M* tritiated serotonin in the presence or absence of 1 μ*M* unlabeled serotonin. Specific binding ranged from 45 to 90 percent, with most samples falling in the middle of this range.

Nucleus	Female		Male	
	Experiments (*N*)	Specific binding (fmole/mg)	Experiments (*N*)	Specific binding (fmole/mg)
Medial preoptic	7	125 ± 12	9	166 ± 15*
Lateral preoptic	8	113 ± 19	11	159 ± 22*
Lateral septum	10	156 ± 18	7	163 ± 28
Anterior hypothalamic nucleus	10	122 ± 15	8	114 ± 17
Arcuate nucleus–median eminence	5	44 ± 10	5	73 ± 21
Ventromedial nucleus of hypothalamus	9	121 ± 18	9	131 ± 22
Corticomedial amygdala	3	136 ± 27	3	138 ± 9
Basolateral amygdala	3	108 ± 3	3	97 ± 7
Ventral subiculum	8	110 ± 15	8	114 ± 26
Central gray	8	122 ± 16	8	128 ± 16
Dorsal raphe nucleus	6	171 ± 27	4	208 ± 38

*Two-tailed paired *t*-test, $P < 0.05$ (*16*).

estrogen administration on serotonin 1 receptor binding were due to changes in the number of binding sites (B_{max}) or the dissociation constant (K_d), saturation analysis was performed on washed membranes obtained from intact, castrated, or EB-treated and castrated male rats. Estrogen treatment decreased B_{max} in the preoptic area and increased it in the midbrain region (Table 2). These effects are consistent with the changes found in serotonin 1 receptor binding obtained in single point assays on microdissected nuclei.

Castration alone lowered the number of binding sites in the septum, hypothalamus, and the medial portion of the midbrain. That the effects in the septum and hypothalamus were not reversible by EB suggests the involvement of nonaromatizable androgens on serotonin 1 receptor binding. Estrogen did reverse the effect of castration in the male midbrain, however. The significance, if any, of the change in K_d in the preoptic area of castrated males is unknown.

Pituitary glucose-6-phosphate dehydrogenase activity (*17*) (Fig. 2) was significantly increased in both sexes with EB treatment, implying along with data for the anterior hypothalamic nucleus that estrogen treatment is effective in castrated males. This observation argues against sex differences in estrogen metabolism and clearance as an explanation for the observed effects.

Our results indicate that peripheral hormones modify the binding of tritiated serotonin to the serotonin 1 receptor in specific regions of rat brain. These responses are sex-specific and due to changes in the number of receptors.

In most of the nuclei in which serotonin content changes over the estrous cycle (*18*), serotonin 1 receptor binding also changes. One notable example is the lateral preoptic area, which exhibits a large change (74 percent) in serotonin content (*18*). We have found a sex difference between males and females in estrogen-induced changes in serotonin 1 receptor binding in the preoptic area and the arcuate nucleus–median eminence region—areas associated with gonadotropin regulation (*9*). These areas may regulate, at least partially, the sex-specific patterns of gonadotropin secretion, especially since sex differences in neuronal structure (*19*) and electrophysiological responses (*20*) have been reported. Whether these effects on serotonin 1 receptor binding are alterable by neonatal hormone manipulation has not yet been determined.

In two other mesencephalic areas that also responded in a sex-specific manner to estrogenic stimulation—the central gray and the dorsal raphe nucleus—the functional significance is less clear. The electrophysiological response of the central gray differs between male and female rats with stimulation of the ventromedial nucleus of the hypothalamus (*14*). With regard to the dorsal raphe, we speculate that estrogen effects on a primary source of serotonergic fibers to the forebrain may have consequences for more generalized changes in overall brain excitability.

Hormonal effects on serotonin 1 receptor binding are not limited to estrogens. Compared with intact males, castrated male rats exhibited changes in receptor number in the septum and hypothalamus, which are not reversible by estrogen; this was not so in the midbrain region. These findings indicate that nonaromatizable androgens may also affect serotonin 1 receptor binding.

The mechanism of steroid-induced changes in serotonin 1 receptor binding is unknown. These effects might be primary—those directly caused by the hor-

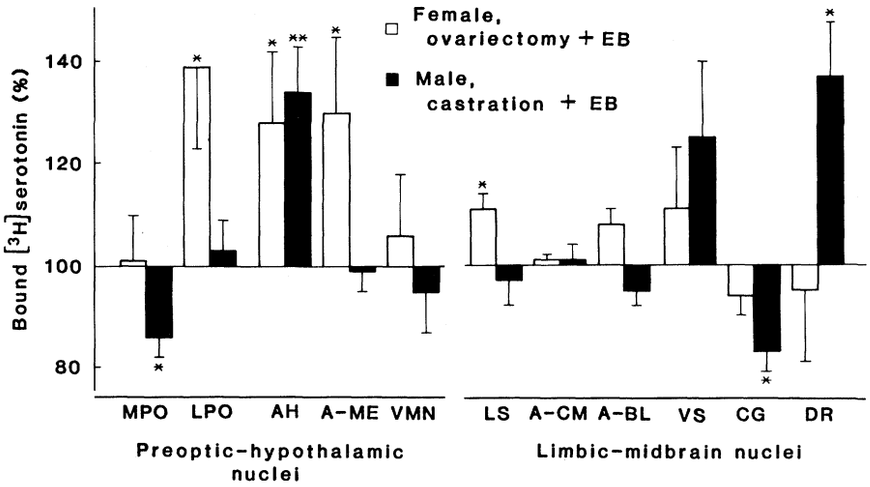

Fig. 1. Mean (± standard error of the mean) tritiated serotonin specifically bound (as a percentage of control values) obtained from rats subjected to gonadectomy in microdissected preoptic, hypothalamic, and limbic-midbrain nuclei. Conditions and number of experiments are listed in Table 1. Abbreviations: *MPO*, medial preoptic area; *LPO*, lateral preoptic area; *AH*, anterior hypothalamic nucleus; *A-ME*, arcuate–median eminence; *VMN*, ventromedial nucleus of the hypothalamus; *LS*, lateral septum; *ACM*, corticomedial amygdala; *ABL*, basolateral amygdala; *VS*, ventral subiculum of the hippocampal formation; *CG*, central gray; *DR*, dorsal raphe nucleus. In comparison with control rats subjected to gonadectomy, $*P < 0.05$, $**P < 0.01$ (two-tailed paired *t*-test).

Table 2. Effect of castration and estradiol benzoate (EB) on serotonin 1 receptor binding in several brain regions of male rats. Results are means ± standard error of the mean of three to eight repeated experiments, each representing six or seven animals in each treatment group. Binding capacity (B_{max}) and dissociation constant (K_d) were analyzed according to the method of Scatchard (*26*). Linear regression coefficients of the Scatchard plots for each structure ranged from 0.81 to 0.91. The midbrain (medial portion) was dissected to maximize binding to the dorsal and median raphe nuclei; hence two vertical cuts were made at the lateral borders of the central gray, and one horizontal cut at its dorsal border. The remaining block of tissue was used for binding experiments.

Brain region	Intact	Castrated	Castrated + EB
	B_{max} *(fmole per milligram of protein)*		
Septum	633 ± 43	384 ± 70*	367 ± 60*
Preoptic area	521 ± 77	589 ± 68	398 ± 69†
Hypothalamus	501 ± 75	391 ± 65*	323 ± 58*
Amygdala	763 ± 143	637 ± 110	554 ± 250
Midbrain	729 ± 207	499 ± 190*	787 ± 289†
Cortex		302 ± 100	234 ± 79
	K_d *(nM)*		
Septum	4.0 ± 0.6	3.2 ± 0.3	2.5 ± 0.1
Preoptic area	2.8 ± 0.4	5.1 ± 0.7*	3.9 ± 1.5
Hypothalamus	3.5 ± 0.7	3.2 ± 0.6	3.0 ± 0.7
Amygdala	5.0 ± 1.0	3.6 ± 0.7	3.0 ± 0.6
Midbrain	5.1 ± 1.2	3.3 ± 0.9	5.7 ± 1.6
Cortex		4.5 ± 1.7	3.0 ± 0.3

*Versus intact, $P < 0.05$. †Versus castrated, $P < 0.05$.

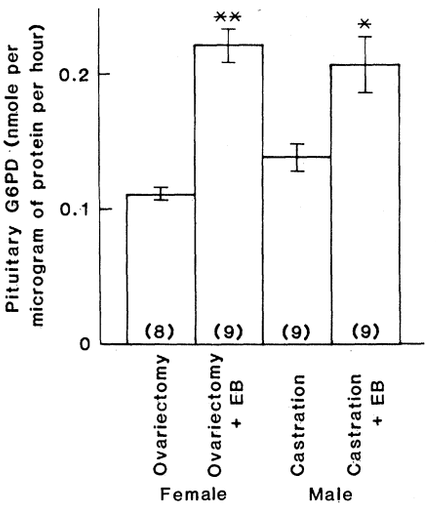

Fig. 2. Mean (± standard error of the mean) pituitary glucose-6-phosphate dehydrogenase (G6PD) activity assayed in duplicate from same animals as those used for Fig. 1. The number of animals is indicated in parentheses. In comparison with control rats subjected to gonadectomy, $^{*}P < 0.01$, $^{**}P < 0.001$ (two-tailed t-test of independent samples).

mone—or secondary—up-or-down regulation as a consequence of changes in local neuronal firing rate. Primary effects might be mediated directly, by steroids acting on membrane receptors (*3, 21*), or indirectly, such as by estrogen acting on the genome mediated by estrogen receptor complexes (*1*) with resultant changes in the relative rates of serotonin 1 receptor synthesis and degradation. The different response pattern of serotonin 1 receptor binding in males and females to estrogenic stimulation indicates that certain nuclei are processing the same information in a manner specific to sex. This might be accomplished by a difference in intracellular programming of responses to hormones or by a difference in neuronal circuitry.

The hormonal influence on neurotransmitter receptor binding may partially explain changing sensitivities of the central nervous system to incoming stimuli, such as drugs or hormones. Changes in brain sensitivity have been hypothesized to account for the onset of puberty and menopause and menstrual and estrous cycles, along with their resultant changes in gonadotropin secretion. In some affective disorders and premenstrual tension, hormone-induced changes in neurotransmitter receptors may cause the response to an incoming stimulus to vary as a function of the state of the neurotransmitter receptor at a particular time. Ultimately, hormone modulation of neurotransmitter receptors may represent another facet of hormone action on the brain; we speculate that this may represent a new level of control of hormone secretion.

In addition to the sex differences found in the serotonin 1 receptor system, sex differences have also been found in the "serotonin behavioral syndrome" (*22*), which is modulated by peripheral androgens through androgen receptors (*23*). The behavioral syndrome is linked to the serotonin receptor system (*24*). The sex differences observed in the serotonergic receptor system (serotonin 1, 2, or both) may be important in the reproductive capacity of an organism, as well as in the etiology of depressive illness in humans (*25*) and their subsequent clinical response to antidepressant drugs (*8*).

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16. We used the Palkovits punch technique (a method characterized by restricted anatomical dissection) to assay homogenates obtained from medial or lateral preoptic nuclei of animals subjected to gonadectomy; males bound more tritiated serotonin than females did. However, saturation experiments and subsequent Scatchard analysis on washed membrane preparations obtained from grossly dissected preoptic area (which unavoidably includes surrounding areas) indicated that females have more binding sites than males {(i) number of binding sites (B_{max}): female, 646 ± 106 fmole/mg; male, 484 ± 88 fmole/mg; [$t(4) = 4.70$, $P < 0.01$ (two-tailed, paired)]; (ii) dissociation constant (K_d): female 3.2 ± 0.6 nM; male 3.1 ± 0.7 nM ($N = 5$ separate experiments)}. Preliminary analysis of autoradiograms obtained from males and females without gonads indicate that the binding of [^{3}H]serotonin to its receptor sites within this region was not homogeneous. Marked differences occurred within the preoptic area itself. The substantia innominata, including the ventral pallidum, was an area of intense [^{3}H]serotonin binding. The olfactory tubercle, including the islands of Calleja, bound [^{3}H]serotonin more moderately. Analysis at this more localized level by autoradiographic techniques has not yet been completed.
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