Pharmacology Biochemistry & Behavior, Vol. 14, pp. 707–711, 1981. Printed in the U.S.A.

Dispensability of Spinal Monoaminergic Systems in Mediating the Lordosis Reflex of the Female Rat

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Received 18 October 1980

KOW, L.-M., C. M. PADEN AND D. W. PFAFF. *Dispensability of spinal monoaminergic systems in mediating the lordosis reflex of the female rat.* PHARMAC. BIOCHEM. BEHAV. **14**(5) 707–711, 1981.—The possibility that bulbospinal monoaminergic systems are essential for expression of the estrogen-induced lordosis reflex in female rats was tested by studying the effects of reserpine and monoamine antagonists on the lordosis responses to mating and manual stimulation. Lordosis performance of five groups of ovariectomized rats, either treated (groups II through V) or not treated (group I) with estrogen, were measured before and after pharmacological treatments. Immediately after post-treatment measurements, females (except group V) were decapitated and the lumbosacral cord quickly removed, frozen, and later assayed for norepinephrine, dopamine and serotonin. Group I females (n=7) did not show any lordosis before or after a control saline injection, and their spinal monoamine contents were not different from those of group II (n=8), who showed near maximal levels of lordosis both before and after a saline injection. Reserpine at low (2 mg/kg; group III, n=6) or high (10 mg/kg, group IV, n=10) doses depleted spinal monoamines severely but had no significant effect on lordosis, except for a slight decline of lordosis intensity after the high dose treatment. A combination treatment of reserpine (10 mg/kg) and a serotonin receptor blocker, methiothepin (10 mg/kg), did not affect lordosis performance (group V, n=6). Further injection of an α-adrenergic antagonist, phenoxybenzamine (20 mg/kg), in addition to this combination, had no effect on the lordosis reflex in response to manual stimulation, although the response to mating stimulation was reduced. The reduction was probably a result of a drug-induced abnormal posture, which made most of the reflexogenic skin area of the female inaccessible to mating stimuli. These results showed that estrogen treatment sufficient to induce a near maximal level of lordosis performance did not alter spinal monoamine contents, and that the monoamines in the spinal cord are not necessary for mediating the estrogen-stimulated lordosis reflex.

Lordosis reflex Reserpine Monoamines Spinal cord

A VARIETY of evidence indicates that estrogen acts directly on the ventromedial hypothalamus of the rat to permit lordosis, a feminine mating reflex to sensory stimulation [16]. This estrogenic influence descends through relays in the midbrain and lower brainstem to the spinal cord where it is integrated with lordosis-triggering sensory inputs. From brainstem to spinal cord the lordosis-inducing effect of estrogen is mediated by bulbospinal fibers traversing in the anterolateral column (ALC) [17]. This is indicated by the abolition of lordosis by spinal cord transections that cut both ALC's. In contrast, transecting all *but* the ALC's of the cord had little or no effect [17].

There are indications that some of the descending ALC fibers are monoaminergic. First, complete spinal cord transection abolishes all the spinal monoamines caudally [20], while sparing ALC's leaves caudal norepinephrine (NE) and serotonin (5-HT) concentrations at about 50% of normal levels. The lordosis reflex was not affected by such a 50% reduction in NE and 5-HT (Kow and Zemlan, unpublished data). Second, descending monoaminergic fibers have been visualized in the ALC of rats [11]; and in cats 5-HT was quite evenly distributed in the white matter of the cord [28]. Third, using the horseradish peroxidase technique, it was found that some of the cells of origin for descending ALC fibers were located in regions known to give rise to bulbospinal monoaminergic fibers [36]. Further, electrolytic lesions of these regions, the medial medullary reticular formation (mMRF), the locus coeruleus-subcoeruleus region (LC-SC), and the nucleus raphe magnus (NRM), depleted 38% of 5-HT, 46% of NE, and 21% of 5-HT in the lumbosacral cord, respectively (Zemlan, Kow and Pfaff, unpublished data). Also, both the depletions of 5-HT and NE induced by lesions of mMRF and LC-SC, respectively, were associated with lowering of lordosis performance, suggesting that both monoamines facilitate lordosis at the spinal level.

Since some of the descending ALC fibers, which are essential for the lordosis reflex, are monoaminergic, and depletions of spinal NE and 5-HT are both associated with reduction in lordosis, it is possible that the lordosis-inducing effect of estrogen is mediated to the spinal cord by the

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facilitating effects of both bulbospinal 5-HT and NE fibers in the ALC. This possibility was tested by investigating the effects of reserpine and monoamine antagonists on the lordosis reflex. Reserpine, which can deplete both NE and 5-HT as well as other monoamines, was chosen because elimination of one type of monoaminergic fiber may not be sufficient to abolish lordosis. In the present experiments we focused our attention on the pharmacological effects in the lumbosacral cord. This section of the cord not only receives sensory inputs essential for triggering the lordosis reflex [14], but also contains motor neurons responsible for executing dorsiflexion [10], the most prominent characteristic of the reflex.

METHOD

Subjects

Adult, ovariectomized Sprague-Dawley rats (Hormone Assay) were used. They were housed individually in cages with ad lib supply of rat chow and water, and were kept in a room under a light:dark (12:12) cycle with light off at 9:00 a.m. Subjects were randomly divided into 5 groups (I through V), and allowed to adapt to the new environment for 2 weeks. Then, females in groups II through V were implanted subcutaneously with estrogen (Progynon pellet, Schering Co.), while group I females were identically surgically processed without estrogen implantation.

Tests for Lordosis

Two weeks after estrogen or sham implantation, all females were subjected to weekly tests for lordosis performance, measured as lordosis intensity and lordosis quotient. For measurement of lordosis intensity, each female was subjected to a manual stimulation, in which the experimenter's hand was used to palpate the female's flanks and then to apply pressure on the flanks, rump, lower back, and perineal region in order to elicit lordosis (see [15,18]). Lordosis intensity was scored on the basis of the extent of dorsiflexion (the most prominent characteristic of lordosis) elicited by the stimulation using a 0 (no dorsiflexion) to 7 (maximum dorsiflexion) point scale. The manual stimulation was administered 5 times to obtain an average score. Measurement of the lordosis quotient was accomplished by placing each female in a testing arena containing a vigorous stud male until she was mounted (with thrusts) 15 times or for 15 min. The lordosis quotient was calculated as the percentage of mounts eliciting lordosis in the female. Females received pharmacological treatment between the third and fourth weekly tests. Squads of 4 to 5 females composed of members from various groups were tested at one time. All tests were carried out in the middle of the dark phase (2:00–4:00 PM), and the tester did not know what treatment the animals had received.

Pharmacological Treatment

Treatments for each group are summarized in Fig. 1. Females in groups I (n=7) and II (n=8) were given a control injection of physiological saline (0.08 or 0.4 ml/100 gm B.Wt.) 5 or 24 hrs before post-treatment tests. Group III females (n=6) were given a low dose (2 mg/kg) of reserpine (Serpasil, CIBA), and tested either 5 or 24 hrs later (n=3, each). Group IV females (n=10) received a high (10 mg/kg) dose of reserpine 24 hrs before the post-treatment test. Group V females (n=6) were subjected to two post-treatment tests for lordosis. They were given reserpine (10 mg/kg) 24 hrs, and Methiothepin maleate (10 mg/kg, Roche), a 5-HT receptor blocker [9, 25, 26], 1 hr before the first test. Immediately after the first test, each female was given phenoxybenzamine (20 mg/kg, Smith, Kline and French), an α-adrenergic receptor blocker [2, 4, 21], and tested again for lordosis 90 min later. The monoamine antagonists were dissolved in physiological saline. All treatments were given IP injection.

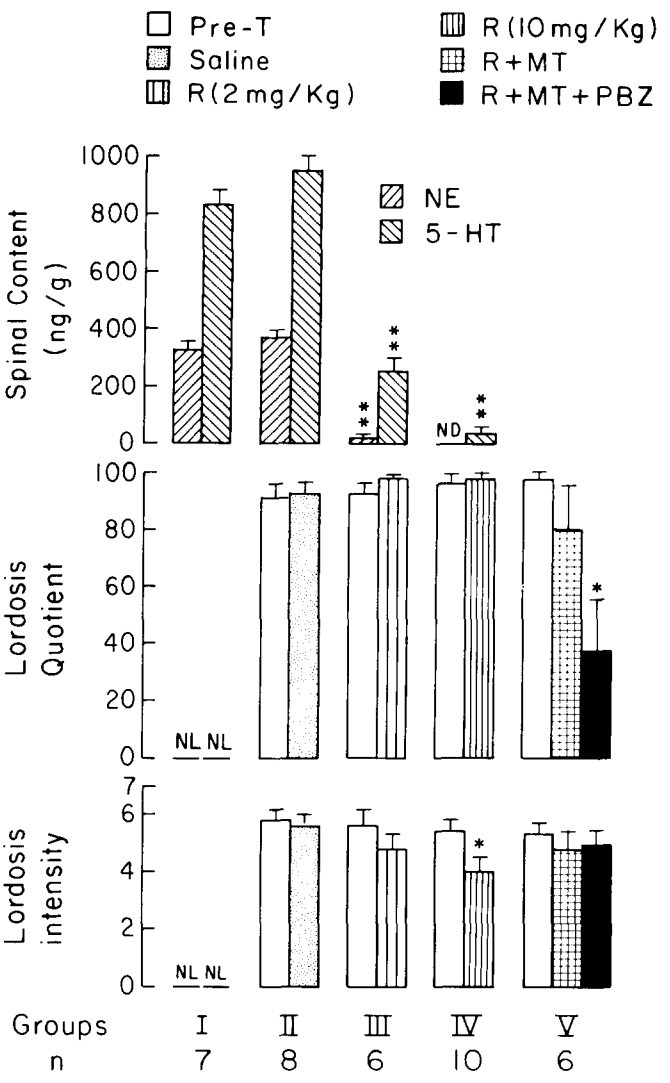

FIG. 1. Results from tests for lordosis and chemical assays, presented as mean±SEM. Abbreviations: Pre-T, pre-treatment; R, reserpine; MT, methiothepin (10 mg/kg); PBZ, phenoxybenzamine (20 mg/kg); NE, norepinephrine; 5-HT, serotonin; ND, not detectable; NL, no lordosis. Spinal content of dopamine, which was very low or not detectable, is not shown in this figure but is presented in the text. $^{**}p<0.005$, Mann-Whitney U-test, comparing with group II. $^{*}p<0.05$, Wilcoxon test, comparing with pre-treatment results.

Spinal Cord Removal

Upon completion of post-treatment tests, females from groups I through IV were immediately decapitated, and the vertebral column together with the spinal cord was severed between vertebrae T_{11} and T_{12}, which correspond to spinal

level T_{12} [14]. Laminectomy was then performed on vertebrae T_{12} through L_4 to expose the lumbosacral cord. Dura and all the spinal roots were removed to free the cord. The isolated cord was then wrapped in a small sheet of clean aluminum foil, frozen with dry ice, and stored in a freezer (−40°C) until assay. The entire process took 4 to 5 min.

Monoamine Assays

To assay for serotonin (5-HT), norepinephrine (NE), and dopamine (DA), the tissue was weighed and homogenized in five volumes or 1.2 ml, whichever was greater, of ice-cold 0.4 N perchloric acid containing 1.0 g sodium metabisulfite and 0.5 g disodium EDTA per liter. The homogenates were centrifuged at 25,000×G for 20 min, and two 0.1 ml aliquots of the acid supernatant taken for determination of serotonin (5-HT). Duplicate 0.4 ml aliquots were adjusted to pH 8.2–8.6 by addition of 0.5 M sodium hydroxide containing 0.1 M tricine (N-tris(hydroxymethyl)methyl glycine; Sigma). Approximately 60 mg of aluminum oxide (Woelm neutral activity grade I, from ICN Nutritional Biochemicals, Cleveland, Ohio, purified according to the procedure of Anton and Sayre [5], were added to each sample in 1.5 ml conical plastic centrifuge tubes and the contents shaken for 30 min. The supernatant was discarded and the alumina washed twice with water. Catecholamines were eluted from the alumina by shaking with 0.3 ml of 0.2 N HCl for 30 min.

Monoamines were isolated and quantified on a high pressure liquid chromatograph equipped with a 5 cm×2.1 mm ID precolumn (at room temperature), and either a 15 cm × 2.1 mm ID column (for indoleamines) or a 50 cm × 2.1 mm ID column (for catecholamines) at 60°C. Columns were packed with Vydac SC pellicular cation exchange material (Applied Science Lab., State College, PA.). The mobile phase for indoleamine analysis was 0.3 M potassium acetate pH 3.0, pumped at 0.4 ml per min. 5-HT was detected by mixing the effluent with concentrated HCl and measuring native fluorescence by the method of Meek [22]. For catecholamine analysis, a mobile phase of pH 5.2 acetate-citrate buffer [13] was pumped at 0.4 ml per min and amines were quantified using an electrochemical detector with a working potential of −0.72 V (Bioanalytical Systems Inc., West Lafayette, Ind.). Peak heights of tissue samples were compared to those of amine standards (5-HT, NE, and DA; Sigma) taken through the assay procedure to correct for recovery and sampling losses. Minimum detectable levels of amines in tissue were between 30 and 50 ng/g wet weight in all assays. Reagent grade materials and double-distilled, deionized water were used throughout.

RESULTS

The results of NE and 5-HT assays and lordosis tests are summarized in Fig. 1. Injection of different amounts of saline in groups I and II, and the different time course of testing and sacrifice in groups I, II, and III did not make a difference in the results. Therefore, for these three groups, data were pooled within group. For statistical analyses, the spinal content for a monoamine which was not detectable was regarded as zero.

Group I females, the only subjects not receiving an estrogen implant, did not show any sign of lordosis before or after the control saline injection. In contrast, all females in the other groups showed near-maximal levels of lordosis performance before pharmacological treatment, and no difference among groups was seen (Fig. 1). In group II, lordosis performance was not affected by the saline injection. Despite the striking difference in lordosis performance, groups I and II did not differ in spinal content of 5-HT, NE or DA, although levels tended to be higher in the estrogen implanted group (Fig. 1). Spinal content of DA was very low in both groups (mean±SEM; 8.6±8.6 ng/g for group I, and 11.3±8.4 ng/g for group II), and was detectable in only three animals. These results show that the lumbosacral cord of the rat contains substantial amounts of 5-HT and NE (886±36 and 343±19 ng/g, respectively, groups I and II combined), but very little DA (10±6 ng/g). Measurements for 5-HT and NE are comparable to, though they tend to be higher than, those reported for rats (ranges in ng/g: 5-HT, 220–1200; NE, 190–265) by other investigators [1, 6, 7, 20, 27, 29, 32, 33]. The slight differences may be attributed to the fact that in the present experiment more efficient assay methods were employed, and also to the exclusive use of the lumbosacral cord, which has a higher monoamine content [3,20]. The DA content was lower than the readings (24–26 ng/g) reported by others [20,29]. However, since spinal DA was barely detectable (present experiment; see also [29]), the difference is probably artificial.

Various behavior effects of reserpine treatment were noted. Soon after treatment the females became immobilized in a hunch-back posture, often with eyes closed. Some also showed catatonia. However, they were unusually sensitive to tactile and auditory stimuli. These effects lasted for at least 24 hrs. Within 24 hrs of treatment, animals became aphagic and adipsic and many developed diarrhea.

In group III, spinal monoamines were severely depleted by the low (2 mg/kg) reserpine dose. DA was no longer detectable in any subject; NE was detected in only 2 of the females, although small amounts of 5-HT could still be found in every rat. Spinal contents of NE and 5-HT were only 5 and 26% of their respective levels in group II animals and these differences were significant (Fig. 1). In spite of such a severe depletion of spinal monoamines and the appearance of the behavioral effects noted above, both measures of lordosis performance remained unchanged. In group IV, after reserpine (10 mg/kg) injection, neither NE nor DA was detectable in any rat, and a residual amount of 5-HT was detected in only 2 of the 10 females. Even with such near-complete depletion, the lordosis quotient remained unchanged, although lordosis intensity declined slightly (Fig. 1).

In group V, the combination treatment of reserpine and methiothepin did not cause a significant change in either measure of lordosis performance. One female, however, did not show lordosis in response to a male's mounting, but responded well to manual stimulation. After treatment with all three drugs, the lordosis quotient declined significantly (Fig. 1). Three females failed to respond to mating stimuli and two others showed a reduction in the lordosis quotient. However, when subjected to manual stimulation, every female responded with lordosis, and the intensity was as high as that scored before treatment. Administration of monoamine antagonists also caused further changes in posture. After methiothepin, and especially phenoxybenzamine, females became more immobilized in an extreme ventroflexed posture. They curled up like a ball, with their rump and lower back resting on the floor, making these body regions inaccessible to a male's stimulation during mating.

DISCUSSION

The fact that no differences in spinal monoamine content was observed between groups I and II despite the striking

difference in behavior indicates that treatment with estrogen, sufficient to induce a near-maximal level of lordosis performance, does not change overall monoamine levels in the cord.

Results from the experimental groups show that reserpine can severely (group III) or almost completely (group IV) deplete 5-HT, NE, and DA in the lumbosacral cord, which is crucial for mediating the lordosis reflex. Since reserpine can also deplete brain epinephrine [8], and the rat spinal cord contains only a small amount (40–60 ng/g) of this amine [29], it can be assumed that the reserpine treatment in the present experiment also depleted spinal epinephrine. Yet, in spite of the depletion of monoamines to an undetectable level, and the concurrent disturbances to locomotion, posture, feeding and gastrointestinal function, lordosis was practically unaffected. It is therefore very unlikely that the bulbospinal monoamine systems are essential for mediating the lordosis reflex.

However, since reserpine may not deplete the functional pool of monoamines [31], which in turn may exist in amounts too small to be detected by chemical assays, one may argue that lordosis could persist because of this residual amount of monoamines. In view of this consideration, monoamine receptor blockers, methiothepin and phenoxybenzamine, were applied in addition to the high dose (10 mg/kg) of reserpine to antagonize the action of any residual 5-HT and NE. Methiothepin has been regarded as the most potent blocker of central 5-HT receptors [26]. In rats [9], and cats [30], this drug has an immediate effect (within 5 min) that lasts more than 5 hrs. This rapid effect has been confirmed in our laboratory. Injection of methiothepin (10 mg/kg) alone to two estrogen-implanted females sedated the animals in a few minutes, but lordosis was not abolished although they remained sedated for at least 5 hrs. Thus, in group V females, which received the combination treatment, 5-HT receptors were blocked at both the first and second post-treatment tests, and at the second test α-adrenergic receptors were also blocked. Lordosis tests revealed that, with the combination treatment, only the lordosis response to stimuli from male rats was reduced, and only after administration of all three drugs. Even the reduction in response to mating stimuli (i.e., lordosis quotient) was apparently non-specific. Treatment with the monoamine antagonists, especially after both were given, enhanced the ventroflexion induced by reserpine (see Results), and made the female's rump, lower back, and perineal regions inaccessible to the male's mating stimuli. Since deafferentation of these body regions, either surgically or pharmacologically, reduces or abolishes lordosis performance [15], it appears that the reduction in lordosis quotient was due simply to the fact that the females received little or no stimulation necessary for eliciting lordosis. This is supported by the finding that every female responded well to manual stimulation, which was not hampered by the extreme ventroflexion. This fact further indicates that the pathway or neural mechanism mediating the lordosis reflex remained intact after inactivation of the bulbospinal monoamine systems, suggesting that the lordosis-inducing effect of estrogen was not mediated by monoaminergic fibers descending in the ALC.

Consistent with the above conclusion is the result from another experiment [19] which employed an opposite approach. In this experiment, attempts were made to reinstate the lordosis reflex in spinal rats with monoamine agonists, and the result was essentially negative. However, agonists of 5-HT, though not NE, facilitated vertebral dorsiflexion, a component of lordosis. This, together with the lesion study (Zemlan *et al.*, unpublished data), suggests that though not essential for mediating lordosis, spinal 5-HT and NE may normally have a facilitatory role. While the present results do not exclude the possibility that spinal monoamines could have an inhibitory effect on lordosis, there is no evidence for such an effect and the results of the agonist [19] and lower brainstem lesion (Zemlan, Kow and Pfaff, unpublished data) experiments argue against this possibility. Even if this possibility were valid, depression of monoamine-mediated inhibition is unlikely to be the principal mechanism by which the estrogen effect is expressed, for depleting and inhibiting monoaminergic systems systemically could not substitute for estrogen in inducing lordosis [23].

Finally, since the drugs were administered systemically and would affect the brain as well as the spinal cord, and since some brain monoamines may inhibit lordosis (e.g., [12, 24, 34, 35]), one may argue that the effect of spinal monoamine depletion may be compensated or neutralized by the release of inhibition due to depletion of brain monoamines. This argument does not apply to the present experiments which tested whether bulbospinal monoaminergic systems were essential for mediating lordosis, because if they were then the depletion would abolish lordosis despite the release of inhibition. The fact that lordosis was practically unaffected by the drug treatments indicated that the estrogen effect is mediated by other bulbospinal fiber systems, and that the presence of monoamine activity in the spinal cord, and the brain as well, is not essential for the expression of the estrogen-induced lordosis reflex.

ACKNOWLEDGEMENT

The authors would like to thank Dr. F. P. Zemlan for his valuable suggestions, Mr. D. Chattman and Ms. J. Habermann for their technical assistance, and Ms. G. Zummer for preparing the manuscript. C. M. P. acknowledges the support of USPHS Postdoctoral Fellowship MH 07493.

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