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17β Estradiol-Induced Increase in Brain Dopamine D-2 Receptor: Antagonism by MIF-1[1]

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RAJAKUMAR, G., P. CHIU, S. CHIU, R. L. JOHNSON AND R. K. MISHRA. *17β Estradiol-induced increase in brain dopamine D-2 receptor: Antagonism by MIF-1.* PEPTIDES 8(6) 997–1002, 1987.—Animal behavioral and neurochemical studies implicate dopaminergic systems in the neurological sequelae induced by estrogen. In the present study, we demonstrated for the first time that MIF-1, a neuropeptide unrelated to classical dopamine agonists, when given prior to, concurrently with, and after 17β-estradiol, antagonized significantly the estrogen-induced increase in the density of dopamine D-2 receptor both in the striatum and the mesolimbic area of male rat brain. The current findings have implications for the prophylactic and therapeutic potential for MIF-1 in extrapyramidal motor disorders caused by estrogen imbalance in humans.

17β Estradiol Striatum Mesolimbic area Dopamine receptor Density increase Antagonism MIF-1

OVER the past decade, sporadic clinical experiences suggest that perturbations in the estrogen homeostasis of the brain can give rise to a variety of extra-pyramidal motor disorders in women. The syndrome of chorea gravidarum [17, 21, 31] and the chorea associated with estrogen-containing oral contraceptives [2, 6, 36] have been well described. On the other hand, post-menopausal women on neuroleptic therapy also exhibit a high incidence of tardive dyskinesia [23]. Clinically it has also been observed that oral estrogen decreases the intensity of both L-dihydroxyphenylalanine (L-Dopa) and neuroleptic-induced dyskinesia in women [2,3]. Although the exact mechanism remains unknown, several lines of experimental evidence suggest that the changes in the central dopaminergic neuronal activity may be relevant to the occurrence of extrapyramidal motor dysfunction. Many investigators have demonstrated that estrogen administration in pharmacological doses to adult male and female ovariectomized rats results in the increase of density (B_{max}) of striatal dopamine receptors without change in the affinity (K_D) [15, 29, 30]. Furthermore, the increase in the density of dopamine receptors consequent upon estrogen treatment is manifested behaviorally by the enhanced stereotyped response towards the prototypal dopamine agonist apomorphine [8,30]. On the other hand, estrogen has been shown to attenuate the apomorphine stereotypy and reduce D-2 receptor density as labelled by [^{3}H]-spiroperidol in the haloperidol-treated rats [18, 22, 23].

Previous studies from our laboratory [10,11] and from other investigators [5] have documented the desensitizing effect of MIF-1 [MIF-1: Melanocyte inhibiting factor-1 identified as L-prolyl-L-leucyl-glycinamide (PLG)] a tripeptide, which is structurally unrelated to classical dopamine agonists, in different animal paradigms of dopaminergic supersensitivity. In the present report, we chose the rodent model of chronic estrogen treatment to evaluate the effect of MIF-1 when administered prior to, concurrently with, or after the estrogen administration. The results indicate that MIF-1 significantly prevented, suppressed and reversed the enhancement in estrogen-induced dopamine D-2 receptor density without altering the affinity of [^{3}H]-spiroperidol in the striatum and mesolimbic areas of the male rat brain.

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TABLE 1
TREATMENT CONTROL

Group	Pre-E_2 Treatment	E_2 Treatment	Post-E_2 Treatment
		Vehicle Control	
I	5 days sesame oil	10 days sesame oil	5 days sesame oil
		Estrogen Control	
II	5 days sesame oil	E_2 for days: (5 days 20 μg/kg followed by 60 μg/kg for 5 days)	5 days sesame oil
		Concurrent Treatment - MIF-1 Control	
IIIA	5 days sesame oil	MIF-1 and sesame oil— concurrent treatment for 10 days	5 days sesame oil
		Post-Treatment - MIF-1 Control	
IIIB	5 days sesame oil	10 days sesame oil	5 days MIF-1
		Pretreatment with MIF-1	
IVA	5 days PLG	E_2 for 10 days	5 days sesame oil
		Post-Treatment with MIF-1	
IVC	5 days sesame oil	E_2 for 10 days	5 days MIF-1

Drug dosage: E_2: (17β Estradiol/Esrogen E_2) The dose and duration of E_2 treatment in Groups IVA-C was the same as in Group II. MIF-1–20 mg/kg/day: In our earlier study [11], this dose effectively prevented, suppressed and reversed the haloperidol-induced increase in [^{3}H]-spiroperidol binding in striatal and mesolimbic areas of the rat brain. Estradiol and MIF-1 were given through subcutaneous routes (SC) in a total volume of 0.1 ml/100 g body weight/day.

METHOD

Animal Preparation

Male Sprague-Dawley rats weighing 200–250 g and having a negligible endogeneous level of estrogen (Canada Breeding Farm, P.Q.) were used throughout the study. They were housed in groups of 3 animals in a temperature-controlled room maintained on a 12 hr-light/12 hr-darkness cycle and were allowed free access to food and water. Six rats were assigned randomly to each of the seven different groups (Table 1).

Drug Sources

Estradiol 17β was obtained from Calbiochem, USA. Sesame oil and MIF-1 were purchased from Sigma Chemical Co. (St. Louis, MO, USA); L-phenyl-4-[^{3}H]-spiroperidol (specific activity 25 Ci/mmol) was obtained from New England Nuclear. All other chemicals used were of the finest reagent grade available. Estradiol was suspended in the sesame oil and the MIF-1 was dissolved in the normal saline solution for administration.

Protocol for Drug Treatment and Dissection of Tissues

The treatment protocols with different drugs for the various groups are summarized in Table 1.

Preparation of Tissues

The rats were sacrificed by decapitation 72 hr after the last drug session. The brains were removed rapidly and the striata and mesolimbic area (olfactory tubercle and nucleus accumbens) were dissected out on ice according to the method of Akiyama *et al.* [1].

[^{3}H]-Spiroperidol Binding Assay

The procedure for [^{3}H]-spiroperidol binding was carried out as described earlier [11]. The striatum and the mesolimbic area were homogenized separately in 50 volumes of iced 50 mM Tris-HCl (pH 7.4, 25°C) using a Brinkman polytron for 20 seconds, and the homogenate was centrifuged at 40,000×g for 10 minutes. The pellet was washed once with 50 volumes of buffer, recentrifuged and resuspended in standard assay buffer (Tris-salt buffer) containing 50 mM Tris-HCl (10 μM pargyline, 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$ at pH 7.4) and assayed. The assay mixture contained 250 μl of tissue homogenate (20 mg tissue/ml of assay medium), 100 μl of [^{3}H]-spiroperidol and an appropriate volume of the assay buffer to give a final volume of 2.0 ml. Several different concentrations of [^{3}H]-spiroperidol (0.05–1.0 nM) were used for incubation. Specific binding was determined in the presence of unlabelled ketanserine 200 times the concentration of

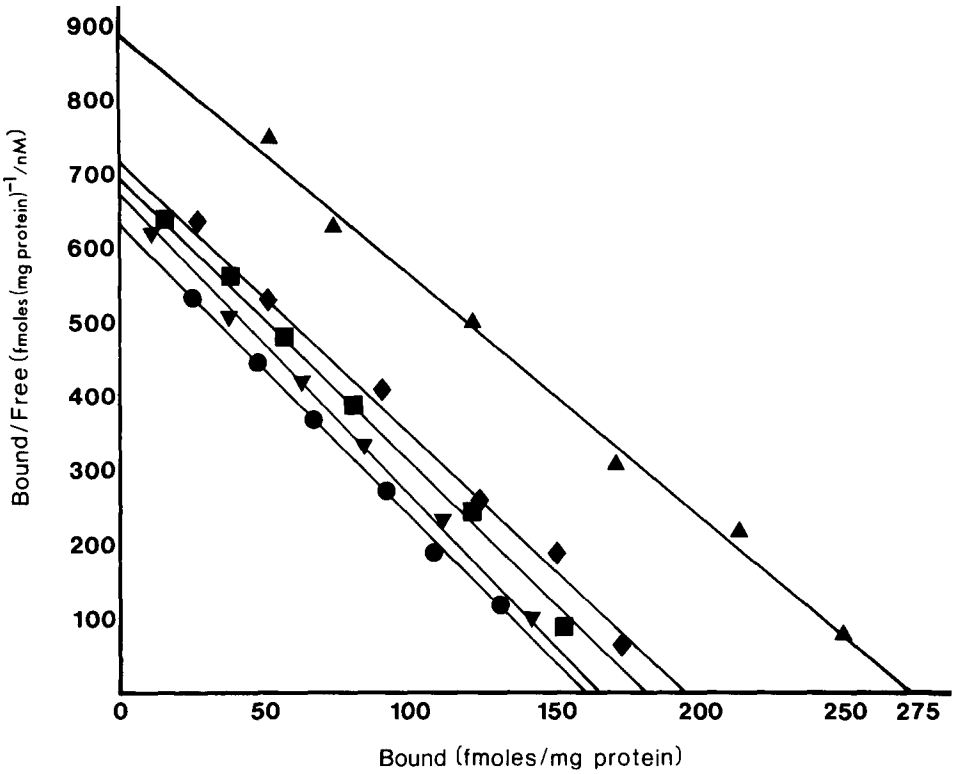

FIG. 1. Representative Scatchard plot for [^{3}H]-spiroperidol binding to striatal dopamine receptors. Estrogen significantly increased the density (B_{max}) (with unchanged affinity-K_D) of the dopamine receptors. Data presented here is from one representative experiment (one rat) from each group. For group mean, see Tables 2 and 3. ● Sesame oil; ▲ Estrogen; ▼ PLG concurrent treatment; ◆ PLG pretreatment; ■ PLG post-treatment.

[^{3}H]-spiroperidol (to block 5-HT receptors) [24] and 1 μM (d)-butaclamol. After incubating 37°C for 15 minutes, the samples were rapidly filtered under vacuum through Whatman GF/B glass fiber filters and washed six times with 3.5 ml of ice-cold 50 mM Tris-HCl. The filters were monitored for radioactivity with a Beckman Scintillation counter after having been immersed in 10 ml of PCS containing cocktail for six hours at 0°C. All assays were run in triplicate. Protein was measured by the method of Lowry *et al.* using bovine serum albumin as the standard [34].

Statistical Analysis

The binding parameters, namely the receptor density (B_{max}) and the affinity constant (K_D) were derived from the Scatchard analysis using the Linear Regression analysis [40] for individual animals in each treated group. The mean biochemical results from the different groups were analyzed by One-way Analysis of Variance followed by Duncan's multiple range test.

RESULTS

Scatchard analysis of the binding parameters revealed a single class of D-2 receptor subtype as illustrated in Fig. 1.

Striatal Dopamine D-2 Receptor Density

Estradiol 17β (Group II) caused significant increase in the (B_{max}) of [^{3}H]-spiroperidol binding when compared to sesame oil group (Group I). MIF-1 significantly prevented (Group IVA), suppressed (Group IVB) and reversed (Group IVC) the estrogen-induced increase in the dopamine D-2 receptor density (Table 2). MIF-1 per se did not have any appreciable effect on the density of striatal dopamine receptors as the B_{max} of D-2 receptors in Groups IIIA and IIIB (MIF-1 control groups) were essentially the same as the vehicle/sesame oil control group (Group I). Similarly, no statistically significant differences were observed among the means of the equilibrium dissociation constants (K_D) of the various groups studied, thus suggesting neither the estrogen nor the MIF-1 treatments altered the affinity of the D-2 receptors (Table 2).

Mesolimbic Area Dopamine D-2 Receptor Density

Estradiol 17β produced significant increase (Group II) of dopamine receptor density in the mesolimbic regions (Table 3). The proportion of the increase observed was approximately the same as that seen in the striatal area. All the treatment regimens with MIF-1: the pretreatment (Group IVA), concurrent treatment (Group IVB) and post-treatment (Group IVC) regimens have significantly ($p<0.05$) reduced the density of dopamine D-2 receptor in comparison with the estrogen treatment (Group II). However, MIF-1 per se did not affect B_{max} or K_D of the D-2 receptor (MIF-1 control groups—Groups IIIA–B). The differences in K_D values among the seven groups were not statistically significant when tested by the Duncan's multiple range test.

The differences among the three treatment regimens (pretreatment, concurrent treatment, and post-treatment) with

TABLE 2

PROPHYLACTIC, SUPPRESSIVE AND REVERSAL EFFECTS OF MIF-1 ON ESTROGEN-INDUCED INCREASED IN [^{3}H]-SPIROPERIDOL BINDING IN RAT STRIATUM

Group	Treatment	N	B_{max} (fmoles/mg protein) Mean±S.E.M.	K_D (nM) Mean±S.E.M.
I	Sesame Oil	6	141.13±13.61	0.39±0.12
II	E_2 for 10 days	6	286.11±27.08*	0.42±0.12
IIIA	MIF-1 and sesame oil concurrent treatment for 10 days	6	148.20±33.07	0.41±0.13
IIIB	Sesame oil followed by MIF-1	6	154.02± 7.15	0.49±0.10
	Pretreatment			
IVA	MIF-1 followed by E_2	6	198.08±11.59	0.48±0.16
	Concurrent treatment			
IVB	(MIF-1 + E_2) both together for 10 days	6	159.73±12.93	0.44±0.11
	Post-treatment			
IVC	E_2 followed by MIF-1	6	169.29±14.27	0.53±0.17

B_{max}: $F(6,35)=6.955$ $p<0.0001$ by one-way analysis of variance.
*Group II—Significantly different ($p<0.05$) from treatment groups (I, IIIA-B and IVA-C). No statistically significant difference among groups I, III-A-B and IVA-B and IVA-C, by Duncan's Multiple range test.
K_D: $F(6,35)=0.156$, $p>0.98$. No statistically significant difference among groups by Duncan's Multiple Range test.

TABLE 3

PROPHYLACTIC, SUPPRESSIVE AND REVERSAL EFFECTS OF MIF-1 ON ESTROGEN-INDUCED INCREASE IN [^{3}H]-SPIROPERIDOL BINDING IN RAT MESOLIMBIC AREA

Group	Treatment	N	B_{max} (fmoles/mg protein) Mean±S.E.M.	K_D (nM) Mean±S.E.M.
I	Sesame Oil	6	151.27±10.34	0.39±0.10
II	E_2 for 10 days	6	289.78±29.10*	0.43±0.16
IIIA	MIF-1 and sesame oil concurrent treatment for 10 days	6	161.68± 8.13	0.45±0.12
IIIB	Sesame oil followed by MIF-1	6	155.85± 7.10	0.37±0.10
	Pretreatment			
73IVA	MIF-1 followed by E_2	6	152.03±12.32	0.41±0.09
	Concurrent treatment			
IVB	(MIF-1 ± E_2) both together for 10 days	6	168.04± 8.16	0.45±0.10
	Post-treatment			
IVC	E_2 followed by MIF-1	6	180.52± 9.30	0.48±0.11

B_{max}: $F(6,35)=12.488$ $p<0.0001$ by one-way analysis of variance.
*Group II—Significantly different ($p<0.05$) from treatment groups (I, III-A-B and IV-A-C). No statistically significant difference among groups I, III-B and IVA-C by Duncan's Multiple range test.
K_D: $F(6,35)=0.114$, $p>0.99$. No statistically significant difference among groups by Duncan's Multiple Range test.

respect to B_{max} and K_D in striatal and mesolimbic area were, however, statistically insignificant.

DISCUSSION

The interaction of estrogen with the central neurotransmitter systems is implicated in the uncommon occurrence of choreiform disorders in hyperestrogen states such as pregnancy and the usage of oral contraceptives. In an attempt to eliminate the fluctuations in the endogenous levels of estrogens, we have chosen the male rats to study the specific effects of exogenous estrogens on central dopamine receptor function as the plausible animal paradigm of hyperestrogenic states in females. A more plausible model of choreiform disorders as a function of the hormonal status in females admittedly may be found in the ovariectomized female rats, or in the primate species. To our knowledge, this is the first report describing the antagonistic activity of MIF-1 against the estrogen-induced increase in the density of dopamine receptor in CNS. We have observed that MIF-1 prevented (by pretreatment), suppressed (by concurrent treatment) and reversed (by post-treatment) the increase in dopamine receptor density in both striatum (Table 2) and mesolimbic area (Table 3).

In the present study, we have also confirmed that in male rats chronic treatment by estradiol 17β (priming dose of 20 μg/kg/day SC for 5 days followed by 60 μg/kg/day SC for 5 days) increased the density of dopamine D-2 receptors approximately 100% over the control group (Tables 2,3). This increase is much higher than reported by earlier workers in the rat (14.3% [15], 23% [14] and 19.2% [29]). The reason for this may be due to higher total dose used in our protocol (20 μg/day/5 days followed by 60 μg/day/5 days). The other investigators used varying lower doses (20 μg/kg/2 weeks [15], 10 μg/kg/twice daily/2 weeks [14] and 125 μg/single dose [29]) incorporated either in oil vehicle or in subcutaneous pellet implants. We gave estrogen by the subcutaneous route in an oil (sesame oil) base/vehicle. Injection of estrogen in an oil vehicle ensures 'depot' formation and sustained release to maintain pharmacologically efficacious *in vivo* level.

Our findings on the enhancement in the density of the dopamine receptor confirm the recent findings of others [1, 13, 28] regarding the supersensitized striatal and mesolimbic area dopamine receptors developing after the administration a high dose of estrogen. Most of the other investigators [15, 16, 29] likewise reported only the upregulation of the dopamine receptor population (B_{max}) without a change in the affinity (K_D). It has been argued that the enhancement of dopamine receptor is believed to be due to the genome-directed *de novo* synthesis of dopamine receptors [7,29], rather than a conformational change in the functional state of the receptor macromolecular complex. Both *in vivo* [13] and *in vitro* [39,41] studies suggest that the effect of estrogen on the central dopamine receptors can be attributed to its metabolite 2-hydroxyestrogen (a catechol estrogen).

The mechanisms of the antagonistic action of MIF-1 with respect to estrogen-induced supersensitivity of dopamine receptors are subject to different possible interpretations. MIF-1 may conceivably inhibit the biotransformation of estrogen to the active metabolite, 2-hydroxyestradiol. Alternatively, MIF-1 may interfere with the genome-directed *de novo* synthesis of the new receptor protein. In relation to the reversal effects, the post-administration of MIF-1 would be highly unlikely to influence either the metabolic or the genetic events associated with the induction of the supersensitivity of dopamine receptors. Hence, these two hypothetical mechanisms albeit intriguing, cannot adequately account for the reversal effects of MIF-1. It has also been shown that estrogen can mediate changes in the rat brain DA system independent of the pituitary [14]. Hence, it is unlikely that in the present male rat models, either the upregulating action of estrogen or its ameliorating MIF-1 action of estrogen is mediated through the pituitary. A unifying heuristic hypothesis can be proposed to the effect that MIF-1 modulates the sensitivity of pre- and post-synaptic dopamine receptors through specific receptor-receptor interactions. Several lines of evidence suggest the interaction of peptides (substance P, CCK and neurotensin) with neurotransmitter receptors [25–27]. MIF-1 selectively enhances the binding affinity of dopamine agonists, [^{3}H]-apomorphine and [^{3}H]-ADTN, to dopamine receptors in the striatum in a peculiar bell-shaped manner [4, 9, 32, 33] and increases the turnover of dopamine in the striatum [20, 35, 42]. It has been shown [37] that [^{3}H]-MIF-1 localizes in various parts of the brain after intracarotid administration of [^{3}H]-MIF-1, thus suggesting that MIF-1 penetrates the blood brain barrier. In addition, the reports from our laboratory, as well as from others, regarding the effects of MIF-1 on CNS clearly indicate that after parenteral administration MIF-1 exerts its effect on the striatal and mesolimbic area. The facilitatory effect of MIF-1 on dopamine receptor binding exhibits some degree of stereochemical requirements highly suggestive of receptor-mediated neuronal events [32,33] which seem to endure beyond the half life (26.4 min in rat plasma and 5–6 days in human plasma) of MIF-1 [43]. In this regard, specific saturable, high-affinity binding sites for MIF-1 [12], for the tyrosine analogue of MIF-1 have been identified by us [12] and by others [44] in various areas of the mammalian brain. Furthermor, MIF-1 may restore any change in the degree of coupling of dopamine receptor to the effector mechanisms as exemplified in adenylate cyclase through interacting with the G/F protein [38]. Recent evidence from our laboratory indicates that MIF-1 enhances the interaction of striatal dopamine receptor with the G/F protein (manuscript submitted).

In conclusion, this report regarding the modulatory effect of MIF-1 on the estrogen-induced influence on the dopamine receptors augur well for a class of small peptides like MIF-1 to emerge as potential prophylactic and therapeutic agents in clinical conditions featuring upregulation of the central dopamine receptor. The pragmatic approach of pharmacological modulation of neurotransmitter receptor sensitivity as suggested by Friedhoff and his coworkers [19] can conveniently be applied to MIF-1 and its active analogues.

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