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Estrogen Treatment to Ovariectomized Rats Modifies Morphine-Induced Behavior[1]

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NOMIKOS, G., C. SPYRAKI, A. KAZANDJIAN AND A. SFIKAKIS. *Estrogen treatment to ovariectomized rats modifies morphine-induced behavior.* PHARMACOL BIOCHEM BEHAV 27(4) 611–617, 1987.—Two weeks after surgery, ovariectomized (OVX) rats were treated for 3 days with either 17β-estradiol (10 or 100 μg/kg, SC, per day) or the oil vehicle. They were then tested for morphine-induced hyperactivity (4 mg/kg, IP), analgesia and catalepsy (15 and 20 mg/kg, IP) 24 or 72 hr after the last steroid or oil injection. Estradiol treatment did not affect the locomotion or the sensitivity to nociceptive stimuli of OVX rats and did not induce a cataleptic state in animals. Estradiol- (100 μg/kg) treated OVX rats exhibited attenuated morphine-induced hyperlocomotion regardless of the time that had elapsed after estradiol treatment cessation, attenuated morphine-induced catalepsy at 24 hr after estradiol treatment and unaltered morphine-induced analgesia. OVX rats treated with a lower estradiol dose (10 μg/kg) exhibited significantly increased morphine-induced analgesia and slightly increased catalepsy. The results show that the sensitivity of brain opiate systems controlling some of the behavioral effects of morphine is modified following estradiol treatment to OVX rats.

Estradiol Morphine Locomotion Catalepsy Analgesia

OPIATES are known to influence a variety of physiological processes, i.e., pain, blood pressure, temperature, food intake, and behavior [7,25], and to exert diverse effects on various endocrine systems [15,33]. Evidence suggests that gonadal steroids may influence endogenous opioid systems activity [14,19]. Brain and pituitary levels of β-endorphin and methionin-enkephalin fluctuate during the estrous cycle [2, 20, 28]. Estradiol treatment reduces the resting levels of β-endorphin in the plasma and the intermediate lobe of the pituitary [34] as well as the hypothalamic β-endorphin content [41]. Besides the mutual interactions of estrogen and opioids at the hypothalamic-pituitary level, several lines of evidence suggest that gonadal steroids also modulate those actions of morphine, which appear to have a neuroanatomical substrate beyond the hypothalamic area [18, 21, 37]. For instance, estrogen increases methionin-enkephalin levels in the striatum [12]; the intensity of morphine-induced analgesia and catalepsy appears to be related to the stage of the estrous cycle and to the presence of ovaries [1]. Furthermore, reduced opiate receptors in the CNS seems to correlate with the decrease in the functional status of the ovary in ageing female rats [32]. These findings implicate gonadal steroids (estradiol and progesterone) as regulators of central opioid systems activity.

In this study, using a behavioral approach, we sought to investigate specifically the role of estrogen in the modulation of opioid systems sensitivity. To this end we studied morphine-induced behaviors in OVX rats treated with estrogen. The interest in this issue derives from the fact that estrogen modulates basal ganglia function (cf: [40]) and from the suggestion that dopaminergic mechanisms may be involved in some of the behavioral effects of morphine [9, 24, 27]. The clinical dimension of such an interest would be relevant to recent attempts to add estrogen [31,35] or opiates [7,15] in the treatment of neuropsychiatric diseases with dopaminergic dysfunction.

METHOD

Animals

Experimentally naive Wistar rats (N=165), bred in our laboratory, 70–75 days old, weighing 180 g were used. They were housed in groups of 8/cage in a light (12 hr cycle) and temperature controlled room with free access to food and water. Female animals, anaesthetized with ether, underwent bilateral ovariectomy 15 days before experimentation. Each animal was assigned to one of 18 groups (N=8–12/group) and was tested only once in each behavioral task. The assignment

[1]A preliminary report of this work was made at the 9th Annual Meeting of the ENA (Oxford 1985).

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TABLE 1

BODY WEIGHT (b.wt.) AND UTERINE WEIGHT (u.wt.) IN VEHICLE AND ESTRADIOL TREATED RATS 24 AND 72 HR FOLLOWING CESSATION OF THE TREATMENT

Treatment	Vehicle			Estradiol 100 μg/kg × 3			10 μg/kg × 3		
Time After Treatment Cessation	b.wt. (g)	u.wt. (mg)	u.wt. (mg)/ 100 g b.wt.	b.wt. (g)	u.wt. (mg)	u.wt. (mg)/ 100 g b.wt.	b.wt. (g)	u.wt. (mg)	u.wt. (mg) 100 g b.wt.
24 hr	182.50±2.3 (42)	133.8±9.9	72±4	180.0±3.0 (37)	243.9±8.1*	135±4*	185.6±6.4 (22)	252.9±15.5*	132±7*
72 hr	181.25±2.4 (26)	90.3±7.2	48±3	166.6±4.1* (38)	155.7±4.7*	96±3*			

b.wt.: body weight; u.wt.: uterine weight.
Data represent mean±SEM. Number of animals in parentheses; *$p<0.01$ with respect to vehicle treated animals.

TABLE 2

THE EFFECT OF ESTRADIOL TREATMENT (3 × 100 μg/kg) ON ACTIVITY, CATALEPSY AND PAIN THRESHOLD OF OVX RATS

Behaviour	Treatment	Withdrawal Time 24 hr	72 hr
Activity (c.e.)	oil	14.00 ± 8.0	12.00 ± 5.0
	estradiol	27.00 ± 12.0	22.00 ± 10.0
Catalepsy (sec)	oil	3.90 ± 1.0	4.70 ± 0.3
	estradiol	4.30 ± 0.8	4.40 ± 0.2
Pain Threshold (sec)	oil	14.95 ± 3.76	16.20 ± 1.5
	estradiol	11.79 ± 2.40	14.60 ± 2.2

c.e.: Compartments entered: seconds.
Activity represents the total 15 min activity in the open field. Catalepsy and pain threshold represent the mean ± SEM of three determinations. N=20–25/group.

of animals to groups was performed according to a 2×2×2 factorial design. The two levels of the first factor concerned a different treatment (oil/17β-estradiol, 100 μg/kg). In some cases a third level was introduced (17β-estradiol, 10 μg/kg). The two levels of the second factor corresponded to a different time which had elapsed between the last estradiol or vehicle injection and the behavioral test (24/72 hr). The third factor comprised also two levels each corresponding to a different dose of morphine (15/20 mg/kg) or to saline/morphine, 4 mg/kg injection. The same animals were tested for morphine-induced catalepsy and analgesia (9 groups, N=9–12/group) but different groups of animals were tested for morphine-induced activity (9 groups, N=8–10/group).

Estrogen Treatment

Estrogen treatment to OVX rats started 15 days following surgery. 17β-Estradiol (10 and 100 μg/kg/ml), dissolved in sesame oil, was injected SC to OVX rats for 3 consecutive days.

Behavioral Testing

The animals were tested 24 or 72 hr after the last estradiol or vehicle injection. The rationale for choosing those particular times to perform the behavioral test is the following: Previous studies have established that estradiol treatment induces dopamine receptor hypo- and hyperresponsiveness. The former is seen 24 hr after the last estradiol injection while the latter requires 40 hr or longer to develop (cf. [40]).

Behavioral experiments were performed between 10.00 and 16.00 hr. The effect of morphine on locomotor activity, catalepsy and analgesia was tested in estrogen- and vehicle-treated animals.

Locomotor Activity

Locomotor activity was measured in circular open fields, measuring 100 cm in diameter, with a wall of 40 cm in height and floor divided by radii and concentric cycles in 17 sections. The rats were individually placed in the open field for 15 min habituation, removed and injected IP with morphine or vehicle and immediately returned for an additional 1 hr test period. Locomotor activity was recorded as number of crossings performed by each rat from one section of the open field to another.

Catalepsy

Catalepsy was measured by gently putting the animal on a vertical wire mesh such that all four feet contacted the wire and recording the time in sec before the animal moved any one of its feet. After morphine injection, estrogen- and vehicle-treated rats were observed for 2 hr at 30 min intervals. The animals were kept in the home cage between measurements.

Analgesia

Analgesia was measured by the hot plate procedure with the plate maintained at 56°C. The rats were placed individually on an electrically controlled metal plate (Ugo Basile).

A Plexiglas cylinder, 30 cm high and open at the top, confined the rat to a defined area of the hot plate. The time that elapsed before animals licked one paw or jumped off the plate was recorded (response latency). If the animal failed to respond within 2 min the test was terminated. Tests were carried out before (pain threshold) and at 30 min intervals after

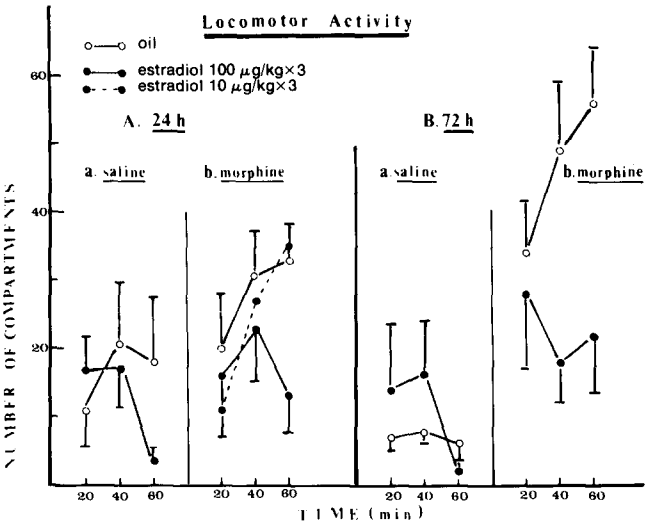

FIG. 1. Locomotor activity of OVX rats treated with 17β-estradiol or oil vehicle and challenged 24 or 72 hr following treatment cessation with morphine (4 mg/kg) or saline. Before the morphine or saline injection the rats were habituated in the open field for 15 min.

the injection of morphine. Recordings were made over a 2 hr period.

As the same dose of morphine can be used to induce catalepsy or analgesia, those behaviors were examined in the same animals following morphine injection. The order in which the tests were carried out at the different testing times following morphine injection was catalepsy and analgesia. The animals were kept in the home cage between recordings.

Estimation of Estrogen's Biological Action

Following completion of behavioral testing, animals were sacrificed and the weight of uteri was determined. Absolute and relative (mg/100 g of body weight) uterine weight served as an index of estrogen's biological activity. Results from animals treated with estrogen and not showing heavier (as compared to vehicle-treated rats) uteri were discarded.

Drugs and Chemicals

The following compounds were used: 17β-estradiol (Sigma) was dissolved in sterilized warm sesame oil. Morphine HCl (Drug Administration, Ministry of Health, Greece) was dissolved in 0.9% NaCl. All solutions were freshly prepared immediately before use. For locomotor activity the dose of 4 mg/kg was used, while for analgesia and catalepsy the dose of 15 and 20 mg/kg of morphine were administered.

Statistics

The data for uterine weight, responsivity to thermal stimulus, activity during habituation and catalepsy (before the administration of morphine) were subjected to two-way ANOVA with treatment (estradiol-vehicle) and time between the last estradiol injection and behavioral test (24–72 hr) as independent variables. The data following morphine administration were analyzed separately for analgesia, catalepsy and locomotion by three-way repeated measures ANOVA, using a multivariate analysis of variance program. In these analyses, the observations at different times were transformed into new variables: average, linear, quadratic and (for catalepsy and analgesia) cubic components. The average represented the mean of the three (activity) or four (catalepsy, analgesia) responses on each subject and the other components represented the shape of the profile of responses. The average was tested by univariate three-way ANOVA and the shape component by multivariate three-way analysis of variance, followed by univariate analyses to identify the source of significant differences [8].

RESULTS

The Effect of Estradiol Treatment to OVX Rats on the Uterine Weight

Table 1 shows that the weight of uteri of OVX rats is increased following estradiol treatment. The increase in the weight of uteri led to a two-fold ratio (u.wt./b.wt.) in estradiol-treated animals. Analysis of variance on the u.wt./b.wt. data yielded a highly significant treatment effect, $F(2,144)=80.87$, $p<0.0001$, and a significant effect of time which had elapsed after ovariectomy or after the last estradiol treatment, $F(1,133)=35.42$, $p<0.01$.

The Effect of Estradiol Treatment on the Activity, Catalepsy and Response to Thermal Stimulus of OVX Rats

Table 2 shows that estradiol treatment (3×100 μg/kg) to OVX rats did not modify the behaviors of animals observed 24 or 72 hr following treatment cessation. Specifically, the activity of OVX rats treated with estradiol, although somewhat higher, was not significantly different from the activity exhibited by OVX rats treated with oil. Estradiol-treated OVX rats did not show catalepsy. Their response to thermal pain stimulus was similar to that shown by OVX animals treated with oil (controls).

Morphine-Induced Locomotion in Estradiol- and Oil-Treated OVX Rats

Figure 1 depicts the number of the open field compartments entered by OVX rats treated with estradiol or oil and challenged with saline (a) or morphine (b), 24 hr (A) or 72 (B) following the last treatment.

The presented data show an attenuated effect of morphine in rats treated with estradiol, regardless of the time elapsed between the last estradiol treatment and the injection of morphine (Ab and Bb). They also indicate an increased response to morphine at 72 hr (Bb), as compared to 24 hr (Ab), following treatment cessation. These observations were confirmed by statistical analysis. Univariate 3-way ANOVA on the average locomotion yielded a significant treatment effect, $F(1,55)=5.03$, $p<0.02$, a significant drug (morphine) effect, $F(1,55)=20.96$, $p<0.001$, a significant treatment × drug interaction, $F(1,55)=5.02$, $p<0.002$, a non-significant time after treatment cessation effect, $F(1,55)=1.62$, but a significant drug × time of treatment cessation interaction, $F(1,55)=5.88$, $p<0.01$. The multivariate analysis also revealed a significant effect of treatment, $F(2,54)=5.16$, $p<0.009$, and of the drug, $F(2,54)=4.12$, $p<0.02$, on the shape components of the time response curve.

The data presented on panel Ab show that a low dose of estradiol did not attenuate the response of rats to morphine. These data were evaluated separately to test for possible estradiol dose effect. The statistical analysis yielded a non-significant treatment effect on the average locomotion.

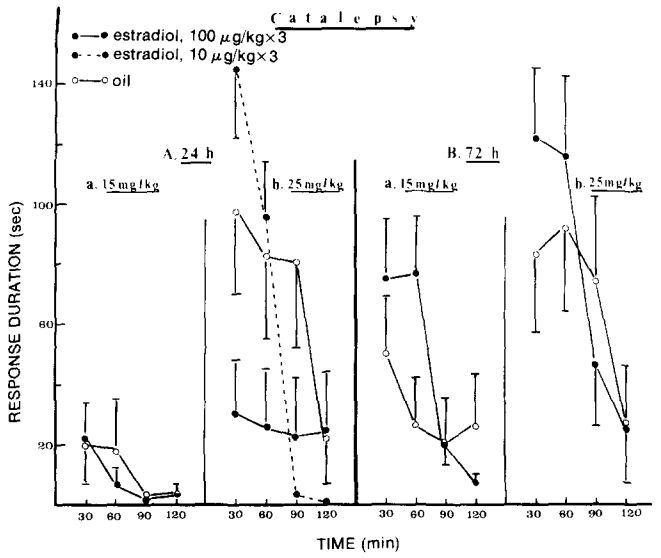

FIG. 2. Duration of a cataleptic state exhibited by OVX rats following 15 or 20 mg of morphine administered 24 or 72 hr after cessation of 17β-estradiol or oil vehicle treatment.

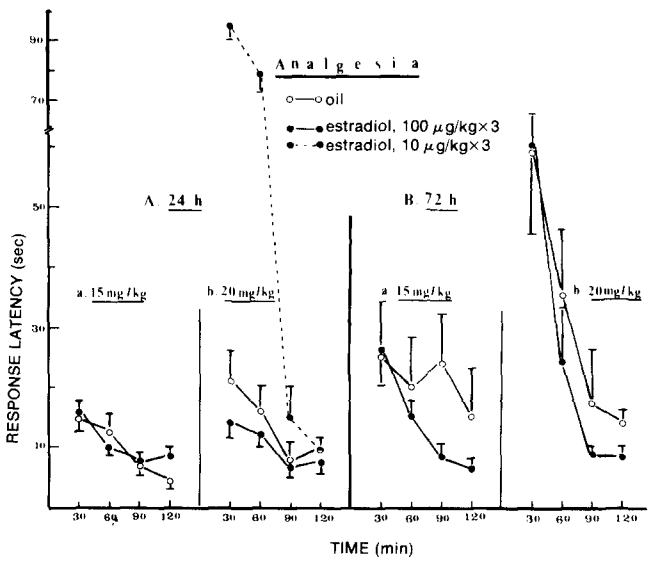

FIG. 3. Response latency to painful thermal stimulus of OVX rats treated with 17β-estradiol or oil vehicle and injected with morphine (15 or 20 mg/kg) 24 or 72 hr following treatment cessation.

$F(2,22)=0.77$, but a significant effect of estradiol treatment on the shape components of the curves, $F(2,21)=4.59$, $p<0.02$.

Morphine-Induced Catalepsy in Estradiol and Oil Treated OVX Rats

Figure 2 depicts the duration of the cataleptic response of estradiol- or oil-treated OVX rats to 15 mg/kg (a) or to 20 mg/kg (b) of morphine administered 24 hr (A) or 72 hr (B) following treatment cessation.

In rats treated with estradiol (100 μg/kg) the effect of morphine on catalepsy was attenuated when tests were carried out 24 hr following treatment cessation, while it appeared slightly increased during tests performed at 72 hr after the last estradiol injection. Three-way analysis of variance on the average cataleptic response yielded a significant time after treatment cessation effect, $F(1,78)=5.56$, $p<0.02$, and a significant treatment (estradiol) × time of treatment cessation interaction, $F(1,78)=3.98$, $p<0.05$. This is due to the fact that estradiol-oil-treated rats differed significantly at 24 hr, but not at 72 hr after treatment cessation. The analysis also yielded a significant effect of the dose of morphine, $F(1,78)=11.23$, $p<0.01$. The multivariate analysis also showed that the shape components of the time response curve to morphine depend significantly on the estradiol treatment, $F(3,76)=3.31$, $p<0.02$, on the time elapsed after treatment cessation, $F(3,76)=4.01$, $p<0.01$, on the interaction of treatment × time, $F(3,76)=4.07$, $p<0.01$, and on the dose of morphine, $F(3,76)=3.53$, $p<0.01$.

The data on panel Ab were also evaluated separately and the multivariate analysis revealed a significant time effect, $F(3,72)=18.55$, $p<0.0001$, and a significant treatment × time effect, $F(6,72)=7.34$, $p<0.0001$. Indeed, the cataleptic response to morphine is attenuated during the first hr after the administration of the drug in the group treated with 100 μg/kg, but not in that treated with 10 μg/kg estradiol.

Morphine-Induced Analgesia in Estradiol- and Oil-Treated OVX Rats

Figure 3 depicts the response latency to a thermal pain stimulus of estradiol- and of oil-treated OVX rats challenged with 15 mg/kg (a) or 20 mg/kg (b) of morphine injected 24 hr (A) or 72 hr (B) following treatment cessation. The analgesic action of morphine did not appear significantly modified by estradiol (100 μg/kg) treatment, regardless of the time elapsed between the last estradiol injection and the administration of morphine.

Three-way analysis of variance on the average of the response latency yielded a significant effect of the time elapsed after the treatment cessation, $F(1,76)=13.82$, $p<0.0001$, due to an increased analgesic effect of morphine at 72 hr following treatment cessation in both oil- and estradiol-treated OVX rats. The effect of the dose of morphine was also significant, $F(1,76)=4.43$, $p<0.03$. The multivariate analysis revealed that the shape components of the time response curves are significantly dependent on the interaction effect of time after treatment cessation × the dose of morphine, $F(3,74)=5.22$, $p<0.02$, and on the interaction effect of time following morphine administration with both the time after treatment cessation, $F(3,74)=6.34$, $p<0.001$, and the dose of morphine, $F(3,74)=5.92$, $p<0.001$. The data on panel Ab show that the analgesic action of morphine is increased in rats treated with 10 μg/kg of estradiol. Analysis of variance on the data of the average response to thermal stimulus revealed a highly significant effect of treatment, $F(2,22)=100.27$, $p<0.0001$. The multivariate analysis showed also that the shape components of the curves are significantly dependent on time, $F(3,66)=71.66$, $p<0.0001$, and on the interaction effect of treatment × time, $F(6,66)=37.91$, $p<0.0001$. In fact, the response latency to thermal stimulus appeared increased during the first hr following morphine administration.

DISCUSSION

The present results indicated that estradiol treatment to

OVX rats can modify the behavioral actions of morphine. This modification varied with the specific behavior under study and it was dependent on the dose of estradiol and on the time elapsed between the last estradiol injection and the administration of morphine. Specifically, the increased locomotion seen following administration of low doses of morphine appeared attenuated in estradiol-treated rats regardless of the time after estradiol treatment that the behavioral test was carried out. The time during which the animals remained cataleptic, following high doses of morphine, was attenuated in estradiol-treated rats. However, this attenuation was observed at 24 hr following termination of the estradiol treatment, while a slightly increased cataleptic response to morphine was apparent at 72 hr after estradiol treatment. The increased latency response to a thermal pain stimulus, observed following administration of high doses of morphine, was not significantly affected in estradiol-treated rats. However, with a low estradiol dose an increase of the effect of morphine was apparent. The low estradiol dose failed to influence significantly the effect of morphine on activity or on catalepsy, although a tendency to increased duration of catalepsy was apparent in rats treated with a low dose of estradiol. The response to thermal stimulus (paw licking, jumping) is dependent on the integrity of motor functions. Therefore, it is plausible that the increased response latency to pain represents a secondary phenomenon due to a slightly increased duration of catalepsy. If this is true, it could be suggested that estradiol treatment may affect specific behavioral effects of morphine, i.e., those related with motor functions (activity, catalepsy). Such a suggestion is not supported by the results of Banerjee *et al.* [1] who concluded that both the altered analgesic and the altered cataleptic response to morphine observed in OVX rats are not estradiol dependent. Methodological differences existing between the Banerjee *et al.* [1] and our study make comparisons problematic. Banerjee *et al.* [1] administered one single estradiol injection 2 hr before the administration of morphine, while we used a 3-day estradiol regimen followed by a 24 or 72 hr washout period before the administration of morphine. They used OVX rats one week after surgery, while we started estradiol replacement 2 weeks after ovariectomy. There are reports indicating that the above differences may be critical in the expression of estrogen-induced modulation of drug action (cf. [40]). From our own results it is also evident that the sensitivity to morphine of both oil- and estradiol-treated rats increases with the passing of time after surgery.

We have considered several mechanistic possibilities which might account for our observation of an attenuated response to morphine by OVX rats following estradiol treatment. Both pharmacokinetic and pharmacodynamic factors are of concern.

On pharmacokinetic grounds, several reports indicate that treatment with steroids can alter the metabolism of drugs [5]. Although, drug-drug interactions for estrogen-morphine are not, to our knowledge, reported, one could speculate an inhibition of the metabolism of morphine by estrogen as both substances are mainly metabolized by liver microsomal enzymes [16]. Such an inhibition would have led to an increase rather than to an attenuation of the behavioral effects of morphine seen in our study. Furthermore, a competition between estrogen-morphine for the same enzyme substrate does not seem very possible as it is unlikely for estradiol, which is excreted very rapidly [6,16], to be present in the animal's body at the time of the administration of morphine. With high doses of estrogen, the liver microsomal enzymes are induced [26]. Therefore, it is not unlikely that in estradiol-treated animals the metabolism of morphine becomes enhanced, leading to an apparent decrease of the drug action. However, the failure to obtain an attenuated effect of morphine in all behavioral tests argues against this suggestion. It follows then that pharmacokinetic factors do not appear to be crucially involved in the modification of the behavioral effects of morphine by estradiol.

On pharmacodynamic grounds, the simplest explanation could be that estradiol and morphine compete for the same site of action. There is a paucity of information regarding estradiol-morphine competitive actions on binding sites. However, this possibility could be excluded for the following reasons. Firstly, the competition of estrogen-morphine for the same site of action does not appear probable because, as noted above, it is unlikely for estradiol to be present at the time of morphine administration [6,16]. In support of a non-competitive drug-drug interaction between estradiol and morphine is also the finding that the effect of morphine given shortly (2 hr) after estradiol is not altered [1]. Secondly, although the membrane-receptor bound estrogen effect in the brain is still an open question, it is well established that the action of estrogen is mediated by specific cytoplasmic receptors and subsequent genomic processes (cf. [29,30]). On the other hand, opiates bind to specific membrane receptors mediating their effects [36].

A more plausible explanation would be that the opiatergic system controlling morphine-induced behaviors are somehow desensitized following estradiol. There are precedents in the literature supporting short-lasting alterations in the sensitivity of central neurotransmitter systems, including opioids, due to variations of ovarian hormones [1, 22, 23, 30, 40]. The probable estradiol-induced alteration in the sensitivity of opiatergic systems could conceivably be accounted for by changes in opiate receptors or by modification of endogenous opioid levels. It has been reported that estrogen increases methionine-enkephalin-like levels in the striatum [12]. Increased amounts of these substances could constantly stimulate opiate receptors and thus desensitize them. Unfortunately, the existing literature on the influence of gonadal steroids on opiate binding is conflicting. For instance, in aged female rats the minimal ovarian activity parallels with a decrease in opiate receptor binding [32]. However, other studies failed to observe estrogen-dependent alterations in the number or the affinity of opiate receptors [10].

A further possibility to consider is that estradiol may modulate opiatergic systems sensitivity indirectly. For instance, it is well established that estrogen has profound effects on dopamine-related functions (cf. [40]). In addition, several lines of evidence suggest a functional coupling of dopamine-opiate systems [39]. Specifically, the mesolimbic dopamine system has been implicated for opioid-induced activation [25]. In our study, the inhibitory effect of estrogen on morphine-induced hyperactivity, still present 72 hr following treatment withdrawal, appeared longer lasting than that on catalepsy. This would imply that the system mediating opioid stimulation, probably the mesolimbic dopamine system [9], is more sensitive to the estrogen effect. Previous work in our laboratory [22] and in others [38] has led to the speculation that ovarian secretions preferentially modulate the activity of the mesolimbic dopamine system. However, in view of our results on catalepsy, it would be unwarranted not to consider equally the well established estrogen-induced

modulation of nigrostriatal DA-system related functions (cf. [40]) inasmuch as the caudate appears to be involved in narcotic catalepsy [11,42].

The opiate-dopamine interaction provides an intriguing interface by which estrogen could attenuate morphine's action, although this is at best, speculative. Other transmitter systems and hypophysial hormones which are assumed to be altered by estradiol or opiate treatment [3, 4, 17] are likely to be involved.

Additionally, the observation that the high rather than the low dose of estradiol was effective in modifying morphine-induced behaviors, raises the conjecture that morphine-estradiol interaction, if it really occurs, is more likely to be manifested with pharmacological doses of the hormone administered for a therapeutic purpose, rather than with the naturally circulating hormone.

The functional importance of the inhibitory effect of estradiol treatment to OVX rats on morphine-induced behavior remains to be determined. This would be of interest as it may have clinical implications. For instance, it has been suggested that the mood disorders often seen in postmenopausal women might be related to estrogen-dependent alterations in brain and plasma levels of β-endorphin [15]. Furthermore, activation of opiate receptors apparently exerts antidepressant effects [13] while clinical data suggest that gonadotropic systems and sex steroids are involved in depression [20].

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