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Effects of L-prolyl-L-leucyl-glycine Amide (MIF-I) on Dopaminergic Neurons

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KOSTRZEWA, R. M., M. A. SPIRTES, JOAN W. KLARA, C. W. CHRISTENSEN, A. J. KASTIN AND T. H. JOH. *Effects of L-prolyl-L-leucyl-glycine amide (MIF-I) on dopaminergic neurons.* PHARMAC. BIOCHEM. BEHAV. 5: SUPPL. 1, 125–127, 1976. In an attempt to determine the mechanism of action of L-prolyl-L-leucyl-glycine amide (MIF-I) in the treatment of Parkinson's disease, various parameters of dopaminergic neuronal function were studied in rats. It was found that the active uptake of ^{3}H-dopamine (^{3}H-DA) by synaptosome-rich homogenates of the striatum of rats treated with MIF-I (1 mg/kg IP × 3, 24 hr intervals) was unaltered 1 hr after final treatment with MIF-I. Also, neither tyrosine hydroxylase nor dopa decarboxylase activity was altered in the striatum and substantia nigra of rats treated with MIF-I (20 mg/kg IP × 3, 24 hr intervals). Thus, vital functional processes associated with dopaminergic neurons apparently are not altered by MIF-I under the conditions studied. These findings illustrate the importance of concurrent DOPA administration in observing an effect of MIF-I on dopaminergic neuronal function.

L-prolyl-L-leucyl-glycine amide Dopaminergic neurons ^{3}H-DA uptake Tyrosine hydroxylase Dopa decarboxylase

RECENT studies by several groups of investigators [1, 2, 6, 9] have determined that the tripeptide, L-prolyl-L-leucyl-glycinamide (MIF-I) has antiparkinsonian activity. Because of the correlation that has been established between Parkinson's disease and destruction of the nigro-neostriatal dopaminergic pathway [5, 8, 12] it is reasonable to assume that MIF-I acts on dopaminergic neurons. Laboratory studies with animals have shown that MIF-I is able to potentiate the behavioral effects of dihydroxyphenylalanine (DOPA) [13]. Other studies in our laboratory indicate that dopamine (DA) levels are elevated in rat striatum after DOPA, and that endogenous levels of DA rise to an even greater degree after treatment with DOPA + MIF-I [15]. When in vivo studies were done to determine the effect of MIF-I on endogenous levels of DA in rat striatum, little change [7] or no change [10] was found. Also, no change in DA turnover was seen in intact or hypophysectomized rats after MIF-I [10].

In this study, the uptake of ^{3}H-DA was studied in synaptosome-rich homogenates of striatum in order to determine whether MIF-I potentiates the effect of neurally released DA. Blockade of the uptake mechanism would prolong the duration of DA in the synaptic cleft and thereby potentiate its actions. Tyrosine hydroxylase activity and dopa decarboxylase activity were also assayed in the striatum and the substantia nigra, in order to determine whether MIF-I alters these enzymes in vivo.

The present studies show that MIF-I does not potentiate dopaminergic neuronal activity by blocking uptake of DA; or by altering tyrosine hydroxylase activity; or by altering dopa decarboxylase activity.

METHOD

^{3}H-DA Uptake Study

Six Sprague-Dawley male albino rats (Charles River, 165–220 g) received a total of 3 injections of MIF-I (1 mg/kg IP) at 24 hr intervals, and were sacrificed by decapitation 1 hr after the final dose. An equal number of control rats received the diluent saline (0.9%) acetic acid (0.01 M), and were studied simultaneously with the experimental group. The striatum was isolated from both groups of rats, and after weighing, was homogenized in 9 vol. of cold 0.32 M sucrose in a Potter-Elvehjem homogenizer. The homogenate was then centrifuged for 10 min at 1000 xg at 4° C and the supernate was diluted with 0.32 M sucrose to give a concentration equivalent to 20 mg of original tissue per ml. Aliquots (100 μl) were added to 0.8

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ml of a modified Krebs-Ringer bicarbonate medium containing 136 mM NaCl, 5.6 mM KCl, 16.2 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, 1.22 mM $MgCl_2$, 0.54 mM $CaCl_2$, 11 mM glucose, and 0.0124 mM nialamide. After preincubation for 10 min at 37° C, 100 μl of ^{3}H-DA stocks (New England Nuclear) were added to give final concentrations of DA of 4, 2, 1 and 0.5×10^{-7} M. Samples were incubated with the ^{3}H-DA for 5 min at 37° C and then placed in an ice bath before filtration through 0.45 μ pore diameter cellulose-derivative filters (Millipore). Filters were dried before being placed in 10 ml of scintillation cocktail containing Triton X-100. Blanks were processed in an identical manner as the above samples, except that incubation with the ^{3}H-DA was at 0° C.

Enzyme Assays

In these studies, 6 Sprague-Dawley male albino rats received a total of 3 treatments with MIF-I (20 mg/kg IP) at 24 hr intervals, and were sacrificed by decapitation 1 hr after the final dose. An equal number of controls received the diluent saline (0.9%)-ascorbic acid (0.1%). At the time of sacrifice the caudate nucleus and substantia nigra were dissected from the rest of the brain and frozen on dry ice. Tyrosine hydroxylase activity was determined in the tissue specimens by a modification of the method of Coyle [4], as utilized by Reis, Joh and Ross [14]. Dopa decarboxylase activity was assayed by the method of Lamprecht and Coyle [11].

Statistics

In order to determine the lines of best fit for the Lineweaver-Burke plots, a linear regression analysis of the data was performed. The K_m and V_{max} for each animal was determined separately and mean values with SE were determined. Student's *t*-test was used to determine significance between the K_m and V_{max} values of the treatment and control groups and for comparing enzyme activities in treated and control groups.

RESULTS

When rats received consecutive daily treatments of MIF-I (1 mg/kg IP) and were sacrificed 1 hr after the third dose, there was no change in uptake of ^{3}H-DA in the striatum. It can be seen in Fig. 1, by linear transformation of the data in the Lineweaver-Burke plot, that the Michaelis-Menten kinetic constants were unaltered by the treatment. The V_{max} and K_m were identical in both groups.

In the striatum it was also found that MIF-I (20 mg/kg IP × 3, 24 hr intervals) did not alter either tyrosine hydroxylase or dopa decarboxylase activity. As shown in Table 1, activity of both enzymes was unchanged 1 hr after final treatment with MIF-I. Likewise, activity of tyrosine hydroxylase and dopa decarboxylase was unchanged in the substantia nigra after treatment with MIF-I.

DISCUSSION

A report several years ago indicated that MIF-I had the capacity to stimulate activity of tyrosine hydroxylase in vitro in slices of rat striatum [7]. That study thus suggested that the rate-limiting enzyme in the biosynthetic pathway for DA, and hence the rate of DA synthesis, could be stimulated. Such a mechanism could explain the usefulness of MIF-I in the treatment of parkinsonism.

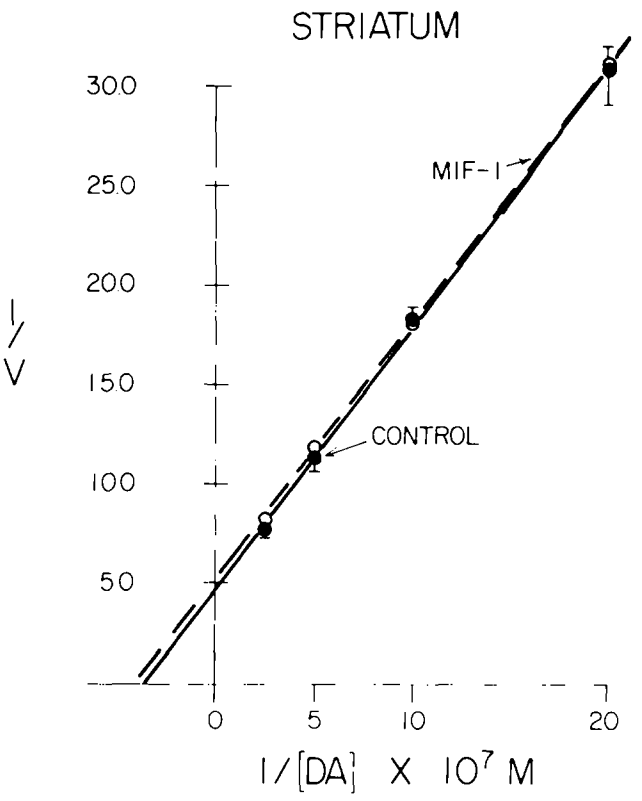

FIG. 1. Lineweaver-Burke plot of ^{3}H-DA uptake into synaptosome-rich homogenates of rat striatum. Rats received 3 treatments of MIF-I (1 mg/kg IP) at 24 hr intervals and were sacrificed 1 hr after the last treatment. Each point with vertical bar represents the mean and SE of 6 control or 6 experimental animals. The ^{3}H-DA uptake at 0° C was approximately 10% of the 37° C value. V_{max} for treated and control groups, respectively, was 2.34 ± 0.08 and 2.54 ± 0.13 nM ^{3}H-DA/mg tissue/hr. K_m for MIF-I and saline groups, respectively, was 2.53 ± 0.14 and 2.71 ± 0.10 × 10^{-7} M.

However, studies in this and a previous report [10] fail to confirm this previous finding. At the dose employed MIF-I (20 mg/kg IP × 3, 24 hr intervals) did not alter tyrosine hydroxylase in either the striatum or substantia nigra of rats. Thus, neither the cell body nor nerve terminal region of the nigro-neostriatal pathway appeared to be stimulated to synthesize DA at a faster rate. Also, dopa decarboxylase activity remained unchanged in the striatum and substantia nigra after treatment with MIF-I. This throws into a query the role of MIF-I in the DOPA potentiation test of Plotnikoff [13]. Recent findings indicate that dopa decarboxylase may be stimulated in vivo by MIF-I, since the level of DA after DOPA + MIF-I increased significantly above that found after DOPA treatment alone [15]. The possibility exists that MIF-I can actually stimulate both tyrosine hydroxylase and dopa decarboxylase at a later time or when the MIF-I is given in a different dose. However, it is also feasible that MIF-I acts in some way to alter the distribution of DOPA in the brain; or alter the metabolism of exogenous DOPA.

Because uptake of ^{3}H-DA is unaltered in synaptosome-rich homogenates of striatum after in vivo treatment of rats with MIF-I, it appears that competition of MIF-I for the DA uptake site at the dopaminergic terminal ending is not

TABLE 1

TYROSINE HYDROXYLASE AND DOPA DECARBOXYLASE ACTIVITY IN RAT BRAIN AFTER TREATMENT WITH MIF-I

Brain Region	Treatment	Tyrosine Hydroxylase Activity (μM/g tissue/hr)*	Dopa Decarboxylase Activity (μM/g tissue/hr.)*
Substantia Nigra	Saline	864.9 ± 79.0	1114.2 ± 120.6
	MIF-I†	782.2 ± 116.7	1041.5 ± 99.5
Striatum	Saline	314.05 ± 24.8	692.6 ± 18.0
	MIF-I	342.65 ± 14.9	704.2 ± 13.3

*Each sample is the mean ± S.E. of 6 determinations.
†Rats received 3 injections of MIF-I (20 mg/kg ip) at 24 hr intervals, and were sacrificed 1 hr after final treatment.

the mechanism of action. Previous study indicated that MIF-I did not alter the turnover of DA, as determined by the disappearance rate of endogenous DA after α-methyl-p-tyrosine administration [10]. Preliminary studies indicate that MIF-I does not alter the release of DA from DA-loaded synaptosomes. The failure of MIF-I to alter apomorphine-induced stereotypy indicates that MIF-I does not have strong postsynaptic activity at the dopamine receptor site [10]. In support of this conclusion is the fact that MIF-I does not alter resting levels of cAMP, a suspected second messenger for DA, in the striatum of intact and hypophysectomized rats [3].

Therefore, the present study indicates that MIF-I alone has little action on dopaminergic neurons, although direct or indirect action is manifested when DOPA administration is combined with the MIF-I. It is suggested that MIF-I produces antiparkinsonian effects primarily through a modification of DOPA metabolism or redistribution of DOPA metabolites in the brain.

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