Opiate regulation of estradiol-2-hydroxylase in brains of male rats: Mechanism for control of pituitary hormone secretion*

(Jack Fishman, Baiba I. Norton, and Elliot F. Hahn)

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ABSTRACT

Treatment of male rats with a single high dose of morphine (10 mg/kg, subcutaneously) results in a dramatic suppression of brain estradiol-2-hydroxylase activity. The suppression is blocked by naloxone and is decreased upon the development of tolerance. The injection of naloxone (0.4 mg/kg) alone produces a significant increase in brain estradiol-2-hydroxylase activity over control levels. The effects of the opiate agonists and antagonists on the activity of this brain enzyme coincide in degree and direction with their effects on plasma luteinizing hormone (luteinizing hormone) concentrations. Because the 2-hydroxyestrogens were shown to induce pituitary lutropin release, the present results indicate that the action of opiates, endogenous or exogenous, on pituitary gonadotropin release can be mediated by brain catechol estrogens.

Administration of a single high dose of morphine or other narcotic agonists to male rats results in a suppression of plasma lutropin (luteinizing hormone) (1). The effect is opiate specific, subject to the development of tolerance, and exerted at hypothalamic rather than pituitary sites (2). Opiate antagonists under the same conditions provoke a sharp increase in concentrations of rat plasma lutropin (3, 4), implying that the endogenous opiate peptides, like the exogenous narcotics, act to inhibit pituitary lutropin secretion (4). The regulation of pituitary gonadotropins is also under the control of the gonadal hormones, which is in part exercised at hypothalamic sites (5), raising the possibility that the opiate effects on gonadotropin secretion are mediated through interaction with central steroid neuroendocrine mechanisms. Identification of such interactions would be of considerable assistance in the elucidation of the mechanisms underlying the physiological regulation of gonadotropins by steroid hormones, the precise nature of which is still obscure. The finding that estrogens are converted to 2-hydroxyestrogens in the brain (6, 7) and that the latter inhibit enzymes involved in catecholamine biosynthesis (8) and metabolism (9) provoked speculation that the 2-hydroxylation of estradiol in central sites participates in the regulation of pituitary hormone release. The augmentation of plasma lutropin by the parenteral administration of 2-hydroxyestrone in male (10) and female rats (11) further suggested that the catechol estrogens may function in the stimulatory mode of the biphasic feedback action of estradiol. The effect of the opiates on lutropin secretion could be expressed by their modulation of the synthesis of the catechol estrogens in the brain. We therefore sought information on the impact of opiate agonists and antagonists on the activity of the estradiol-2-hydroxylase in male rat brains. Existence of such a relationship would not only shed light on the nature of the opiate interaction with the hypothalamic-pituitary axis, but would also provide support for the role of 2-hydroxyestrogens in the control of gonadotropin release.

MATERIALS AND METHODS

Male CD rats (225–250 g) in groups of four were injected subcutaneously with morphine hydrochloride (10 mg/kg). The animals were killed 2 hr later by cervical fracture. Whole brains, except for the cerebellum, were pooled and homogenized in 0.32 M sucrose. The microsomal fraction was obtained by standard procedures. Liver microsome preparations from the same animals were obtained similarly. [2-3H]Estradiol (0.25–0.75 nmol; specific activity 20 Ci/mmol; 1 Ci = 3.7 X 10^10 becquerels) was incubated in triplicate with the microsomal suspensions in 0.1 M Tris-HCl (pH 7.2). The incubations were allowed to proceed for 60 min under air at 37°C, after which the mixtures were frozen and lyophilized. The specific activity of the water lyophilizate was determined in a Packard scintillation spectrometer. Protein determinations were made in triplicate by the method of Lowry. Control animals received saline injections and were otherwise processed identically.

To test the specificity of the morphine effect, the above experiment was repeated except that naloxone (100 μg) was administered simultaneously with the morphine (10 mg/kg) and then three additional injections of 100 μg each of naloxone were given at 0.5-hr intervals until the animals were killed 2 hr after the first injection.

The effect of prolonged morphine treatment on estradiol-2-hydroxylase was studied in rats made tolerant to morphine by successive implantation of morphine pellets at 5-day intervals (12). The animals were killed 72 hr after the second pellet had been implanted, and brain and liver microsomes were obtained as described above. Control tissues were obtained in a similar manner from rats implanted with placebo pellets.

To examine the effect of naloxone alone on estradiol-2-hydroxylase activity in rat brain microsomes, we injected naloxone hydrochloride (100 μg) subcutaneously into naive male rats. To obtain information about the time course of the effect, we killed the animals in groups of four at intervals of 5, 10, 15, and 20 min after the injection.

The assay of the enzyme in the present experiment was limited to the microsomal fraction of the brain because the subcellular distribution of rat brain estradiol-2-hydroxylase is predominantly in that particulate fraction (7, 11). The liver microsomal preparations obtained from each group of rats at the same time were assayed for estradiol-2-hydroxylase in order to provide positive controls except that there is not assurance that the brain and liver enzymes are identical. Enzyme activity was measured by the radiometric procedure (1), which has recently been validated (1) and which has the advantage of superior sensitivity compared to the radioenzymatic assay (13).

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RESULTS AND DISCUSSION

The results of the measurements of enzyme activity in brain microsomes from the different morphine treatment groups are presented in Fig. 1. The administration of a pharmacological dose of morphine (10 mg/kg) results in a highly significant 70% decrease (P < 0.01) in brain microsomal estradiol-2-hydroxylase activity compared to control animals. This inhibition of enzyme activity by morphine is almost completely blocked by the antagonist naltrexone; when naltrexone is coadministered with the morphine dose, the decrease in enzyme activity is only 15% compared to the 70% decrease achieved by morphine alone. The naltrexone antagonism of the morphine-induced enzyme suppression offers evidence that the effect is opiate specific.

Prolonged exposure to the narcotic produces a decrease in enzyme activity that is significantly less than that observed with the single treatment. After a 6-day exposure to morphine the enzyme levels were only 30% lower than controls compared to the 70% reduction after a single treatment. This attenuation in the morphine-induced suppression of enzyme activity upon chronic exposure to the narcotic is consistent with the initiation of tolerance to the effect of the agonist on the hypothalamic-pituitary-gonadal axis, which has been reported by Cicero et al. (14).

In contrast to the large changes in brain estradiol-2-hydroxylase activity under the influence of the narcotic, the activity of the corresponding liver enzyme was only minimally affected in the opposite direction with both a single dose of morphine and prolonged morphine treatment resulting in modest 15% and 19% increases, respectively, in activity relative to controls. The direction and degree of the changes in liver enzyme emphasize the special nature of the fluctuations in the brain enzyme and lends support to the neurobiological significance of the latter.

The results obtained upon injection of the narcotic antagonist naltrexone alone are of particular interest (Fig. 2). Five minutes after the injection of 100 µg of naloxone, a significant 37% increase in the brain microsomal estradiol-2-hydroxylase is observed. The increase in activity is sustained at 10 min, and at 15 min the enzyme activity rises further, reaching 61% above the control level. The stimulation appears to have peaked at 15 min; the brain microsomes obtained 20 min after the injection exhibit estradiol-2-hydroxylase activity only 29% greater than that of the control specimens.

The suppression of estradiol-2-hydroxylase in rat brain by morphine has all the hallmarks of specific opiate agonist action and, hence, presumably could be duplicated by other narcotic agonists. The effect is blocked by naltrexone and is greatly re-

![Graph](image1)

**Fig. 1.** Effect of morphine on microsomal estradiol-2-hydroxylase in male rat brain (Left) and liver (Right). M: 16 male CD rats in groups of four were injected with morphine hydrochloride (10 mg/kg) and killed 2 hr later by cervical fracture. Brains (except for cerebellum) and liver samples were dissected and homogenized in 0.32 M sucrose, and microsomes were prepared. M + N: Injections were morphine-HCl (10 mg/kg) plus naloxone-HCl (100 µg). Naloxone-HCl (100 µg) injections were repeated every 30 min until the animals were killed at 2 hr. TOL: Morphine pellets were implanted subcutaneously. Implantations were repeated after 3 days and animals were killed on day 7. Control animals received placebo pellet implants. Incubations were carried out in 0.1 M Tris-HCl (pH 7.2) under air at 37°C for 60 min. Each incubation mixture contained tissue (1 mg of protein per ml), NAD (1 µmol/ml), NADP (1 µmol/ml), glucose-6-phosphate dehydrogenase (1 unit/ml), glucose-6-phosphate (15 µmol/ml), and [2-3H]estradiol (20 Ci/mmol; 50 pmol/ml). Incubation volumes were 5-15 ml, depending on quantity of tissue available. At termination, incubation mixtures were flash frozen and lyophilized; the radioactivity of 1-ml samples of lyophilized water was measured in triplicate in a Packard scintillation spectrometer. Control incubations were carried out as above except that the tissue was omitted; values obtained were treated as blanks and subtracted from tissue incubation results. Results are shown as means of separate determinations (n = 4) with the bars representing SD and are presented as percent of enzyme activities in control animals, which were 3.75 ± 0.32 and 24.96 ± 1.85 pmol of 3H2O per mg of protein per hr in the brain and liver, respectively (1 pmol of 3H2O = 20,000 cpm). Statistical analysis revealed that in the brain the M and TOL values were significantly different from controls: P < 0.01 and P < 0.05, respectively. In the liver, the M and TOL values were significantly higher than controls (P < 0.05).

![Graph](image2)

**Fig. 2.** Elevation of microsomal estradiol-2-hydroxylase activity in male rat brain by naloxone. Male CD rats were injected subcutaneously with 100 µg of naloxone-HCl. The animals were killed in groups of six at 5, 10, 15, and 20 min after the injection. Brain microsomes were prepared and incubations were carried out as in Fig. 1. Results are presented as means ± SD of three determinations and are recorded as pmol of 3H2O generated per mg of protein per hr. Statistical analysis shows that all time values are significantly higher than controls (P < 0.01). The 15-min value is also significantly higher than the values at other times (P < 0.05).
duced upon the development of tolerance. The stimulation of the brain enzyme activity by naloxone carries the implication that estradiol-2-hydroxylase is also suppressed by endogenous opiates and confers a physiological regulatory role on this interaction. In this communication we report a biochemical link between the opiates and the female sex hormone in the brain. The interrelationship may have considerable relevance to the mechanisms underlying the various behavioral and central physiological actions of both the endorphins and the female sex hormones, but our present concern is with the role of this interaction in the regulation of pituitary lutropin release in male rats. Although there is little doubt that the changes in brain enzyme are a direct consequence of opiate action, there is some question whether they are related to the changes in plasma lutropin concentrations. The direction of the changes in brain enzyme activity and plasma lutropin concentrations induced by opiate agonists and antagonists in male rats and their temporal relationship argue forcibly that the fluctuations in brain estradiol-2-hydroxylase participate in the mechanisms by which opiates regulate pituitary lutropin release. The agonists, which suppress pituitary lutropin release, also suppress the formation in the brain of 2-hydroxysterogens, catechol estrogens that have been implicated in the stimulation of lutropin secretion (10, 11, 12). Conversely, opiate antagonists, which elevate plasma lutropin levels, also increase the activity of the brain enzyme responsible for the catechol estrogen formation. The cause-effect relationship between the changes in brain enzyme activity and lutropin secretion is further strengthened by the sequence of these events. Two hours after morphine administration, at which time a large decrease in brain microsomal estradiol-2-hydroxylase activity is observed, is also a time at which morphine-induced plasma lutropin suppression has been reported to be at a maximum (15). For naloxone administration, the peak brain enzyme elevation observed occurred at 15 min after injection, whereas the peak elevation of plasma lutropin content is reported to occur at 20 min (3). This time sequence is well within the context of a 2-hydroxyestrogen stimulation of luteolin (lutropin-releasing hormone) release and the subsequent augmentation of pituitary lutropin secretion. The scheme that emerges from the present studies is that the opiates, endogenous or exogenous, regulate pituitary lutropin release by modulating the biosynthesis in the brain of the 2-hydroxyestrogens which act to stimulate lutropin release. The outlined mechanism requires the presence of estrogens as enzyme substrates at the neuronal sites of action, and it is probably concerned more with the control of acute rather than prolonged lutropin release. The opiate control is exercised by modulation of the stimulatory feedback action of catechol estrogens and it is thought to be independent of the mechanism of lutropin inhibition by testosterone. The blockade by naloxone of the inhibitory action of testosterone in castrated rats (3) and the inhibition by morphine of the elevation of plasma lutropin after castration (2) can readily be accommodated by postulating that these opiate effects represent the input of the catechol estrogen-mediated mechanism which compensates for, but does not interact with, the testosterone inhibitory action. The estrogens required to participate in the catechol estrogen pathway are available in the castrated rats from nongonadal sources and also from the central nervous system aromatization of the administered testosterone (16).

The present studies were limited to measuring enzyme changes in the microsomal fraction of whole brains. It is likely that changes in localized sites, such as the hypothalamus, may be more informative. The results already obtained, however, provide a plausible mechanism for the regulation of pituitary hormone secretion by the opiates and further support the role of in situ 2-hydroxylation of estrogens in the positive feedback action of the female sex hormone. Although the reported studies and their interpretation are directly applicable only to male rats, it is not unreasonable to extrapolate them to female rats. The blockade of the pre-ovulatory lutropin surge by narcotics (17) may very well proceed by their interference with the proestrous rise in brain estradiol-2-hydroxylase. The action of the opiate agonists and antagonists on concentration of human plasma lutropin (18) invites comparison with the results in rats and suggests that a commonality of underlying mechanisms may also exist.

The recent discovery that 2-hydroxyestrone greatly suppresses prolactin secretion in women (19), together with the established stimulation of prolactin release by agonists and its inhibition by antagonists (20) provides a link between the opiates and the estrogens in the control of prolactin release. The present results lend further credence to the concept that the effects of endorphins on prolactin release are mediated via their effect on brain estradiol-2-hydroxylase activity.

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