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Modulation by Estrogen and Progesterone of the Effect of Muscimol on Nociception in the Spinal Cord

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McCARTHY, M. M., M. CABA, B. R. KOMISARUK AND C. BEYER. *Modulation by estrogen and progesterone of the effect of muscimol on nociception in the spinal cord.* PHARMACOL BIOCHEM BEHAV **37**(1) 123–128, 1990.—The $GABA_A$ agonist, muscimol, administered intrathecally (IT) to the spinal cord at a dose (1 μg) that was subthreshold for affecting pain thresholds (vocalization-threshold-to-tail-shock: VTTS, and tail-flick latency: TFL) in ovariectomized, hormonally untreated rats, showed a significant increase in VTTS up to 30 min postinjection in intact females only in proestrus or estrus. This treatment produced no significant effect on TFL at any stage of the estrous cycle. IT muscimol produced a significant increase in VTTS (but not TFL) in ovariectomized rats primed with estradiol benzoate (EB) for 2 days and tested 40 hr after the second injection but had no effect in females primed with a single EB injection and tested 15 min later. By contrast, ovariectomized females primed with progesterone (P) for 15 min exhibited a significant increase in pain thresholds after IT muscimol in both the VTTS and TFL tests. When EB-primed females (2 days) received P 4 hr prior to muscimol there was no analgesia produced by IT muscimol, in contrast to EB-primed females receiving P 15 min prior to IT muscimol in which there was significant analgesia. These results suggest a mechanism for antagonistic effects of estrogen and progesterone.

Analgesia Steroids Rats

GABAERGIC transmission has been implicated in the control of nociception at the spinal cord. Intrathecal (IT) administration of $GABA_A$ agonists produces analgesia, whereas $GABA_A$ antagonists produce hyperalgesia (21). This suggests that GABAergic spinal neurons exert a tonic inhibition over pain-sensitive pathways. There is a high concentration of GABAergic neurons in the spinal cord, principally in laminae I to III, and ultrastructural examination suggests a role for GABA terminals in both pre- and postsynaptic inhibition (1,15).

In vitro studies suggest that both estrogen (6, 12, 25) and progesterone (10,27) modulate the GABA/benzodiazepine receptor system. Although steroids have been implicated in the control of opioid- and nonopioid-modulated nociception (5,23), there are no studies on modulation of GABA-induced analgesia by steroids. Therefore, in the present studies we examined the analgesic response to the intrathecal administration of muscimol in the female rat under diverse endocrine conditions, i.e., various stages of the estrous cycle, ovariectomy and exogenous administration of estrogen and progesterone. The results indicate that ovarian steroids interact with the $GABA_A$ agonist, muscimol, in modulating the response of the rat to noxious stimuli.

GENERAL METHOD

Adult female Sprague-Dawley rats weighing 250–350 g (Charles River, Kingston, NY) were housed individually at 23°C and maintained on a reverse day-night cycle (lights on: 20:00 to 10:00 hr). Food and water were provided ad lib. Rats were either intact, or ovariectomized under Ketamine anesthesia (0.1 mg/kg) and allowed to recover for at least two weeks before implantation

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of an intrathecal catheter. A catheter (Clay Adams PE-10 tubing; Fisher Chemical, Springfield, NJ; 7.5 cm insertion length) was implanted chronically in the subarachnoid intrathecal space, extending to the lumbar level of the spinal cord (28). Testing did not begin for at least two weeks postimplantation and was conducted at the mid-point of the dark phase of the cycle. At the completion of the experiment, rats were injected with an overdose of the anesthetic, Chloropent, perfused with 4% formalin, and the spinal cord was dissected to verify correct placement of the catheter. Testing consisted of two nociceptive threshold measurement paradigms.

Vocalization-Threshold-to-Tail-Shock (VTTS) was determined by placing rats in a Plexiglas restrainer and taping two stainless steel electrodes to the tail after applying conductive gel. Electrical shocks (100 msec train of 60 Hz symmetrical, biphasic square waves) with an intertrain interval of 3 sec, were delivered by a constant current shock generator (Coulbourn Instruments Programmable Shocker; Lehigh Valley, PA). This current was increased stepwise in 10 μA steps (range: 0.25 to 0.75) until vocalization was elicited ("upper shock level") and then decreased stepwise until vocalization ceased ("lower shock level"). This was repeated three times. Upper and lower shock levels were averaged to provide an estimate of vocalization threshold [modified from (4)].

Tail-Flick-Latency (TFL) was determined with a IITC Model 33 Analgesia-meter (Woodland Hills, CA; at 90% intensity). Rats were placed in a Plexiglas restrainer with the tail exposed to a radiant heat lamp. Tail-flick latencies were measured automatically by activation of a photocell upon tail withdrawal. A cutoff time of 10 sec was employed to avoid tissue damage.

Each rat was pretested twice for VTTS and once for TFL (average of three trials) to determine predrug baseline values. After pretesting, rats were injected intrathecally (IT) with either drug or saline in a 5 μl volume delivered to the subarachnoid space with an additional 2 μl of saline flushed from the catheter. Testing was done at 3, 10, 20, 30 and 60 min postinjection and the VTTS test was followed by the TFL test in all cases. Nonparametric statistical comparisons between groups were made using the two-tailed Mann-Whitney U-test.

In all experiments, muscimol (1 μg) or saline were injected IT under the following hormone conditions: For Experiment One, intact rats were monitored daily by vaginal lavage for at least one week to determine the presence of a normal estrous cycle beginning two weeks postsurgery. Separate females were tested during three different phases of the estrous cycle (proestrus, estrus and diestrus). The phase of the cycle for a particular rat was unknown to the investigator at the time of testing. In the remainder of the experiments, all rats were ovariectomized and injected SC with steroids dissolved in sesame oil. Rats in Experiment Two were either treated with 10 μg estradiol benzoate (EB) for two consecutive days 40 hr prior to testing (long-term estrogen) or injected once and tested 15 min later (short-term estrogen). In Experiment Three, rats received a single injection of progesterone (P) and were tested 15 min later. In Experiment Four, all rats received long-term estrogen treatment and a single injection of P either 4 hr or 15 min prior to testing. The muscimol and steroids were purchased from Sigma Chemical Co. (St. Louis, MO).

RESULTS

The results of VTTS testing for each experiment are summarized in Table 1. Results of the TFL testing when significant or relevant are found in Figs. 2 and 5.

EXPERIMENT ONE

Effect of Phase of Estrous Cycle

There were significant differences in the response of rats at different stages of the estrous cycle to 1 μg muscimol infused IT. As shown in Fig. 1, females in estrus showed significant analgesia in the VTTS test at 10, 20 and 30 min postinfusion. Females in proestrus were analgesic at 10 min postinfusion as compared to control rats receiving saline, or diestrous rats infused with muscimol. Diestrous rats were not analgesic at any time postinfusion. The control group consisted of nine females, three from each phase of the cycle, that were infused with saline. None of the control females exhibited analgesia at any time post infusion. Consequently, they were combined into a single control group for comparison with females receiving muscimol at the three specific phases of the estrous cycle. Pretest baseline values for the VTTS test did not differ significantly among groups (range of means = 0.43 to 0.52 microamps). There was no significant effect of phase of the estrous cycle on TFL after 1 μg muscimol or saline (Fig. 2).

EXPERIMENT TWO

Effect of Estradiol Benzoate

Experiment 2A: Long-term estrogen. Rats receiving long-term EB and infused IT with 1 μg muscimol exhibited significant analgesia in the VTTS test at 10, 20 and 30 min postinfusion as compared to females treated with EB and infused with saline. Rats not treated with EB and infused IT with muscimol were not different from those treated with EB and infused IT with saline (Fig. 3). Pretest baseline values for the VTTS test did not differ significantly among groups (range of means = 0.34 to 0.43 microamps). There was no significant effect on TFL produced by any of the treatments.

Experiment 2B: Short-term estrogen. Females infused with 1 μg muscimol 15 min after EB did not differ from females infused with saline after EB in either the VTTS or TFL tests. Neither group differed significantly from ovariectomized hormonally untreated females infused IT with muscimol. Therefore, in contrast to long-term exposure, short-term exposure to EB had no significant effect on nociceptive responses to 1 μg muscimol administered IT.

EXPERIMENT THREE

Effect of Progesterone

There was a significant increase in VTTS at 3, 10, 20, 30 and 60 min in females receiving 2 mg P and infused IT 15 min later with muscimol, as compared to females receiving oil plus 1 μg muscimol or 2 mg P plus saline IT (Fig. 4). There was also significant analgesia in the VTTS test in females that received 0.4 mg P plus 1 μg muscimol at 3 min postmuscimol compared to females receiving oil plus muscimol. Again, there was no significant difference in pretest baseline values for VTTS (range of means = 0.42 to 0.50). Despite high variability, there was a significant increase in TFL at 10, 20 and 30 min in rats receiving 2 mg P plus 1 μg muscimol as compared to females receiving oil plus 1 μg muscimol (Fig. 5). There was no significant difference in pretest baseline TFL values among groups (range of means = 2.93 to 3.30 sec).

EXPERIMENT FOUR

Effect of Combined Treatment With EB and P

Experiment 4A: Effect of EB plus P 4 hr prior to muscimol. IT infusion of 1 μg muscimol or saline had no significant effect on nociceptive responding of rats treated with long-term EB (2 days) and P 4 hr prior to muscimol. Thus, the ability of long-term EB exposure to induce analgesia after 1 μg muscimol IT was evidently

TABLE 1

	Percent Change From Baseline at				
	3′	10′	20′	30′	60′
Effect of Estrous Cycle					
Muscimol					
1) estrus (9)	+7.7 ± 9	+26.7 ± 7†	+29.1 ± 9†	+13.2 ± 8*	+13.8 ± 7
2) proestrus (9)	+3.0 ± 5	+17.8 ± 9†	+4.0 ± 10	−1.2 ± 9	+0.3 ± 1
3) diestrus (9)	−5.0 ± 6	−2.2 ± 5	−14.7 ± 6	−10.1 ± 7	−4.1 ± 7
4) ovariectomized (10)	−12.6 ± 6	−13.7 ± 5	−14.2 ± 7	−15.1 ± 6	−14.4 ± 6
Saline					
5) gonadally intact (9)	−6.2 ± 7	−11.5 ± 4	−11.6 ± 5	−9.9 ± 5	−11.6 ± 7
Effect of Estrogen					
Muscimol					
6) long-term EB (11)	0.0 ± 9	+4.7 ± 6*	+11.2 ± 7*	+0.3 ± 6*	+6.2 ± 5
7) short-term EB (8)	−11.6 ± 5	+4.0 ± 6	−1.8 ± 5	−6.9 ± 4	+1.5 ± 1
8) ovariectomized (8)	−12.5 ± 5	−12.9 ± 7	−11.8 ± 6	−12.6 ± 5	−13.8 ± 8
Saline					
9) long-term EB (8)	−18.8 ± 5	−18.6 ± 7	−24.5 ± 7	−26.3 ± 6	−10.4 ± 9
10) short-term EB (8)	−11.9 ± 4	−10.1 ± 3	−0.3 ± 5	−6.3 ± 5	−4.1 ± 3
11) ovariectomized (8)	−12.0 ± 5	−14.1 ± 6	−14.6 ± 5	−14.8 ± 6	−14.3 ± 5
Effect of Progesterone					
Muscimol					
12) 0.4 mg P (12)	+6.6 ± 5*	+1.0 ± 6	+5.9 ± 5	+7.5 ± 4	−1.5 ± 5
13) 2.0 mg P (14)	+7.9 ± 6*	+24.4 ± 8*	+18.6 ± 8*	+27.2 ± 15*	+22.9 ± 12*
14) ovariectomized (18)	−9.5 ± 5	−3.6 ± 6	−3.1 ± 5	−5.5 ± 6	−3.3 ± 6
Saline					
15) 2.0 mg P (12)	−11.4 ± 7	−7.2 ± 8	−0.2 ± 9	−1.6 ± 10	−4.9 ± 7
Effect of EB + P					
Muscimol					
16) EB+P; 4 hr prior (9)	−12.5 ± 4	−7.6 ± 5	−3.5 ± 8	+9.0 ± 3	+0.1 ± 1
17) EB+P; 15 min prior (9)	+6.8 ± 4*	+20.1 ± 9*	+11.6 ± 5*	+12.6 ± 5*	+3.0 ± 9
Saline					
18) EB+P; 4 hr prior (8)	−12.5 ± 4	−11.1 ± 5	−10.3 ± 6	−2.6 ± 10	−1.4 ± 6
19) EB+P; 15 min prior (8)	−13.1 ± 4	−13.6 ± 5	−13.4 ± 5	−21.1 ± 7	−23.1 ± 7

Values are mean percent change from baseline ± standard error of the mean for all groups in the VTTS test (*$p<0.05$, †$p<0.01$ compared to corresponding saline controls for each hormone condition; Mann-Whitney U).

antagonized by administration of P 4 hr prior to the test (Fig. 6).

Experiment 4B: Effect of EB plus P 15 min prior to muscimol. In the females receiving EB plus P, there was a significant increase in VTTS at 10, 20, 30 and 60 min postinfusion of muscimol as compared to controls infused with saline, and at 10, 20 and 30 min compared to hormonally untreated females receiving muscimol (Fig. 6). There was no effect of this treatment on TFL.

Although there was a significant increase in VTTS after long-term EB and short-term P followed by muscimol, the effect does not seem to be additive. Furthermore, without EB, but in combination with muscimol, short-term P induced a significant increase in TFL. The effect of P on TFL was absent when the rats were pretreated with EB.

DISCUSSION

Effect of Estrous Cycle

Our findings indicate that gonadal steroids can modulate nociceptive thresholds after IT administration of muscimol. Previous studies showed that the dose of muscimol (1 μg) used in the present study administered IT to ovariectomized hormonally untreated rats either does not produce analgesia or produces hyperalgesia in the VTTS test (11,21). However, the response of intact females to the same dose of muscimol varies over the estrous cycle. Females in estrus infused IT with 1 μg muscimol showed a significant and prolonged analgesia on the VTTS test. Rats in proestrus given the same dose of muscimol were analgesic only at 10 min after muscimol. By contrast, diestrous rats displayed no analgesia after muscimol.

Effect of Estrogen

Long-term EB exposure (2 days) resulted in significant analgesia after IT muscimol with a time course similar to that observed in estrous females, suggesting that two days of exposure to estrogen increases the analgesia-producing efficacy of muscimol. Most likely, this effect is genomic since short-term exposure to estrogen (about 15 min) was without effect on the analgesic response to muscimol. Potential genomic mechanisms include

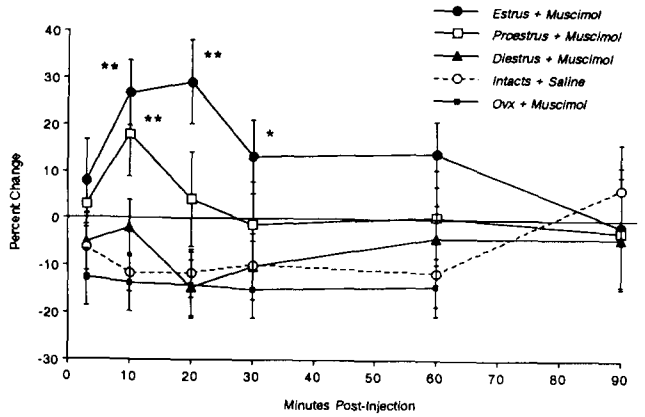

FIG. 1. Influence of phase of estrous cycle on response to muscimol administered intrathecally—Percent change of VTTS. In this and all other figures, values = mean ± S.E.M.; range of n's = 8–14; $^{**}p<0.01$, $^{*}p<0.05$ by comparison with saline controls (Mann-Whitney U-test).

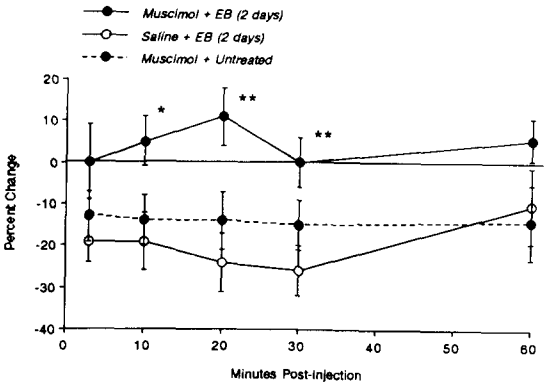

FIG. 3. Influence of long-term estrogen treatment on response to muscimol administered intrathecally—Percent change in VTTS. Females were treated with EB (10 μg) for two consecutive days and infused with muscimol 40 hr after the last EB injection ($^{**}p<0.01$, $^{*}p<0.05$).

changes in receptor number and/or alteration in GABA synthetic and degradative enzymes. Estrogen has been reported to influence all these parameters of GABAergic transmission (12, 14, 19), although both the direction and significance of these effects remains controversial (25). Apparently, there have been no reports to date of nongenomic effects of estrogen on the GABA system.

Estrogen-concentrating neurons are present in the lumbosacral spinal cord of the female rat, particularly in lamina II of the substantia gelatinosa and in the area surrounding the central canal (8,18). While relatively few in number, the distribution of estrogen-concentrating neurons in the spinal cord parallels that of benzodiazepine binding sites (26) and glutamate decarboxylase-containing axon terminals (13). Thus, the spinal cord may be a site of action of estrogen on GABA-induced analgesia. However, there are also high concentrations of estrogen-concentrating neurons in higher CNS areas, particularly the medial hypothalamus and midbrain central gray (17). Both these regions have been implicated in processing of nociceptive activity ascending from the spinal cord. The response of single lumbar dorsal horn neurons to radiant heat applied to the hind paw of the rat was consistently and powerfully inhibited by electrical stimulation of the medial hypothalamus (2). In addition, the heat-evoked activity of single spinal dorsal horn neurons was abolished by blockade of $GABA_A$ receptors in the midbrain central gray. This led to the conclusion that the activity of descending pain-inhibitory neurons, that originate in central gray and terminate in spinal cord, is increased when these neurons are released from tonic GABAergic inhibition (24). It is possible that estrogen exerts its effect on GABA-induced analgesia by modulating input to the spinal cord from these higher brain centers. Estrogen treatment has been demonstrated to have a genomic effect on the GABA receptor in the medial hypothalamus and midbrain central gray (14, 19, 25).

Effect of Progesterone

Short-term exposure to P (15 min) greatly increased nociceptive thresholds after IT muscimol in both the VTTS and TFL tests. In contrast to the effect of estrogen, short-term exposure to P appears to exert a rapid and nongenomic enhancing effect on GABAergic transmission. P has been demonstrated to increase muscimol binding in cerebral cortex of the male rat (10) and to enhance GABA-mediated inhibition in Purkinje cells of the cerebellum (27). Both of these effects are nongenomic since they have very short latencies and occur in the absence of nuclear

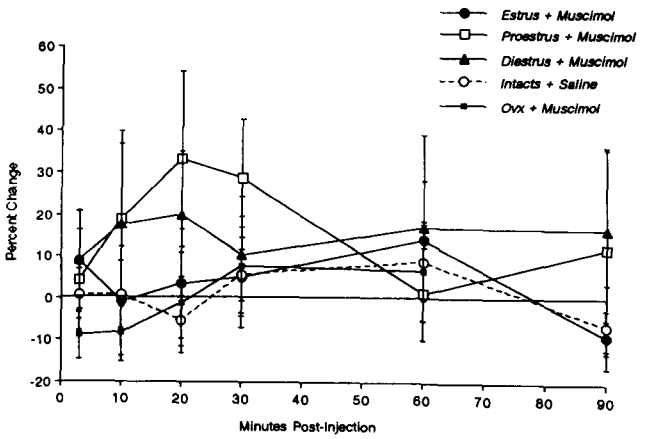

FIG. 2. Influence of phase of estrous cycle on response to muscimol administered intrathecally—Percent change of TFL. The same females as in Fig. 1 were tested also for tail-flick latency to radiant heat. There was no significant effect of 1 μg muscimol infused intrathecally during any phase of the estrous cycle.

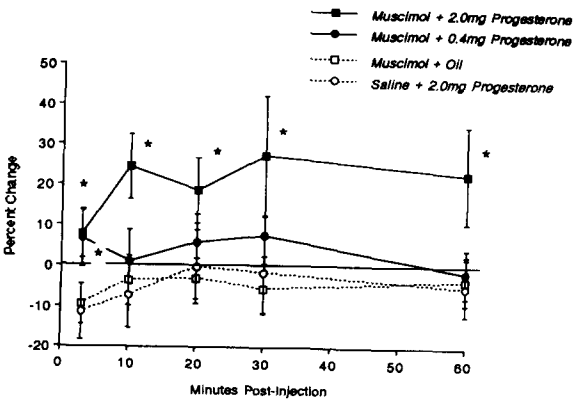

FIG. 4. Influence of progesterone treatment on response to muscimol administered intrathecally—Percent change in VTTS. Females were treated with P 15 min prior to infusion with muscimol ($^{*}p<0.05$).

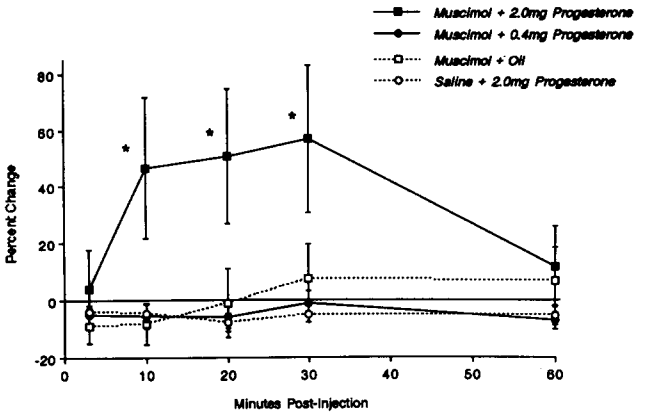

FIG. 5. Influence of progesterone treatment on response to muscimol administered intrathecally—Percent change in TFL. The same females as in Fig. 4 were also tested for TFL to radiant heat (*$p<0.05$).

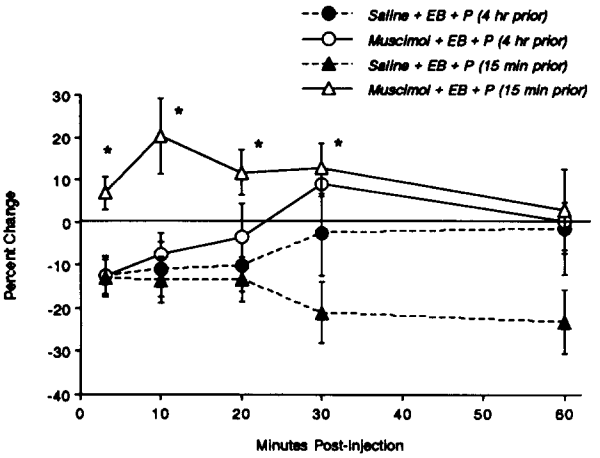

FIG. 6. Influence of combined EB+P treatment on response to muscimol administered intrathecally—Percent change in VTTS. Ovariectomized rats given long-term exposure to EB (2 days) received P either 4 hr or 15 min prior to infusion with muscimol (*$p<0.05$). The same females were also tested for TFL and there was no significant effect (data not shown).

progesterone receptors. Several ring A-reduced metabolites of progesterone also exert a nongenomically mediated enhancing effect on the GABA system (6,10), and production of these metabolites may play a role in the effects observed in the present study.

Effect of Combinations of Estrogen and Progesterone

When P was injected 4 hr prior to muscimol in long-term EB-treated females, analgesia was not produced. However, when P was injected 15 min prior to muscimol in EB-primed females, significant analgesia was produced, but only in the VTTS test. This is in contrast to the effect of 15-min exposure to P in the absence of EB in which case there was significant analgesia in both the VTTS and TFL tests after IT muscimol. These two measures of nociception have previously been reported to be differentially affected by analgesia-producing procedures (3, 7, 9).

It is of interest that combined exposure to EB and P exerted different effects on pain thresholds depending on the schedule of hormone administration. When long-term EB exposure was combined with P treatment 4 hr prior to muscimol there was evidently an antagonism of the effect of EB on nociception. In contrast, when long-term EB was combined with P treatment 15 min prior to muscimol administration a significant enhancement of the effect of 1 μg muscimol was observed. There are at least two interpretations of these findings: 1) estrogen exerts an antagonistic effect on the enhancement of GABAergic transmission produced by progesterone, and 2) progesterone antagonizes the effect of estrogen on the GABAergic system. Evidence exists to support both of these possibilities. P treatment increases benzodiazepine binding in the dorsal horn of the spinal cord of the rat, but this increase disappears if the animals are pretreated with EB (26). In addition, EB treatment decreases the number of high-affinity muscimol binding sites in the brain, but this decrease is selectively inhibited in the ventromedial nucleus of the hypothalamus and the midbrain central gray if EB treatment is followed by P under the same injection schedule used in the present study (25). Furthermore, EB pretreatment enhanced vaginal-stimulation-produced-analgesia (VSPA), but combined treatment with EB plus P antagonized this effect (3). GABAergic antagonists attenuate VSPA (22) and steroid effects on the GABAergic system may play a role in the observed antagonism of the effect of estrogen that is produced by P. Similarly, nonopioid-mediated tailshock-induced analgesia in ovariectomized EB-treated rats was assessed by the hot-plate test 48 hr after hormone treatment. EB only enhanced pain inhibition by comparison with vehicle-treated animals; however, this enhancement was partially attenuated when P was administered 6 hr prior to the test (23). Thus, the present findings suggest that with regard to the production of analgesia, antagonistic effects of EB and P may be the result of modulation of GABAergic neurotransmission by the steroids. The genomic effect of estrogen on the GABAergic system is antagonized by four hours, but not 15 min of exposure to P. By contrast, the apparent nongenomic enhancement of GABAergic transmission by P is attenuated by long-term exposure to EB, particularly with regard to the increase in TFL observed after P in the absence of EB. Finally, both EB and P exert effects on a variety of other neurotransmitter systems (20). Thus, it is possible that the antagonistic effects between EB and P observed in the present study are the result of alterations in other neuronal systems in addition to the GABAergic system.

In conclusion, the present results demonstrate a behavioral manifestation of steroidal influences on GABAergic transmission. Both EB and P appear to enhance GABAergic transmission in such a manner that IT administration of the specific $GABA_A$ agonist, muscimol, produces significant analgesia after steroid treatment, in contrast to ovariectomized hormonally untreated rats receiving the same dose of muscimol. The current results also suggest that EB and P modulate the GABAergic system through different mechanisms that are antagonistic under appropriate temporal conditions.

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