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Participation of Opiatergic, GABAergic, and Serotonergic Systems in the Expression of Copulatory Analgesia in Male Rats

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GONZÁLEZ-MARISCAL, G., P. GÓMORA AND C. BEYER. *Participation of opiatergic, GABAergic and serotonergic systems in the expression of copulatory analgesia in male rats.* PHARMACOL BIOCHEM BEHAV **49**(2) 303–307, 1994.—Copulation in the male rat provoked an abrupt and significant rise in the threshold to induce vocalization by electrical shock to the tail (copulatory analgesia, CA). The possible effect on CA of the intrathecal (IT) administration of receptor antagonists to neurotransmitters participating in nociception was ascertained in this study. CA was significantly reduced, though not abolished, by IT injections of either naloxone, picrotoxin, or methysergide, but not by strychnine or yohimbine. This analgesic effect was achieved without significantly altering copulatory behavior. Results suggest that both brain and spinal systems participate in the development of CA. Brain effects would be mediated by descending serotonergic fibers, although intrinsic spinal systems would involve both opiate and GABA interneurons.

Analgesia Nociception Pain Male sexual behavior GABA Glycine Opiates Serotonin Noradrenaline Spinal cord Intrathecal

SZECHTMAN et al. (39) found that during copulation male rats vocalize less frequently to the application of intense electric shocks than before copulation. We (14) measured the vocalization threshold to tail shock (VTTS) in sexually inactive and actively copulating rats and showed that intense copulatory analgesia (CA) already occurred following the first intromission. Because prolonged periods of copulation reduce the concentration of midbrain opiates in male rats, Szechtman et al. (39) suggested that these peptides participate in CA. Forsberg et al. (11), using the hot plate method for assessing pain, found an increase in the latency to paw licking in the rat during the postejaculatory interval (PEI). This analgesia was apparently produced by endogenous opiates because the IP injection of naloxone prevented it. On the other hand, Cruz-Morales and Noble (8) found in the male hamster a slight analgesia immediatly after interrupting copulation, but this effect was not inhibited by naltrexone.

Except for these conflicting results on the role of opiates, no other studies have been made on the neurochemical basis of CA. Male copulation is a complex phenomenon involving the integrated activation of many neural systems related to vigilance, motor activity, genital tract functioning, and endocrine secretion (24). Some of these copulation epiphenomena, e.g., general arousal, alter pain perception (19). Moreover, activation of genital or perineal nerves, a situation normally occuring during copulation, produces strong and persistent analgesia in female rats (22). These data suggest that CA, as other environmental analgesias (18), depends on the combined action of brain and spinal mechanisms involving various neurotransmitters or neuromodulators. In the present study we assessed the effect of intrathecal (IT) injections of some specific antagonists of neurotransmitters or neuromodulators, known to control nociceptive transmission at the spinal cord (23,41,42), on the expression of CA. Because the spinal cord is the final common outflow of a variety of neurotransmitter systems, the IT administration of drugs allows us to investigate which of them may participate in nociception modulation regardless of the origin of the corresponding cell bodies.

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METHOD

General Procedures

Subjects (Ss). Wistar male rats bred in our colony and weighing 350 g were used. They were kept in a rat colony with a reversed light : dark cycle (14 hours light : 10 hours dark, lights off at noon) and maintained at 23°C. Purina rat chow and water were available ad lib. All males received four training sessions with highly estrous females (ovariectomized rats injected with 20 μg estradiol benzoate, followed 40 h later by 2 mg progesterone). Each training session, performed during the dark phase of the light cycle, lasted 20 min and Ss achieved at least one ejaculation per test. Ss showing long latencies to mount were discarded from the observations.

Surgery. Ss were implanted with a catheter (Clay Adams PE-10 tubing; Fisher Chemical, Springfield, NJ; 7.5 cm insertion length) in the subarachnoid space through an incision in the atlanto-occipital membrane (44). The catheter extended along the spinal cord to the lumbar level. Ss were anesthetized during surgery with ketamine (Bristol Laboratories, Syracuse, NY; 80 mg/kg IP) and xylazine (Harver-Lockhart, Shawnee, KS; 15 mg/kg IP), and treated once with Terramycin (Pfizer laboratories, NY; 5 mg IM.). At least 7 days of recovery were allowed before testing postsurgery.

Sexual behavior testing. Sexual behavior was observed under dim red-light illumination in circular Plexiglas arenas (55 cm in diameter) with a sawdust-covered floor. Males were brought to the mating arena from the colony to adapt for 5 min before introducing an estrous female. Rarely were stimulus females used with more than one male. The SBR computer program (7) was used to quantify the various parameters of male sexual behavior, including the hit rate (29).

Nociceptive testing. The vocalization threshold to tail shock (VTTS) was determined in freely moving rats as described previously (14). Two stainless steel electrodes were attached to the tail through an adjustable cylindrical plastic cuff that was then taped onto the tail. Conductive gel was applied to the skin of the tail before attaching electrodes. The electrodes were connected and prevented from tangling by the use of a rotatory connector. This procedure did not hinder the movements of the rats. Electrical shocks (50 ms trains of 60 Hz symmetrical biphasic square waves; pulse duration = 4 ms) were delivered with a constant current shock generator (Nuclear-Chicago, Des plaines, IL). The current was increased step wise in 100 μA units until vocalization was elicited (upper shock level) and then decreased until vocalization ceased (lower shock level). This procedure was repeated three times, shocks being delivered once every 5 s. Upper shock levels were averaged to provide an estimate of vocalization threshold. VTTS tests took from 1-3 min to perform, depending on the threshold of the Ss.

Drugs. Yohimbine and naloxone were purchased from Sigma (St. Louis, MO). Strychnine, picrotoxin and methysergide were purchased from Research Biochemicals, Inc. (Natick, MA).

Treatments and experimental design. The effect of IT administration of naloxone, yohimbine, picrotoxin, strychnine, or methysergide on CA occurring during the first copulatory series was studied. Drugs were dissolved in 5 μl of saline, and delivered to the perispinal space with an additional 7 μl of saline flushed from the catheter. Injections lasted around 1 min. Rostrocaudal diffusion following IT injection of this volume is limited to the spinal cord at least within the first 30 minutes postinjection (44). Groups were as follows: saline, 5 μl (n = 20); naloxone, 10 μg (n = 8); yohimbine, 30 μg (n = 9); picrotoxin, 1 μg (n = 9); strychnine, 5 μg (n = 11); and methysergide, 30 μg (n = 10). These dosages are known to effectively block their corresponding receptors when administered perispinally to rats: i.e., opioid μ (40), α_2-adrenergic (33), $GABA_A$ (34), glycine 1 (28), and 5-HT_2 (17). All injections were administered 3 min before measuring the precopulatory (baseline) VTTS values. Immediately afterwards, an estrous female was introduced into the testing arena and the VTTS was again measured following the first intromission. The time elapsing between the determination of the precopulatory VTTS (baseline values) and initiating the measurement of the copulatory VTTS (experimental values) was usually less than 1 min. During this period males were allowed to continue copulating. Number of Ss in each group is shown in the results section.

Data analysis. An ANOVA was initialy made to compare precopulatory VTTS to determine if some of the antagonists induced a significant effect on the baseline VTTS. The effect of copulation on VTTS was assessed on the various experimental groups by comparing precopulatory with copulatory values with t-tests. An ANOVA was also made to compare the copulatory VTTS to determine the existence of differences between treatment groups. When adequate, t-tests for differences between two independent means (saline vs. experimental groups) were performed. Probability levels of $p < 0.05$ were considered significant.

RESULTS

No significant differences in precopulatory VTTS values were found among the different groups: mean ± SE for saline = 1011 ± 58 μA and range for experimental groups = 835 ± 52 to 1127 ± 163 μA. This means that none of the antagonists per se significantly modified the VTTS within the time period explored. Control Ss receiving only saline presented a clear CA revealed by a 550 μA increase in the VTTS (55% above baseline) following the first intromission ($p < 0.05$, Fig. 1). Figure 1 also shows the effects produced by the IT injection of the various antagonists on CA. None of the treatments totally blocked CA. Yet variance analysis comparing copulatory VTTS revealed a significant difference among treatment groups, $F(5, 61) = 3.2$, $p < 0.05$. Subsequent comparison against saline values revealed that IT naloxone, picrotoxin, and methysergide induced significant decreases in CA. On the other hand, both strychine and yohimbine failed to inhibit CA, individual VTTS values in these two groups being highly variable.

Perispinal administration of strychnine induced allodynia in most Ss, a condition in which innocuous tactile stimulation elicits strong aversive responses. However, this condition did not prevent injected Ss from initiating copulation.

Table 1 shows the effect of the IT treatments on various parameters of male sexual behavior in both control (saline) and experimental rats. It can be seen that, at the doses used, only IT naloxone provoked a significant effect: a decrease in mount frequency, $F(5, 61) = 8.2$, $p < 0.05$.

DISCUSSION

Our study agrees with the suggestion of Szechtman et al. (39) that CA is partially mediated by opiates. This effect is exerted by release of spinal endorphins because perispinal naloxone depressed CA. The spinal opiate system participates in several types of analgesia (42), including that produced by vagino-cervical stimulation (VCA) in the rat (20,38). VCA has in common with male CA, the activation of genital and peri-

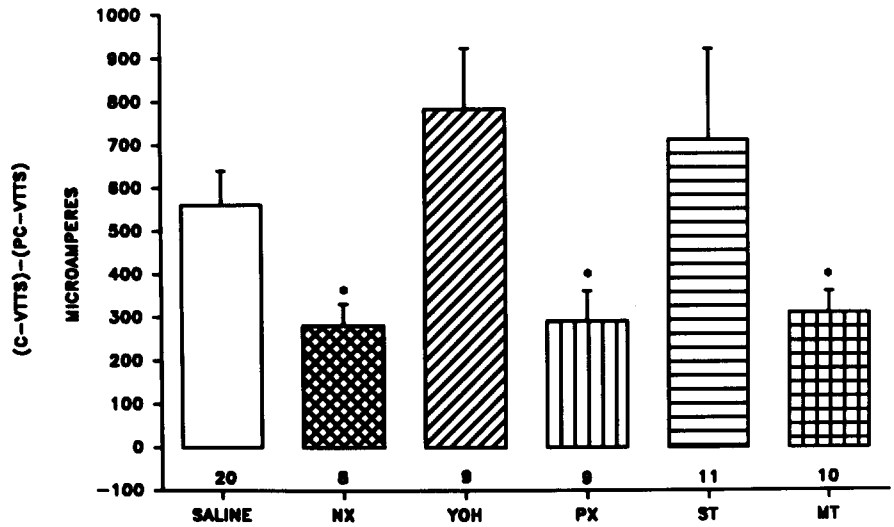

FIG. 1. Effect of the intrathecal (IT) administration of saline, naloxone (NX, 10 μg), yohimbine (YOH, 30 μg), picrotoxin (PX, 1 μg), strychnine (ST, 5 μg) or methysergide (MT, 30 μg) on the magnitude of copulatory analgesia (CA). Experimental subjects (Ss) received the above IT treatments 3 min before determining the precopulatory vocalization threshold to tail shock (PC-VTTS). Ss were then allowed to copulate and, after one intromission, the copulatory VTTS (C-VTTS) was determined. Thus, the value obtained from subtracting PC-VTTS from C-VTTS indicates the magnitude of CA. Data show means + SE. Numbers below bars indicate number of Ss in the corresponding group. *$p < 0.05$ vs. saline group.

neal afferent nerves [pelvic, pudendal, hypogastric; (25-27,32)]. Furthermore, the observation that an opioid-mediated analgesia develops during parturition has suggested that the stimulation of nerves in the pelvic region activates endorphin-containing interneurons (13). However, our observations do not exclude an indirect activation of endorphin interneurons through descending pathways.

The finding that naloxone did not abolish CA, suggests the additional participation of nonopiate systems in this reponse. The inhibitory aminoacids glycine and GABA, which exist in large concentrations in the dorsal horn (1,9,31,45), regulate nociceptive information at the spinal cord. Thus, perispinal strychnine, a Gly 1 antagonist, and bicuculline or picrotoxin, $GABA_A$ antagonists, produce allodynia and hyperalgesia in rats (2,4,34,43). Moreover, $GABA_A$ agonists (10,36) as well as glycine itself, when Gly 2 receptors are blocked (3), produce analgesia in several nociceptive tests. Our finding that picrotoxin decreased CA, strongly supports the participation of GABA in this effect. Release of spinal GABA may also participate in VCA, because this response is depressed by the IT injection of $GABA_A$ antagonists (34). In contrast to this coincidence, strychnine, which also inhibits VCA (33), failed to inhibit CA in male rats. However, a major difference between these studies, is that in the present study, we tested the effect of strychnine within seven minutes of IT injection, while in the female study VTTS were measured at 15 min postinjection (33). This diference in the testing schedule may be important because within the first 10 min after IT injection of strychnine intense sensory disturbances occur [allodynia, scratching, self-biting; (4,33)], which may, in turn, have provoked a rise in VTTS.

The finding that IT methysergide, a 5-HT_2 antagonist, sig-

TABLE 1

EFFECT OF INTRATHECAL ADMINISTRATION OF NEUROTRANSMITTER ANTAGONISTS ON MALE SEXUAL BEHAVIOR

Sexual Parameter	Saline $n = 20$	Naloxone 10 μg $n = 8$	Yohimbine 30 μg $n = 9$	Picrotoxin 1 μg $n = 9$	Strychnine 5 μg $n = 11$	Methysergide 30 μg $n = 10$
Mount frequency	11.4 ± 1.9	4.8 ± 1.3*	8.8 ± 1.6	6.2 ± 1.5	16.7 ± 2.9	6.7 ± 0.5
Intromission frequency	10.6 ± 1.3	7.1 ± 1.6	9.0 ± 0.8	7.8 ± 1.6	8.9 ± 0.5	5.5 ± 0.9
Mount latency (s)	21.7 ± 10.8	52 ± 47	14.6 ± 4.5	19.3 ± 5.6	7.1 ± 1.2	8.4 ± 3.4
Intromission latency (s)	77 ± 33	32 ± 14	58 ± 34	41 ± 20	42 ± 12	32 ± 23
Ejaculation latency (s)	461 ± 93	290 ± 71	340 ± 67	329 ± 60	498 ± 107	302 ± 111
Interintromission interval (s)	42 ± 5.0	31 ± 5.9	39 ± 5.0	50 ± 13	76 ± 19	59 ± 13
Hit Rate	0.54 ± 0.04	0.62 ± 0.05	0.56 ± 0.05	0.67 ± 0.05	0.42 ± 0.06	0.53 ± 0.08

*$p < 0.05$, vs. saline group. Values in the table are means ± SE

nificantly reduced CA suggests a role of serotonin in this effect. Indeed, methysergide reduces the analgesia provoked by vaginocervical stimulation in female rats and VCA increases the release of serotonin into spinal cord superfusates (38). Serotonin is released in the spinal cord through the activation of descending fibers originating in the raphe magnus nucleus (16,37). Electrical stimulation of this nucleus provokes analgesia, which is partially blocked by IT serotonin antagonists (15,17). It is possible that serotonin is unspecifically released as a result of the intense excitement associated with male copulation, because some raphe neurons increase their firing during arousal (21). It is, however, possible that this system is activated along with that involved in the initiation of copulatory behavior. Stimulation of the medial preoptic area (mPOA) elevates pain thresholds (5), most likely through activation of raphe neurons (6). Similarly, stimulation of the mPOA facilitates copulation (28,30). Spinal serotonin itself, however, is probably not responsible for triggering copulation because the IT administration of methysergide, its antagonist, failed to block or delay copulation. It would be interesting to determine if the same mPOA neurons whose activation induces analgesia through serotonin release also facilitate copulatory behavior by enhancing the release of other neurotransmitters. Stimulation of raphe magnus neurons in the cat, which reduces nociception through serotonin and glycine release, also provokes the release of excitatory aminoacids (37) which could be involved in triggering copulation.

In summary, the present data suggest that opiatergic and GABAergic interneurons, activated by impulses arising from the genital area (penile erection, penile insertion, perineum, etc.) during copulation contribute to the production of CA. Our findings also suggest that descending serotonergic fibers, possibly activated by unspecific arousal or by neuronal discharge from the mPOA during copulation, may also contribute to CA. The possible participation of the adrenergic and glycinergic systems in male CA cannot, however, be ruled out from our results that were obtained by using a single dose of the corresponding antagonists.

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