The Neuropeptides
Pharmacology Biochemistry and Behavior, Vol. 5, Suppl. 1, pp. 121–124. ANKHO International Inc., 1976.

Possible Association of Increased Rat Behavioral Effects and Increased Striatal Dopamine and Norepinephrine Levels During the DOPA-Potentiation Test[1]

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SPIRTES, M. A., N. P. PLOTNIKOFF, R. M. KOSTRZEWA, C. T. HARSTON, A. J. KASTIN AND C. W. CHRISTENSEN. *Possible association of increased rat behavior effects and increased striatal dopamine and norepinephrine levels during the DOPA-potentiation test.* PHARMAC. BIOCHEM. BEHAV. 5: SUPPL. 1, 121–124, 1976. – Previous reports have indicated that α-MSH release inhibiting hormone (MIF-I) increased the behavior occurring as a result of the dihydroxyphenylalanine (DOPA) potentiation test [3,7]. This study was undertaken to see whether dopamine (DA) or norepinephrine (NE) levels likewise increased in the test animals. The DOPA potentiation test was performed as follows: 2–4 hr before behavior measurement, 40 mg/kg of the monoamine oxidase inhibitor pargyline HCl was given orally. Two hr later this was followed by the intraperitoneal (IP) injection of MIF-I at doses of 0.1, 0.3 or 1.0 mg/kg. Behavioral measurement was begun after the IP injection of 200 mg/kg of dl-DOPA 1–2 hr after the MIF-I. The parameters included social interaction, aggressiveness, fighting, ataxia, jumping, defecation, urination and salivation. The animals were beheaded while the behavior was still increased and the striatal area removed, placed in aluminum foil, and kept at −50° C until assayed. In general, especially among the younger animals, a significant correlation ($p = 0.05$ to $p = 0.01$) was found between the increased behavioral responses to MIF-I and the rise in DA. Because of a few exceptions to this correlation the possibility is suggested that MIF-I might also affect behavior by acting directly on the postsynaptic membrane thus bypassing any change in NE or DA which is known to increase cyclic AMP in the striatum.

α-MSH MIF-I Dopamine Norepinephrine Rat brain

UNTIL recently, interest in the hypothalamic hormones centered on their ability to promote or inhibit the release of pituitary peptide hormones [10]. The latter then induced synthesis or release or both of various peripheral hormones in a number of endocrine glands. However, during the last few years, evidence has been accumulating that a number of the hypothalamic hormones also exert primary effects within the central nervous system (CNS). The work of Plotnikoff and Kastin [7], in which α-melanocyte stimulating hormone release inhibitory factor (MIF-I) injections were followed by behavioral increases both in unoperated and hypophysectomized (hypox) animals, was the first indication of such a direct CNS action. These experiments have been corroborated by others [4]. Accordingly, to avoid secondary peripheral hormonal effects via the pituitary gland, our experimental procedures were performed on both unoperated and hypox animals using the dihydroxyphenylalanine (DOPA) potentiation behavioral test [3] as a model. No known hypothalamic hormone has been shown to affect peripheral glands of internal secretion directly [8] except possibly somatostatin [1,5].

METHOD

The DOPA potentiation test brings about changes in complex social behavior such as aggressiveness, fighting and repetitive jumping, as well as autonomically mediated behavior (urination, defecation, salivation, increased motor activity). It was carried out by placing 3 or 4 male, Charles River rats, together in a metal cylinder (27.5 cm dia. × 20 cm high), at the bottom of which was a fitted circle of paper, marked off into 4 equal quarters. Motor activity, including circling and jumping, was rated (0 to 3+). Other

[1] Supported by grants from Veterans Administration and NIH (NS 07664).

changes in the behavioral parameters investigated, namely salivation, urination, diarrhea and ataxia, were also measured similarly as 0 to 3+. An unbiased observer, unaware of the drug treatment of the experimental animals, was employed to rate the behavior. Behavioral changes began about ½ hr after the last drug injected (dl-DOPA) and lasted about ½ -1 hr. Animals were decapitated at the height of the above-mentioned behavioral changes. The experimental procedure was as follows: Groups of 3 or 4 rats, kept in a room at 25°C, were administered an oral dose (40 mg/kg) of pargyline HCl in 0.9% NaCl or in H_2O. Two hr later these rats received an intraperitoneal (IP) injection of either MIF-I or thyrotropin releasing hormone (TRH) in 0.9% NaCl or H_2O at a dose of 0.1, 0.3 or 1.0 mg/kg for MIF-I and 0.5, 1.0 or 2.0 mg/kg for TRH. Controls received only the same volume of vehicle at this time. One hr after injection of peptide, an IP injection of dl-DOPA in 0.9% NaCl or H_2O, at a dose of 200 mg/kg, was made. This dose of dl-DOPA resulted in an increase of behavior ratings from 0 to the 1 or 1+ level. This would allow for a further increase in behavior, were it to occur, as a result of the treatment with MIF-I or TRH. In tests with normal (unoperated) rats, further control animals received only pargyline, dl-DOPA, a peptide, or a vehicle injected at the time when drugs were given. Hypox animals were purchased from Charles River Laboratories and allowed to acclimate for 3–5 days before being used experimentally. During this time the daily gain in weight was followed and any animals gaining more than 1 g daily were not used. After decapitation, the brains were rapidly removed and the striatal area dissected, frozen on dry ice and stored at −50°C until assayed for dopamine (DA) and norepinephrine (NE). Sella turcica were examined for any remains of pituitary tissue. The catecholamine assays were performed by the hydroxyindole method of Hogans [6,9]. Briefly, tissues were homogenized in acidified butanol and aliquots were added to a phosphate buffer (0.1 M, pH 6.5) for extraction of catecholamines. Iodine oxidation was used for conversion of the NE and DA to the respective trihydroxy- and dihydroxy-indoles. Samples containing NE were read in an Aminco-Bowman Spectrophotofluorometer at 385/485 nm wavelengths (uncorrected activation/emission), and samples containing DA were read at 320/380 nm (uncorrected). Statistical analysis of the results for all animal groups was carried out by an analysis of variance followed by a Scheffé test [11] for the hypox rats and a Dunnett's test [2] for the unoperated animals to determine the effect of saline controls vs the MIF-I injected groups or TRH-treated groups of rats.

RESULTS

If one considers the results for hypox animals weighing 90–100 g (Fig. 1), a correlation was evident between increases in DA levels due to MIF-I and increases in behavior for both normal and hypox animals. There appeared to be a tendency for such an increase in DA levels and behavior to disappear at the highest MIF-I level used (1 mg/kg), especially in the case of the hypox animals. Table 1 indicates that pargyline alone caused a rise in DA in the striatum accompanied by a considerable rise in NE. The behavioral rating of the pargyline treated animals, however, did not change. The dl-DOPA alone increased the DA level in the striatal region more than two-fold over the control value. The NE level was about the same (0.60 ± 0.02) as

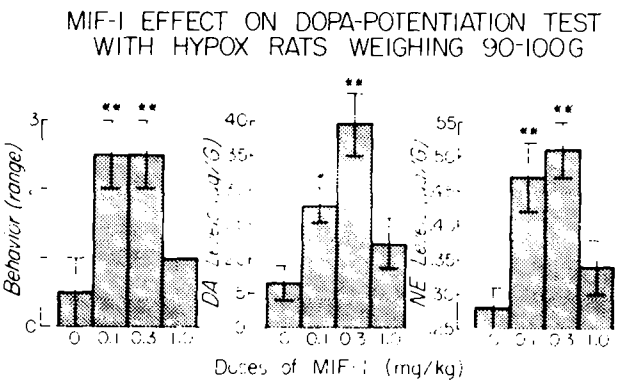

FIG. 1. Mean behavioral rating ± range. The MIF by age statistical interaction from the analysis of variance was significant ($F(6,63) = 35.13$; $p<0.001$). The difference between means needed for significance at the 0.05 level was 0.4 units and for the 0.01 level was 0.47 units (via the Scheffé's test). Mean dopamine levels ± the standard error of the mean (SEM). The MIF by age statistical interaction from the analysis of variance was significant ($F(6,63) = 3.97$; $p<0.002$). The difference between means needed for significance at the 0.05 level was 10.6 μg/g for the 0.01 level was 12.4 μg/g (via the Scheffé's test). Mean norepinephrine levels ± the (SEM). The MIF by age statistical interaction from the analysis of variance was significant ($F(6,63) = 3.21$; $p<0.008$). The difference between means needed for significance at the 0.05 level was 0.14 μg/g and for the 0.01 level was 0.17 μg/g (via the Scheffé's test). $^*p<0.05$; $^{**}p<0.01$.

after the pargyline alone and no behavioral changes were noted. Pargyline + MIF-I gave the same DA level as pargyline alone; however, the behavioral rating rose from 0 to 1. The parameters measured after the combination of pargyline + dl-DOPA were considered to be control values when evaluating the effects of MIF-I + pargyline + dl-DOPA. It was clear that MIF-I increased both DA and NE levels considerably as well as the behavioral ratings. There was relatively little difference in DA or NE levels between the 0.1, 0.3 and 1.0 mg/kg doses of MIF-I. When TRH was substituted at doses of 0.5 and 1.0 mg/kg, no increase in striatal values for DA and NE over the controls (pargyline + dl-DOPA) were noted although behavior was somewhat increased. At a dose of 2.0 mg/kg TRH, there was a considerable rise in levels of DA and behavioral ratings, but no rise in NE levels. However, the rise in DA was associated with a large variance and was not statistically significant at this TRH dose and this may have been due to the larger standard error of the mean.

Experiments similar to those performed with normal rats were also carried out on hypox Charles River rats of three different weight groups, 90–100 g, 140–150 g and 190–200 g. In the case of the animals of the lowest weight range, results were much the same as for normal animals. In 90–100 g hypox rats a correlation was seen between behavioral changes and increases in DA and NE levels (Fig. 1). For animals weighing 140–150 g (Fig. 2), even the controls, injected only with pargyline and dl-DOPA showed greatly increased behavior so that only at 0.3 mg/kg of MIF-I was this behavior notably increased. Neither DA nor NE levels were significantly increased further at any MIF-I dosages. Finally, when 190–200 g animals were employed, the only changes in behavior correlating with DA or NE levels occurred with MIF-I at a dose of 1.0 mg/kg (Fig. 3).

TABLE 1

EFFECTS OF MIF-1 AND TRH ON STRIATAL DOPAMINE AND NOREPINEPHRINE LEVELS AND BEHAVIOR IN NORMAL RATS

Treatment	DA ± SEM§	NE ± SEM§	Behavioral Rating
Saline	8.52 ± 0.48	0.33 ± 0.02	0
Pargyline (45 mg/kg)	11.28 ± 0.74	0.63 ± 0.08	0
MIF-1 (1.0 mg/kg)	10.14 ± 0.44	—	0
dl-DOPA (200 mg/kg)	19.08 ± 0.95	0.60 ± 0.02	0
Pargyline + MIF-1	11.48 ± 0.35	—	0
MIF-1 + dl-DOPA	19.68 ± 4.21	—	0
Pargyline + dl-DOPA	19.60 ± 0.95	0.62 ± 0.03	1.0
Pargyline + MIF-1 (0.1 mg/kg) + dl-DOPA	37.25 ± 3.37*	1.16 ± 0.10*	1.5
Pargyline + MIF-1 (0.3 mg/kg) + dl-DOPA	33.94 ± 4.56	0.81 ± 0.11	1.5
Pargyline + MIF-1 (1.0 mg/kg) + dl-DOPA	38.46 ± 5.95†	1.08 ± 0.14*	2.5
Pargyline + TRH (0.5 mg/kg) + dl-DOPA	17.70 ± 0.28	0.73 ± 0.06	1.5
Pargyline + TRH (1.0 mg/kg) + dl-DOPA	21.29 ± 0.98	0.60 ± 0.03	1.5
Pargyline + TRH (2.0 mg/kg) + dl-DOPA	34.58 ± 6.02‡	0.68 ± 0.05	2.5

* = $p<0.01$; † = $p<0.05$; ‡ = $p<0.10$. Unless otherwise stated all doses of MIF-1 are 1.0 mg/kg.
5 - 6 rats per group, 140-150 g, albino, Charles River animals.
§ - All DA and NE values are μ g/g wet weight.

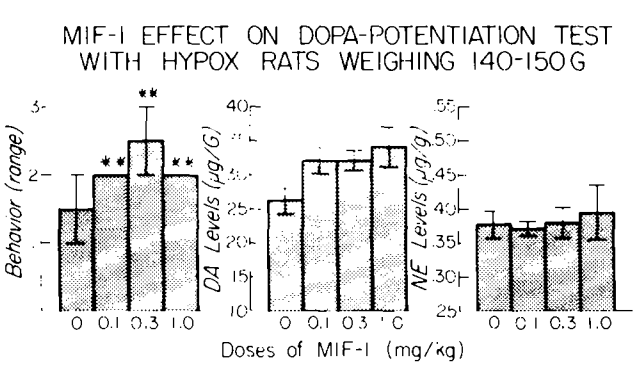

FIG. 2. Each group consisted of 6 male albino rats from Charles River Labs. Statistical analysis analysis of variance followed by the Scheffé test. No statistically significant changes found in behavior DA or NE levels under the condition of the experiment. *$p<0.05$; **$p<0.01$.

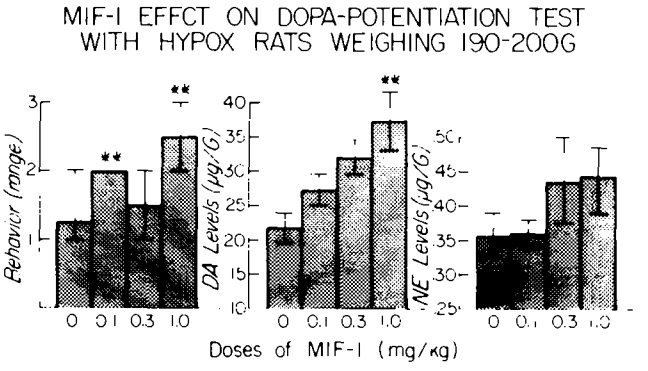

FIG. 3. Each group consisted of 6 male albino rats from Charles River Labs of 190 200 g weight. (1) For behavior difference between means needed for significance at the $p<0.05$ level (*) is 0.40 units; at the $p<0.01$ level (**) it is 0.47 units. (2) For DA levels difference between means needed for significance at the $p<0.05$ level is 10.6 μg/g; at the $p<0.01$ level it is 12.4 μg/g. (3) For NE levels difference between means needed for significance at the $p<0.05$ level is 0.14 μg/g; at the $p<0.01$ levels it is 0.17 μg/g. Statistical analysis: analysis of variance followed by the Scheffé test.

DISCUSSION

In general, the experiments performed indicate a correlation of the effects of MIF-1 on behavior with increases in DA and NE levels in the striatum of both unoperated and hypox animals. Whether this correlation indicates a causal relationship between the rise in neurotransmitter levels and behavior cannot be established at this time. In several instances the correlation was lacking. For example among the normal animals as seen in Table 1, administration of only dl-DOPA more than doubled the levels of DA and NE without causing a noticeable change in the behavioral rating. Furthermore, among one of the groups of hypox rats (weighing 190 200 g each), behavior was definitely increased whereas NE levels were not statistically altered and DA increased only at the highest level of MIF-1. Among another group of hypox animals weighing 140 150 g, all of which showed behavioral responses after injection of MIF-1, none responded with DA or NE increases which were statistically significant. Because of these exceptions, to our general findings, the possibility must be considered that MIF-1 could cause behavioral changes by directly affecting the neuronal postsynaptic membranes via adenylate cyclase and cyclic-AMP without concomitant neurotransmitter alteration.

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