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MIF-1: Effects on Norepinephrine, Dopamine and Serotonin Metabolism in Certain Discrete Brain Regions

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SINGHAL, R. L. AND R. B. RASTOGI. *MIF-1: Effects on norepinephrine, dopamine and serotonin metabolism in certain discrete brain regions.* PHARMAC. BIOCHEM. BEHAV. **16**(2) 229–233, 1982.—A single injection of melanocyte-stimulating hormone inhibitory factor (MIF-1) in a dose of 3 mg/kg IP produced no significant effect on dopamine turnover. However, a dose of 5 mg/kg increased striatal tyrosine hydroxylase activity by 25% and homovanillic acid level by 27% when compared to control values. No change in either parameter was detected in olfactory tubercles. Dopamine levels also were elevated in striatum, pons-medulla and cerebral cortex in rats receiving 5 mg/kg dose of MIF-1. In olfactory tubercles, dopamine levels were however, reduced to 71% of control values taken as 100%. The concentration of norepinephrine tended to increase in several brain areas examined but, the change was statistically significant only in olfactory tubercles and cerebral cortex. The level of norepinephrine metabolite, 3-methoxy-4-hydroxyphenylethylene glycol, was lowered to 63% in whole brain of animals given MIF-1 at the dose of 5 mg/kg. These data suggest that MIF-1 enhances the turnover of dopamine and norepinephrine in the brain. However, MIF-1 treatment seemed to produce no consistent change in brain serotonin turnover. In striatum and cortex, this neuropeptide increased serotonin but elevated the level of its metabolite, 5-hydroxyindoleacetic acid indicating that the release of this brain amine was decreased in these two brain regions. The levels of 5-hydroxyindoleacetic acid were enhanced in hypothalamus and pons-medulla regardless of the dose of MIF-1 administered.

MIF-1 Striatum Dopamine Tyrosine hydroxylase Homovanillic acid Tryptophan hydroxylase Norepinephrine Serotonin

MELANOCYTE-stimulating hormone inhibitory factor (MIF-1) is a hypothalamic neuropeptide which not only controls the release of melanocyte-stimulating hormone (MSH) from the intermediate lobe of the pituitary but also elicits direct effects on the brain itself [15]. Kastin and Barbeau [14] demonstrated for the first time that MIF-1 (L-protyl-L-leucyl-glycine amide; PLG), ameliorated rigidity in patients suffering from Parkinson's disease. Gerstenbrand *et al.* [10] later confirmed these findings. Moreover, in clinical trials, patients with mental depression experienced marked improvement in their symptoms within two to three days [6,7]. However, the mechanism(s) underlying the antidepressant and anti-parkinsonian action of MIF-1 is not entirely clear. Contradictory reports on the effects of MIF-1 have been published on the turnover of DA in the striatum [16, 18, 25, 28, 33], on striatal tyrosine hydroxylase (TH) activity [9,20], endogenous dopamine (DA) levels in the striatum [19,28], as well as on the enhancement of the actions of apomorphine and oxotremorine [3, 4, 18, 25]. However, the reports demonstrating the effects of MIF-1 on DOPA-potentiation in animals appear to be less controversial [11, 12, 26, 27]. In the present study, we have examined the effect of MIF-1 on DA synthesis and release in not only striatal regions of the brain but also in olfactory tubercles, a part of the mesolimbic DA system. Furthermore, the influence of this tripeptide has been investigated on NE and 5-HT metabolism in certain areas of the rat brain.

METHOD

Animals

Male Sprague-Dawley rats weighing 130–150 g were used in this study. Animals were kept in groups of 6 per cage under constant environmental conditions (24°C, 60% relative humidity and regular alternate cycles of 12 hr light and darkness) with food and water ad lib. MIF-1 was injected IP in a dose of either 3 mg or 5 mg/kg and rats killed using the near-freezing technique [32], 1½ hr later. Control animals received an equal volume of physiological saline. MIF-1 in high doses (8 and 16 mg/kg) produces overt sedative effects and elicits a lesser DOPA-potentiation response [26]. Studies of Ehrensing and Kastin [7] showed that MIF-1 produces dose-related biphasic effects; greater improvement was seen in patients treated with a lower dose of MIF-1 than those receiving a higher dose of this neuropeptide. Similarly, less pronounced changes in certain neurochemical parameters have been reported in animals treated with higher doses of MIF-1 [20,27]. Furthermore, Friedman *et al.* [9], using a lower dose of 4 mg/kg, reported a significant increase in the levels of striatal DA. In the present study, we therefore em-

TABLE 1

EFFECT OF ACUTE MIF-1 ON SOLUBLE TH ACTIVITY AS WELL AS DA AND HVA LEVELS IN DISCRETE BRAIN AREAS OF RATS

Regions Examined	Control	MIF-1 (3 mg/kg)	MIF-1 (5 mg/kg)
TH (nmoles DOPA/g/hr)			
Striatum	13.47 ± 1.02 (100)	11.19 ± 1.07 (83)	16.84 ± 1.12 (125)*
Olfactory tubercles	3.24 ± 0.56 (100)	2.95 ± 0.35 (91)	3.30 ± 0.25 (102)
DA (μg/g)			
Striatum	7.93 ± 0.64 (100)	8.25 ± 0.69 (104)	9.83 ± 0.53 (124)*
Pons-medulla	0.44 ± 0.03 (100)	0.53 ± 0.05 (119)	0.60 ± 0.03 (136)*
Cerebral cortex	0.97 ± 0.06 (100)	1.11 ± 0.03 (114)*	1.77 ± 0.09 (183)*
Olfactory tubercles	0.64 ± 0.07 (100)	0.51 ± 0.06 (79)	0.45 ± 0.03 (71)*
HVA (μg/g)			
Striatum	0.68 ± 0.06 (100)	0.82 ± 0.09 (120)	0.86 ± 0.07 (127)*
Olfactory tubercles	0.39 ± 0.02 (100)	0.43 ± 0.03 (110)	0.41 ± 0.02 (104)

Values represent means ± S.E.M. of 6 rats in each group. Rats received a single IP injection of MIF-1 in a dose of either 3 or 5 mg/kg and killed 1½ hours later. Control animals received an equal volume of the physiological saline (vehicle). Data in parentheses express results in percentages taking the control values as 100%.

*Statistically significant difference when compared to control values ($p<0.05$).

TABLE 2

EFFECT OF ACUTE MIF-1 ON BRAIN NE AND MOPEG LEVELS

Regions Examined	Control	MIF-1 (3 mg/kg)	MIF-1 (5 mg/kg)
NE (μg/g)			
Hypothalamus	1.86 ± 0.16 (100)	2.30 ± 0.27 (124)	2.18 ± 0.25 (117)
Mid-brain	0.40 ± 0.01 (100)	0.41 ± 0.02 (104)	0.37 ± 0.03 (93)
Pons-medulla	0.40 ± 0.02 (100)	0.47 ± 0.06 (117)	0.41 ± 0.05 (103)
Olfactory tubercles	0.83 ± 0.06 (100)	1.13 ± 0.07 (136)*	0.90 ± 0.05 (108)
Cerebral cortex	0.72 ± 0.04 (100)	0.65 ± 0.03 (90)	1.10 ± 0.09 (153)*
MOPEG (μg/g)			
Whole brain	0.69 ± 0.06 (100)	0.56 ± 0.07 (82)	0.44 ± 0.03 (63)*

For experimental details, refer to the legend in Table 1.

*Statistically significant difference when compared to control values ($p<0.05$).

ployed a dose 3 and 5 mg/kg MIF-1 to study if there were any dose-related changes in neurochemical parameters related to DA and 5-HT metabolism.

Sample Preparation and Biochemical Assays

Following decapitation, brain was dissected into specific brain regions as published in a previous report [31]. The activity of tyrosine hydroxylase (TH) was assayed in the soluble fraction of striatum and olfactory tubercles in the presence of $FeSO_4$ and the co-factor BH_4 according to the method of Rastogi *et al.* [30]. The activity of tryptophan hydroxylase (TPH) was determined in mid-brain as described by Peters *et al.* [23]. The levels of norepinephrine (NE) and dopamine (DA) were measured according to the radio-enzymatic method of Peuler and Johnson [24], where as serotonin (5-hydroxytryptamine; 5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were assayed by the fluorometric assay of Curzon and Green [5]. The concentration of tryptophan (TP) was estimated in mid-brain as described elsewhere [31]. For determining the levels of homovanillic acid (HVA), striata were pooled from 3 rats and assay carried out fluorometrically according to the method of Murphy *et al.* [22] with minor modification [31]. The 3-methoxy-4-hydroxyphenylethylene glycol (MOPEG) level was measured in the whole brain according to the method of Meek [21]. The blank values were always obtained in triplicate.

Data were evaluated statistically by a two-way analysis of variance using logarithmically transformed raw scores. For those main effects found to be significant beyond the 0.05 level of probability, a Duncan's Multiple Range Test was applied.

RESULTS

Effect on DA Metabolism

Data presented in Table 1 demonstrate that a single injection of MIF-1 (3 mg/kg) produced no change in the activity of soluble TH in striatum as well as olfactory tubercles. However, the 5 mg/kg dose of MIF-1 increased TH activity in striatum by 25% and produced no change in olfactory tubercles. DA levels were increased in striatum and pons-medulla of rats receiving only this higher dose of MIF-1. In cerebral cortex, a significant rise in DA concentration was seen after both the 3 and 5 mg/kg dose of MIF-1. In olfactory tubercles, on the otherhand, MIF-1 treatment produced a decrease in DA although the change was statistically significant only in rats treated with the higher dose of this neuropeptide. The levels of DA metabolite HVA were elevated in striatum of rats treated with the 5 mg/kg dose, whereas no change was observed in olfactory tubercles (Table 1). These data seem to suggest that MIF-1 enhances synthesis and turnover only of the striatal DA system without any such effect in mesolimbic DA pathways.

TABLE 3

EFFECT OF ACUTE MIF-1 ON MID-BRAIN TPH ACTIVITY AND TP LEVELS

Treatment	TPH (nmoles/g/hr)	TP (μg/g)
Control	6.79 ± 0.55 (100)	4.92 ± 0.31 (100)
MIF-1 (3 mg/kg)	4.42 ± 0.34 (65)*	4.68 ± 0.29 (95)
(5 mg/kg)	7.72 ± 0.69 (114)	4.97 ± 0.36 (101)

For experimental details, refer to the legend in Table 1.
*Statistically significant difference when compared to control values ($p<0.05$).

Effect on NE System

MIF-1 treatment (3 mg/kg) tended to elevate NE levels in several brain areas examined (see Table 2), however, the change was statistically significant only in olfactory tubercles. A dose of 5 mg/kg produced no effect on NE levels in hypothalamus, mid-brain, pons-medulla and olfactory tubercles; in cerebral cortex an increase of 53% was recorded (Table 2). The level of NE metabolite, MOPEG was reduced significantly to 63% of control values taken as 100% in brains of rats receiving the 5 mg/kg dose of MIF-1.

Effect of MIF-1 on Biosynthetic Capacity of 5-HT

Data in Table 3 demonstrate that injection of MIF-1 (3 mg/kg) decreased TPH activity in mid-brain by 35%. The MIF-1 dose of 5 mg/kg tended to elevate (14%) TPH activity but the change was statistically non-significant. The TP level remained unaltered after MIF-1 treatment in both groups.

As shown in Table 4, the concentrations of 5-HT and 5-HIAA were changed variably in different brain regions examined. In striatum and cerebral cortex, 5-HT levels were enhanced whereas those of 5-HIAA were decreased suggesting a reduced turnover of this indoleamine in these brain regions. In other brain areas, such as hypothalamus, midbrain and pons-medulla, the 5-HIAA levels were increased by 37, 33 and 120% respectively, in rats given the 3 mg/kg dose of MIF-1. In contrast, the 5 mg/kg dose of MIF-1 reduced 5-HIAA level in pons-medulla by 47%.

DISCUSSION

The present study demonstrates that MIF-1 at the dose of 5 mg/kg produced a small but significant effect on the synthesis and release of striatal DA, a finding consistent with the previous data [9, 28, 33]. A more pronounced increase in DA level was seen in cerebral cortex of rats treated with this dose of MIF-1. Plotnikoff *et al.* [26] had demonstrated that MIF-1 up to a dose of 4 mg/kg IP, greatly potentiated the behavioural effects of L-DOPA in normal as well as hypophysectomized mice suggesting that this effect on DA-ergic system is not mediated by melanocyte stimulating hormone (MSH). Versteeg *et al.* [33] examined several brain nuclei and showed that DA turnover was increased only in striatum of rats given MIF-1, but not in other brain areas including nucleus accumbens. This is in line with our present findings as we observed no change in olfactory tubercles which together with nucleus accumbens constitute a part of mesolimbic DA system. Thus, DA potentiating effect of MIF-1 seems to be limited to striatal and possibly cerebral cortical regions. However, these observations do not coincide with those of Kostrezewa *et al.* [16]. Plotnikoff *et al.* [27] reported no change in caudate HVA 1 hour after a single injection or following 5 day treatment with 100 mg/kg dose of MIF-1. Kostrezewa *et al.* [20] noted a slight, but statistically non-significant increase in striatal TH activity of rats receiving MIF-1 for 3 days at a daily dose of 20 mg/kg. The apparent discrepancies reported in earlier studies [20,27] are probably due to the higher dose of MIF-1 employed by these

TABLE 4

EFFECT OF ACUTE MIF-1 ON 5-HT AND 5-HIAA LEVELS IN DISCRETE BRAIN REGIONS OF RATS

Regions Examined	Control	MIF-1 (3 mg/kg)	MIF-1 (5 mg/kg)	Control	MIF-1 (3 mg/kg)	MIF-1 (5 mg/kg)
	5-HT (μg/g)			5-HIAA (μg/g)		
Hippocampus	0.30 ± 0.02 (100)	0.33 ± 0.01 (110)	0.34 ± 0.02 (112)	0.35 ± 0.04 (100)	0.37 ± 0.05 (106)	0.39 ± 0.05 (113)
Striatum	0.84 ± 0.06 (100)	1.06 ± 0.05 (126)*	1.41 ± 0.08 (168)*	0.93 ± 0.07 (100)	0.80 ± 0.08 (86)	0.72 ± 0.05 (78)*
Hypothalamus	1.37 ± 0.08 (100)	1.60 ± 0.18 (117)	1.53 ± 0.14 (112)	0.96 ± 0.05 (100)	1.32 ± 0.14 (137)*	1.21 ± 0.07 (126)*
Midbrain	1.61 ± 0.12 (100)	1.53 ± 0.14 (95)	1.56 ± 0.10 (97)	1.37 ± 0.07 (100)	1.82 ± 0.09 (133)*	1.56 ± 0.12 (114)
Pons-medulla	0.47 ± 0.02 (100)	0.52 ± 0.04 (111)	0.51 ± 0.03 (108)	0.36 ± 0.02 (100)	0.79 ± 0.05 (220)*	0.19 ± 0.02 (53)*
Cerebral cortex	0.48 ± 0.02 (100)	0.46 ± 0.02 (95)	0.61 ± 0.03 (128)*	0.52 ± 0.03 (100)	0.48 ± 0.02 (92)	0.44 ± 0.01 (85)*

For experimental details, refer to the legend in Table 1.
*Statistically significant difference when compared to control values ($p<0.05$).

investigators. In fact, a dose-related biphasic response has been reported in depressed patients [7], and those suffering from Parkinson's disease [1,2] and tardive dyskinesia [8], where the beneficial effects were observed only with lower doses of MIF-1, but not with the higher doses. Itil [13] reported considerably different computerized EEG profile after low and high doses of MIF-1 given orally. Our own findings as well as those of Friedman *et al.* [9] reporting that MIF-1 elevated the endogenous levels of DA in striatum and other discrete brain areas are at variance with the data of Plotnikoff *et al.* [27] who failed to see any change in whole brain DA.

The mechanism underlying the modulatory action of MIF-1 on striatal DAergic neurons is not well understood. The previous findings that MIF-1 produced no effect on the activity of choline acetyltransferase and glutamic acid decarboxylase [17] suggest that this neuropeptide does not affect the functioning of acetylcholine and γ-aminobutyric acid (GABA) containing neurons which interact with DAergic neurons. Furthermore, MIF-1 neither changes the sensitivity of post-synaptic DA receptor sites nor its neuronal uptake [17]. It remains to be shown whether neurochemical changes are the result of MIF-1 interaction with opiate receptors.

The effects of MIF-1 on other neuronal systems such as NE and 5-HT are not too consistent. In cortex, MIF-1 treatment elevated the endogenous level of NE and decreased that of MOPEG in whole brain suggesting a trend for decreased release of this catecholamine which is in accordance with the report of Pugsley and Lippmann [29]. MIF-1 enhanced 5-HT content but reduced the levels of 5-HIAA in striatum and cortex, indicating that the release of this indoleamine was decreased in these two brain areas. However, in other brain regions, 5-HIAA levels were increased regardless of the dose of MIF-1 injected. Hence no evidence can really be provided for the potentiating effect of this neuropeptide on serotonergic neurons [11,28].

In conclusion, our data demonstrate that MIF-1 significantly enhances the DA synthesis and release in striatum and enhances the level of DA in cerebral cortex. It is believed that nigro-striatal DA regulates the motor behaviour whereas mesocortical DA pathway has been identified as the anatomical site of emotional behaviour. It is therefore likely that beneficial effects observed in Parkinsonism and depressed patients might, at least in part, be associated with increased DA levels in striatum and cerebral cortex, respectively. However, further well controlled clinical studies using small doses of MIF-1 would be desirable in a relatively large population of patients to demonstrate the antidepressant effect of this potentially short-acting drug with no discernible side effects.

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