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Pargyline-Induced Increase in Serotonin Levels: Correlation With Inhibition of Lordosis in Rats

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ALLEN, D. L., K. J. RENNER AND V. N. LUINE. *Pargyline-induced increase in serotonin levels: Correlation with inhibition of lordosis in rats.* PHARMACOL BIOCHEM BEHAV **45**(4) 837–841, 1993.—The effect of intrahypothalamic infusion of the monoamine oxidase inhibitor pargyline on lordosis behavior and monoamine levels in the preoptic area and hypothalamus was examined. Progesterone-facilitated lordosis was blocked by pargyline in half the treated rats. The inhibition of lordosis was correlated with increases in serotonin and dopamine levels in the ventromedial nucleus of the hypothalamus and serotonin levels in the arcuate nucleus-median eminence when compared to controls or pargyline-treated rats with high levels of lordosis responding. Changes in norepinephrine levels were not correlated with changes in behavior. The results provide further evidence for an inhibitory role of basomedial hypothalamic serotonin in the control of female sexual behavior.

Lordosis Pargyline Hypothalamus Ventromedial nucleus Arcuate nucleus Serotonin Norepinephrine Dopamine

SEQUENTIAL placement of estrogen and progesterone into the ventromedial nucleus of the hypothalamus (VMN) is sufficient to activate sexual behavior in female rats (35). Increasing evidence suggests that monoaminergic neuronal systems are critical for the hormonal control of female sexual behavior (lordosis). Systemic injections of monoamine oxidase inhibitors, which raise monoamine levels by inhibiting their degradation, block hormone-dependent sexual behavior (24,29). Further, placement of the monoamine oxidase inhibitor pargyline directly into the VMN rapidly blocks behavior, suggesting that this area may be a locus for monoaminergic regulation of behavior (22). Although a precise role for monoaminergic systems in the control of sexual behavior has not been defined, the use of specific monoaminergic agonists and antagonists has implicated a number of specific monoaminergic receptor subtypes in the regulation of lordosis (1,8,10,11,27,28).

In the present study, the neurochemical effects of intrahypothalamic pargyline and the site(s) where the drug may be acting to inhibit female sexual behavior (lordosis) in hormone-primed female rats were investigated. In these animals, monoamine levels were measured in the VMN and adjacent hypothalamic and preoptic nuclei. The results corroborate the earlier demonstration that intrahypothalamic pargyline inhibits sexual behavior and suggest that this effect is associated with increased levels of serotonin [5-hydroxytryptamine (5-HT)] in the VMN.

METHOD

Adult female rats, 175–225 g, were ovariectomized and housed on a 12 L : 12 D schedule with the lights off after 7:00 p.m. Seven days after ovariectomy, rats were pretreated with estradiol benzoate, 5 μg SC, at 9:00 p.m. Two days later, from 2:00–4:00 p.m. rats were anesthetized with ether. These animals were bilaterally injected with saline (0.5 μl, $n = 9$) or pargyline (Sigma Chemical Co. St. Louis, MO, 35 μg in 0.5 ml saline, $n = 10$) via a 30-ga needle aimed just dorsal to the ventromedial nucleus of the hypothalamus. The stereotaxic coordinates were: incisor bar, −2.4 mm; posterior to bregma, 2.2 mm; lateral, ±0.8 mm; and depth, 8.3 mm from dura. Three hours later, rats received 500 μg progesterone in propylene glycol. Sexual behavior was tested 4 h after progesterone. Female rats were mounted 10 times by a male rat during the test and the lordosis quotients (LQs) and quality scores were calculated (16). Rats were sacrificed immediately after behavioral testing. Their brains were removed, rapidly frozen on dry ice, and sectioned at 300-μm intervals. During sectioning, the location of cannulae tracts and tips was noted.

For analysis of monoamines in discrete brain nuclei, punches were taken of specific brain regions according to Palkovits (32). The brain nuclei sampled were the dorsomedial nucleus (DMN), the lateral portion of the VMN, anterior hypothalamic nucleus (AH), arcuate nucleus-median eminence

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(ARC-ME), and the medial preoptic area (POA). Punched tissue was expelled into 60 μl sodium acetate buffer containing 8×10^{-8} M α-methyldopamine (kind gift of Walter Gall, Merck, Sharp and Dohme) as an internal standard. Samples were frozen and then thawed. Two microliters of ascorbate oxidase (Boehringer Mannheim, W. Germany, 1 mg/10 ml in double-distilled water) was added to each tube to reduce the contribution of ascorbate to the solvent front (25). Levels of monoamines were measured by high-performance liquid chromatography with electrochemical detection as previously described (14). The assay was linear from 20 pg to 2 ng for norepinephrine (NE), 30 pg to 2 ng for dopamine (DA), and 40 pg to 2 ng for 5-HT. Protein was measured using the method of Bradford (5). Levels of monoamines were expressed as pg monoamine per μg protein.

Differences in sexual behavior and levels of monoamines were analyzed by analysis of variance (ANOVA) and Newman-Keuls test after normalization of behavioral data by arcsine transformation. As an additional index of the relationship between monoamine levels and lordosis responding, the correlation between monoamine levels in individual nuclei and lordosis quotients was examined by linear correlation analysis (39).

RESULTS

The use of ether anesthesia in conjunction with intracranial injection of saline did not affect the expected maximal lordosis quotients after estrogen and progesterone in control rats (mean LQ 90 ± 7, $n = 9$). In contrast, intrahypothalamic pargyline injections inhibited lordosis behavior in some but

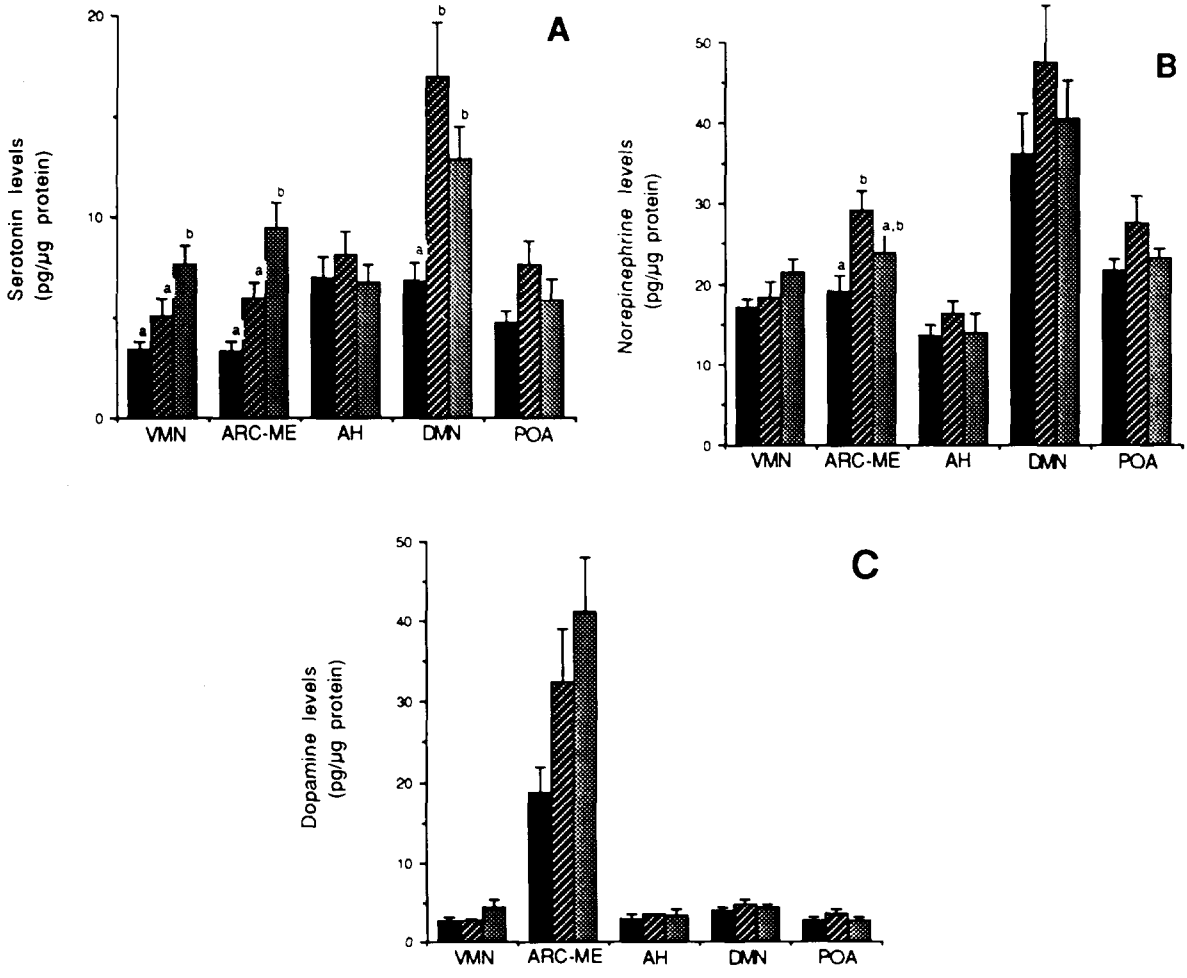

FIG. 1. Levels of monoamines in the hypothalamus after intrahypothalamic infusion of pargyline. Pargyline (35 μg) or saline was bilaterally infused into the basomedial hypothalamus of estrogen-primed female rats. Rats were injected with progesterone (500 μg), sexual behavior was tested, and rats were sacrificed. Levels of serotonin (A), norepinephrine (B), and dopamine (C) were measured in control rats (solid bars, $n = 4$–8), noninhibited rats (hatched bars, $n = 4$–5), and inhibited rats (shaded bars, $n = 3$–5). The number of animals for each point varies due to the loss of some samples during processing for measurement of monoamines. Values are the mean ± SEM. The areas of the brain in which monoamines were measured were the ventrolateral portion of the ventromedial nucleus of the hypothalamus (VMN), arcuate nucleus-median eminence (ARC-ME), anterior hypothalamus (AH), dorsomedial nucleus (DMN), and medial preoptic area (POA). Letters in the figure are used to indicate significant differences between monoamine levels in a specific area of the brain. Different letters within an area indicate groups that are significantly different from each other ($p < 0.05$), while the same letter indicates no significant difference between two groups. Absence of letters indicates that there were no significant differences between treatment groups.

not all rats. These rats were divided into two groups (n = 5 for each group) based upon their lordosis quotients: inhibited (INH, mean LQ 28 ± 7, range 0–40) and noninhibited (NON-INH, mean LQ 96 ± 4, range 80–100). The injection sites in inhibited rats were located just dorsal or caudal to the VMN. The injection sites of noninhibited rats were well rostral or dorsal to the VMN (data not shown).

As expected, pargyline administration increased levels of monoamines in areas near the site of application. In those areas rostral to the cannula, the POA and AH, no differences in monoamine levels between groups (SAL, NON-INH, or INH) were found (Fig. 1A–1C). In the DMN, located directly dorsal to the VMN, pargyline increased levels of 5-HT but the increase was independent of changes in behavior because 5-HT was significantly increased in both INH and NON-INH groups (Fig. 1A). In the ARC-ME, levels of NE in the NON-INH group (Fig. 1B) and DA in the INH group (Fig. 1C) were increased over controls, but the two pargyline groups (INH and NON-INH) did not differ significantly from each other. In the VMN and ARC-ME, levels of 5-HT were significantly higher in the INH group than in the other two groups (Fig. 1A). Thus, only the levels of 5-HT in the VMN and ARC-ME showed a correspondence to the inhibition of sexual behavior by pargyline.

Linear correlation analysis was used to further examine the relationship between lordosis quotients and levels of monoamines (Table 1). In the DMN, POA, and AH, there were no significant correlations between monoamine levels and behavior. Levels of 5-HT in the ARC-ME and VMN and levels of DA in the VMN were negatively correlated with lordosis quotients, that is, higher levels of these monoamines were associated with lower lordosis quotients.

DISCUSSION

Intrahypothalamic injections of pargyline were effective in inhibiting sexual behavior in a subgroup of estrogen- and progesterone-primed female rats, which were characterized by injection sites near the VMN. Pargyline was associated with altered monoamine levels in several brain nuclei, but only changes in 5-HT levels in the ARC-ME and 5-HT and DA levels in the VMN were correlated with the inhibition of sexual behavior. The localized increases in monoamine levels and the inhibition of lordosis behavior were presumably due to the inhibition of monoamine degradation and the subsequent potentiation of monoaminergic postsynaptic activity. Systemic injection of pargyline inhibits MAO activity within 20 min, as shown by increases in 5-HT levels (34). Crystalline implants of pargyline dorsal to or within the ventrolateral portion of the VMN decrease lordosis quotients and inhibit MAO activity 4–6 h after implantation (22).

TABLE 1

CORRELATION COEFFICIENTS BETWEEN LORDOSIS QUOTIENTS AND MONOAMINE LEVELS IN INDIVIDUAL BRAIN NUCLEI

Region	5-HT	NE	DA
VMN	−0.992*	−0.464	−0.553†
DMN	−0.004	0.081	−0.015
POA	0.040	−0.008	0.142
ARC-ME	−0.592*	0.135	−0.264
AH	0.189	0.164	−0.080

n = 16–19 except for the ARC-ME, where n = 14–15.
*$p < 0.01$.
†$p < 0.05$.

Intracranial injection of pargyline did not significantly affect monoamine levels in the AH and POA, possibly due to minimal diffusion into these areas or differences in time or dose requirements for pargyline inhibition of MAO. These results suggest that monoamines in these areas did not contribute to the inhibition of sexual behavior by pargyline in this study.

Intrahypothalamic pargyline was effective in altering monoamine levels in the VMN and in two adjacent brain nuclei, the DMN and ARC-ME. Pargyline increased 5-HT levels in the DMN, but levels were increased to the same extent in both behaviorally inhibited and noninhibited rats. Thus, increases in 5-HT in the DMN are not correlated with the inhibition of behavior. In behaviorally noninhibited rats, levels of the three monoamines in the DMN were increased over levels in inhibited rats. This suggests that the injection sites in noninhibited rats were more dorsal than in inhibited rats and that increases in monoamines in the DMN do not directly inhibit sexual behavior.

Pargyline increased 5-HT, NE, and DA levels in the ARC-ME, but only 5-HT levels were higher in inhibited when compared to noninhibited and control rats. While the changes in 5-HT levels were correlated with the inhibition of behavior, the ARC-ME has not been previously implicated in the regulation of hormone-dependent sexual behavior (33). Instead, strong evidence implicates the ARC-ME in the control of hormone secretion (4,19).

Increases in levels of 5-HT and DA in the VMN were correlated with the inhibition of sexual behavior by pargyline. These pargyline-induced changes in behavior negatively correlated with increases in monoamine levels in a brain nucleus believed critical for the expression of lordosis. In contrast, there was no evidence for a correlation between NE levels in the VMN and the inhibition of behavior.

Pargyline in high doses inhibits both type A and type B monoamine oxidase (24) and inhibits the degradation of 5-HT, NE, and DA. The reported increases in DA and NE after pargyline were smaller than the increases in 5-HT. This is most likely due to differences between the regulation of catecholamine and indoleamine synthesis. Catecholamine synthesis is subject to catecholamine feedback inhibition (20,31). In addition to metabolism by monoamine oxidase, catecholamines are also degraded by catechol-*o*-methyltransferase (3). The rate of 5-HT synthesis, unlike the synthesis of catecholamines, is not inhibited by increased levels of 5-HT (20,30). Pargyline increases levels of 5-HT by blocking degradation but does not inhibit 5-HT synthesis. The inhibition of lordosis following pargyline treatment is most likely due to a potentiation of monoaminergic activity rather than drug-induced alterations in monoamine synthesis or release.

Catecholamines do not appear to make a major contribution to the inhibition of lordosis by pargyline. In the present study, only norepinephrine and dopamine in the ARC-ME and dopamine in the VMN were increased by pargyline, but there was no significant difference in catecholamine levels between the INH and NON-INH groups. Lordosis quotients were negatively correlated with dopamine levels in the VMN. It seems unlikely that the increased dopamine levels in the VMN contribute to the pargyline-induced inhibition of behavior because intrahypothalamic dopamine and dopaminergic agonists facilitate lordosis (7,13). However, systemic injections of dopamine agonists inhibit sexual behavior in hor-

mone-primed female rats, while antagonists facilitate behavior (8,9). Norepinephrine in the hypothalamus is mainly facilitatory for sexual behavior. Infusions of NE into the hypothalamus increase lordosis quotients (10,11), while infusions into the POA inhibit behavior (6). Lesions that eliminate NE innervation of the hypothalamus abolish hormone-facilitated behavior (15). Microdialysis probes in the VMN show dramatic increases in extracellular NE from dialysates of hormone-treated female rats displaying high lordosis quotients (37).

Increasing evidence implicates 5-HT in the hypothalamic regulation of female sexual behavior in the rat. When pargyline was injected systemically, the inhibition of behavior was related to the increase in 5-HT levels in the whole basomedial hypothalamus but not to increases in NE levels (24). Further, intrahypothalamic serotonergic antagonists increase lordosis responding in estrogen-primed rats (12,38,40), and 8-OH-DPAT, an agonist, rapidly inhibits behavior when infused into the VMN (36). Consistent with an inhibitory role of 5-HT in lordosis behavior, lesioning of hypothalamic serotonergic nerve terminals with 5,7-dihydroxytryptamine (5,7-DHT) results in a facilitation of behavior (23), which is lost with reinnervation of serotonergic terminals into the hypothalamus (14). Our results suggest that changes in 5-HT within the VMN are sufficient to dramatically alter lordosis responding.

There are reports of serotonergic facilitation of lordosis (2,17,26,27). Facilitation by systemically administered serotonergic agents may be mediated at sites other than the VMN. Indeed, recent studies suggest the POA as a site of 5-HT facilitation of lordosis. Progesterone enhances 5-HT turnover in the POA (18), and serotonergic activity in the POA also increases on proestrus when lordosis is induced (21). In addition, the stimulation of lordosis by TFMPP, a 5-$HT_{1B/1C}$ agonist, is enhanced in centrally lesioned 5,7-DHT females (2), suggesting a POA-hypothalamic locus for the facilitatory effects of 5-HT on lordosis. Further studies could assess whether pargyline implants in the POA of estrogen-primed female rats facilitate behavior.

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