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# CHANGES IN RAT BRAIN OPIATE RECEPTOR CONTENT UPON CASTRATION AND TESTOSTERONE REPLACEMENT

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## Summary

The saturable binding of naltrexone-$^{3}H$ in the brains of castrated male rats exceeds that found in intact animals by a factor of two. This increase is androgen dependent since testosterone replacement reduced the binding to control levels. Scatchard analysis of the saturation curves revealed that the change in binding reflects increased available binding sites and is not due to increased binding affinity. This relationship between testosterone and brain opiate receptors provides for the participation of endorphins in the regulation of pituitary gonadotropins by gonadal hormones. The increased content of opiate receptors in the brains of castrated rats correlates with the greater brain N-demethylation of morphine establishing a further link between this biotransformation and agonist action.

Narcotic agonists exert profound effects on the hypothalamic-pituitary-gonadal axis in both experimental animals and in man. In the male rat it has been established that the interaction occurs at the hypothalamic levels (1,2) and that it is expressed in a suppression of pituitary LH secretion resulting in a reduction of testicular testosterone production (1). More recently it has been reported (3) that the opiate antagonists induce an increase in LH secretion. This action of the antagonists which occurs also at the hypothalamic level (3) indicated that the endogenous opiate peptides at that central site participate in the physiological control of pituitary gonadotropin secretion. Gonadotropin secretion is also suppressed by the testicular hormone testosterone acting at central sites in a negative feedback mode (3). The similarity in the inhibitory action of the opiate agonists and testosterone on LH secretion and the high concentration of both androgen and opiate receptors in the hypothalamus suggested the possibility that the physiological control of gonadotropin secretion by testosterone may be modulated by the endorphins. We now present evidence of

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such an interrelationship between testosterone and the endorphins in that we demonstrate that the male hormone has a profound effect on the opiate receptors in the male rat brain.

## Materials and Methods

Male CD rats (Charles River Laboratories) were sacrificed at least three weeks post castration by cervical fracture. The brain minus cerebellum was homogenized in 5 volumes cold Tris buffer 0.05M, pH 7.4 by means of a motor driven teflon pestle, and centrifuged at 1000 x g for 10 minutes. The supernatant was decanted and centrifuged at 17500 x g for 20 minutes and the resulting pellet was resuspended in 60 volumes of Tris buffer 0.05M, pH 7.4. Brain tissue suspensions from intact CD rats were prepared exactly as described above. In another series of experiments castrated male rats were injected subcutaneously with testosterone propionate (2.5 mg/kg) for seven days. Animals were sacrificed on the eighth day and brain suspensions were prepared as above.

Tissue preparations (2.0 ml) were incubated with or without unlabelled naltrexone ($10^{-6}$M) and with increasing concentrations of naltrexone-$^{3}$H (S.A.= 25 Ci/mmole, National Institute on Drug Abuse) ranging from 2 x $10^{-10}$M to 7.5 x $10^{-9}$M for 15 minutes at 37°. Each experiment was performed in triplicate. After the incubation the samples were allowed to sit in ice for 30 minutes after which they were filtered through Whatman GF/B filters under vacuum. The filters were washed two times with 4 ml cold Tris buffer then shaken in 15 ml Aquasol-2 (New England Nuclear) and assayed for radioactivity using liquid scintillation counting. The binding observed in the presence of $10^{-6}$M unlabelled naltrexone (non-saturable binding) was subtracted from that observed in the absence of unlabelled drug (total binding) to calculate the saturable binding. Protein concentrations were determined by the method of Lowry et al. (4).

## Results and Discussion

The results of the experiments in which the effect of castration and testosterone replacement therapy on binding to the opiate receptor are measured are presented in Fig. 1. In each case the difference between total and nonsaturable binding of naltrexone-$^{3}$H to brain particulate fractions (P-2) showed a saturation effect with increasing concentration of naltrexone-$^{3}$H. Saturable binding was increased almost 100% in the brain preparations from castrated rats relative to intact animals. This increase in saturable binding was abolished in the brain preparation from castrated animals treated with testosterone propionate for seven days indicating that the observed effect is androgen related. Scatchard analysis of the data in Fig. 1 reveals that there are two saturable binding sites for $^{3}$H-naltrexone in each of the brain preparations assayed (Fig. 2). Previous reports have demonstrated the existence of high

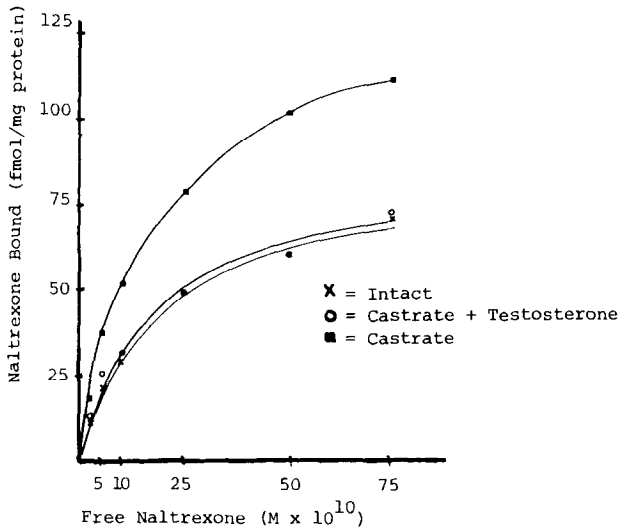


Fig. 1. Saturation curves for stereospecific binding of naltrexone-$^{3}$H with rat brain P-2 fractions.

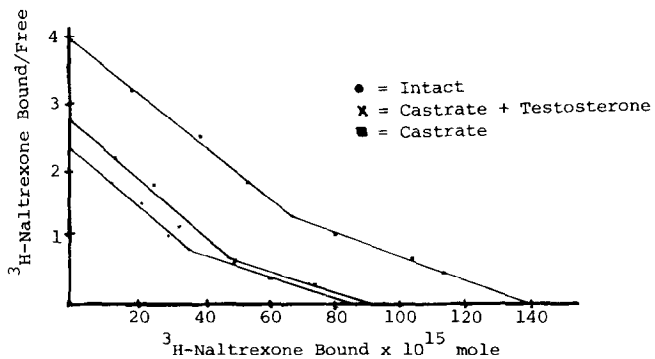


Fig. 2. Scatchard analysis of naltrexone-$^{3}$H binding with P-2 fractions in the brains of intact, castrate and castrate rats receiving testosterone replacement. $B_{max}$ high affinity site (fmol/mg protein): intact = 54.7 ± 5.1; castrate = 100.2 ± 7.4; castrate + testosterone = 58.8 ± 4.8. $B_{max}$ low affinity site: intact = 88.8 ± 6.5; castrate = 142.1 ± 8.3; castrate + testosterone = 94.8 ± 7.3.

and low affinity opiate binding sites in the brain although their significance at this time is still obscure (5,6). In our binding studies the dissociation constants (Kd) do not differ significantly for either of the two binding sites between the various brain preparations. The maximal saturable binding ($B_{max}$) was however significantly higher in the castrated animals for both the high and low affinity binding sites, being almost double that in the intact animals.

This difference was not observed in brain preparations from the castrated animals that were subjected to testosterone replacement. The significance of the increases in opiate receptor content in the brains of the castrated rats is elusive. Direct competition of testosterone for the opiate receptor is not being considered because of the absence of any affinity of the steroid for the opiate receptor (7). The increase in opiate binding sites may then reflect either new synthesis of receptor molecules, or it may reflect an increase in vacant receptors due to a reduction in endogenous ligand concentrations. The latter appears to be the more likely rationale and it supports the speculation that the negative feedback effect of testosterone on LH secretion is mediated by an induction of opiate peptides in the appropriate central site by the androgen. This concept conforms to the experimental observation that naloxone blocks the negative feedback effect of testosterone in castrated rats (3). The present results provide the first evidence of a direct relationship between the gonadal hormones and the endorphins in the brain and they offer a biological link for the neuroendocrine functions of the two substances.

It is important to add that we have previously observed that the N-demethylation of morphine is greatly increased in the brains of castrated animals compared to intact males and that this effect is also testosterone dependent (8). We have elsewhere presented evidence that such N-demethylation in the brain may be related to opiate receptors and the mechanism of agonist action (9-11). The present results show that the changes in brain opiate receptor content and morphine N-demethylation upon castration and upon testosterone replacement are coincident in direction and degree, and offer further support for the intimate relationship of brain N-dealkylation to narcotic agonist activity.

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