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Short communication

# Gonadal steroid hormones upregulate medial preoptic μ-opioid receptors in the rat

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**Abstract**

The density of μ-opioid receptors is dependent upon the levels of gonadal steroid hormones in the medial preoptic area of ovariectomized female rats. Because the region containing these cyclical μ-opioid receptors is relatively small, autoradiographic analysis of the binding of a saturating concentration of μ-selective radioligand was used to determine whether hormone treatment affects maximal binding capacity of these receptors. The results reveal that gonadal steroid hormones upregulate μ-opioid receptor binding capacity specifically in the medial preoptic area 27 h later, without affecting binding in adjacent brain regions. This suggests that hormones induce a functional increase of medial preoptic area μ-opioid receptors which could serve to regulate reproductive activities.

*Keywords:* μ-Opioid receptor; Estrogen; Progesterone; Medial preoptic area; Upregulation

## 1. Introduction

Endogenous opioid peptides in the medial preoptic area of the hypothalamus reportedly regulate reproductive behavior (Sirinathsinghji, 1986) and gonadotrophin secretion (Kalra, 1981). The density of μ-opioid receptors, which readily bind ß-endorphin (Paterson et al., 1983), is cyclical across the estrous cycle in the medial preoptic area of female rats (Hammer, 1990), and is dependent upon the levels of gonadal steroid hormones (Mateo et al., 1992). Homogenate studies have shown that chronic estrogen exposure increases the binding capacity of μ-opioid receptors in the anterior hypothalamus (Wilkinson et al., 1985). However, the hormone-induced alteration of medial preoptic μ-opioid receptors has not been pharmacologically characterized. Therefore, the present autoradiographic study was undertaken to determine whether hormone treatment alters the maximal binding capacity of μ-opioid receptors using a saturating concentration of a μ-selective radioligand in brain sections containing the medial preoptic area.

## 2. Materials and methods

### 2.1. Animals and hormonal treatment

Female Sprague-Dawley rats weighing 150–175 g were obtained from the National Cancer Institute (Frederick, MD, USA) and were maintained in light- (on from 05:00 to 19:00 h) and temperature- (21–24° C) controlled rooms with ad libitum access to food and water. After 14 days acclimation to the laboratory environment, rats were ovariectomized at 60–70 days of age under fluothane anesthesia and allowed to recover for 2 weeks. Silastic capsules (2 mm long, O.D.: 0.125 in, I.D.: 0.078 in.; Dow Corning Corp., Midland, MI, USA) were either filled with crystalline 17ß-estradiol or remained empty, and were soaked in absolute ethanol for 1 h followed by sterile 0.05 M phosphate buffered saline solution overnight. Animals were then divided into two groups of five rats per group, and capsules were implanted subcutaneously under

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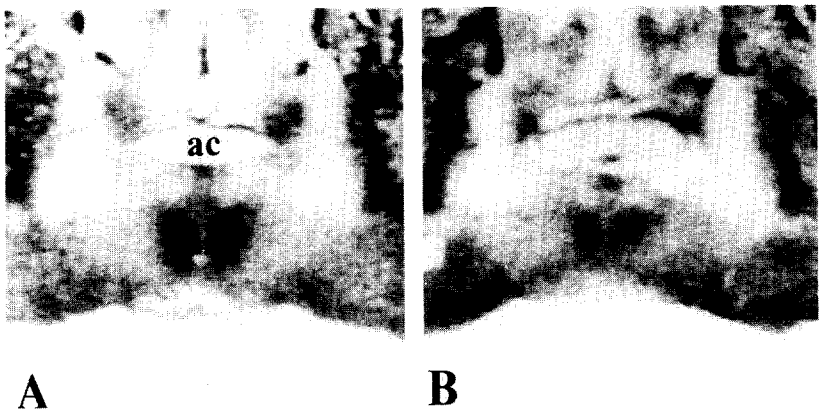


Fig. 1. Binding of 10 nM [$^3$H]DAGO to $\mu$-opioid receptors in autoradiographs of sections taken through the medial preoptic area of the hypothalamus. Binding density is greater in the medial preoptic area following gonadal steroid hormone treatment (A) than after vehicle treatment (B) in ovariectomized rats. Binding density in surrounding regions is unaffected. ac, anterior commissure. Calibration bar: 1 mm.

fluothane anesthesia. Forty-eight hours later, the capsules were surgically removed under fluothane anesthesia and 2.5 mg progesterone or 250 $\mu$l propylene glycol vehicle was administered subcutaneously. Animals were anesthetized and decapitated 27 h after progesterone injection. This hormonal treatment paradigm yields similar plasma hormone levels to those present during diestrus, and increases $\mu$-opioid receptor binding density in the rat medial preoptic area (Mateo et al., 1992).

### 2.2. *In vitro receptor autoradiographic procedures*

Following decapitation, brains were removed, frozen in $-35°$ C 2-methylbutane, and sectioned at 20 $\mu$m in a $-14°$ C cryostat. Sections were thaw-mounted onto gel-coated glass slides, dried in an evacuated desiccator jar at 0° C, and stored at $-70°$ C during collection. Slides were preincubated for 30 min at 4° C in 15 mM Tris HCl (pH 7.4), 150 mM NaCl and 1 mg/ml bovine serum albumin, then incubated for 60 min at 23° C in 50 mM Tris HCl (pH 7.4), 3 mM MnAc, 1 mg/ml bovine serum albumin and 10 nM [$^3$H][D-Ala$^2$, MePhe$^4$,Gly-ol$^5$]enkephalin (DAGO; 55 Ci/mmol; New England Nuclear). Slides were then rinsed $2 \times 1$ min in 50 mM Tris HCl (pH 7.4) at 4° C, gently dried with cool air, and exposed to X-ray film (Hyperfilm-$^3$H; Amersham) for 8 weeks at 23° C. Nonspecific binding in the presence of 10 $\mu$M levallorphan was determined to be less than 9% of total binding. Following film development, serial autoradiographs of sections containing the preoptic and adjacent septal regions were analyzed by computer-assisted quantitative film densitometry (NIH-Image, Wayne Rasband, NIMH, Bethesda, MD, USA). Regional receptor density in terms of fmol/mg was determined using calibrated autoradiographic standards co-exposed with tissue sections (Geary et al., 1985). The calculated values represented total ligand binding across the brain region of interest. Statistical analyses of the data were performed using Student's $t$-test.

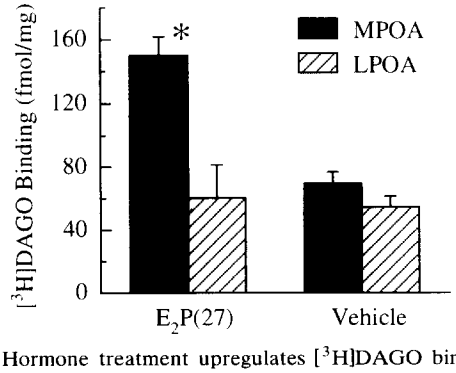


Fig. 2. Hormone treatment upregulates [$^3$H]DAGO binding in the medial preoptic area (MPOA), but not in the adjacent lateral preoptic area (LPOA). Data shown represent mean ± S.E.M. from five animals in each experimental group.

## 3. Results

The distribution of $\mu$-opioid receptors in autoradiographs of the medial preoptic area is similar to that reported previously (Hammer, 1990). A central core of dense receptors, located adjacent and lateral to the preoptic periventricular nucleus and midway between the anterior commissure and optic chiasm in a coronal section (Fig. 1), appears to extend caudally and dorsolaterally toward the bed nucleus of the stria terminalis.

Quantitative comparison of [$^3$H]DAGO binding density revealed that $\mu$-opioid receptors located in the central medial preoptic area are significantly ($P \leq 0.01$) denser in the hormone-treated group than in the vehicle-treated group (Fig. 2), in which binding density was 56% lower. Hormonal treatment did not alter [$^3$H]DAGO binding density in either the lateral preoptic area (Fig. 2), or in the medial septum (67.7 ± 4.1 vs.

63.1 ± 1.8 fmol/mg after hormone or vehicle treatment, respectively) or the lateral septum (62.8 ± 0.4 vs. 61.5 ± 2.1 fmol/mg after hormone or vehicle treatment, respectively) in the same coronal sections. Opioid binding density in these latter brain regions did not differ significantly between the hormone and vehicle treatment groups.

## 4. Discussion

### *4.1. Effects of gonadal steroid hormones on maximal binding density of medial preoptic μ-opioid receptors*

The binding conditions and ligand utilized herein to demonstrate opioid receptors are recognized to selectively label μ-opioid receptors (Mansour et al., 1987). Saturation analyses using these conditions in brain sections reveal a single component of binding with a $K_D$ of 1.3 nM (Hammer, 1990). Thus at the concentration utilized, approximately 90% of available receptors are occupied by radioligand. This saturating concentration is optimal to detect changes in binding capacity rather than binding affinity (Brady et al., 1989). Therefore, our results suggest that this paradigm of gonadal steroid hormone treatment increased the maximum binding capacity ($B_{max}$) of μ-opioid receptors in the medial preoptic area. However, since the observed hormone-induced increase of binding capacity is actually much greater than that observed using a lower non-saturating concentration of radioligand to assess the effect of the same hormone treatment paradigm (Mateo et al., 1992), a compensatory reduction of binding affinity might also exist.

These results support the hypothesis that gonadal steroid hormones directly regulate the expression of medial preoptic μ-opioid receptors, which have relatively high affinity for endogenous ß-endorphin (Paterson et al., 1983). The density of ß-endorphin-like immunoreactive fibers in the medial preoptic area is cyclicity and gonadal-steroid hormone dependent (Cheung et al., 1995), and the pattern of hormonal regulation of ß-endorphin-like immunoreactive fiber density is quite similar to that of medial preoptic μ-opioid receptors. For instance, ß-endorphin-like immunoreactive fiber density increased remarkably 27 h after experimental estradiol/progesterone treatment of ovariectomized females (Cheung et al., 1995) as did medial preoptic μ-opioid receptor density (Mateo et al., 1992). Thus, ß-endorphin and its putative receptor are apparently regulated independently by gonadal steroid hormones in the medial preoptic area, rather than the endogenous ligand inducing compensatory alteration of its own receptor.

Gonadal steroid hormonal control of medial preoptic μ-opioid receptor expression may produce an initial decrease (Jacobson and Kalra, 1989) followed by a delayed increase (Mateo et al., 1992) in response to progesterone following estradiol priming. Protein synthesis is apparently required for the hormonal upregulation of medial preoptic μ-opioid receptors, which can be prevented by anisomycin treatment, but only if protein synthesis is inhibited immediately following progesterone treatment (Hammer et al., 1994). The delayed increase of binding capacity could be due to hormonal induction of μ-opioid receptor expression at a distant site, with subsequent transport of the protein to synaptic terminals located in the medial preoptic area. For instance, opioid modulation of norepinephrine release in the medial preoptic area is hypothesized to act via μ-opioid receptors located presynaptically upon noradrenergic terminals (Dyer and Grossmann, 1986). Opioids could thus provide a rapid or tonic control of noradrenergic function in this brain region.

### *4.2. Functions of medial preoptic μ-opioid receptors related to gonadal steroid hormones*

Our results reveal that hormone-induced μ-opioid receptor upregulation is regionally specific. It is only observed in the central medial preoptic area; no significant difference exists in the lateral preoptic area, the medial septum or the lateral septum, suggesting that these regions might not, therefore, be involved in the regulation of gonadal steroid-dependent reproductive function. Opioid suppression of lordosis and gonadotropin release can be reversed by naloxone (Kalra, 1981; Sirinathsinghji, 1986), suggesting that these effects are mediated by opioid receptors. Our results reveal that estradiol/progesterone priming ultimately increases the binding capacity of medial preoptic μ-opioid receptors. Thus, the functional activation of medial preoptic μ-opioid receptors could serve to modulate reproductive behavior during the late portion of the estrous cycle. A rapid hormone-induced reduction of medial preoptic μ-opioid receptors (Jacobson and Kalra, 1989) could also be permissive toward stimulation of gonadotropin release by other factors. Furthermore, homogenate binding assays of hypothalamic μ-opioid receptors have revealed increased receptor concentration during parturition compared to lactation, without a change in binding affinity (Dondi et al., 1991). We have suggested that this alteration might be related to opioid regulation of maternal behavior (Hammer et al., 1992).

In summary, the results suggest that gonadal steroid hormones increase medial preoptic μ-opioid receptor number. The actual mechanism of this receptor upregulation remains to be determined. The data confirm that this hormonal effect is regionally specific, suggesting that μ-opioid receptors in the medial preoptic area might be involved in the regulation of gonadal steroid

hormone-dependent reproductive function in female rats.

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