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# Intensification of Central Catecholaminergic and Serotonergic Processes by the Hypothalamic Factors MIF and TRF and by Angiotensin II

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HUIDOBRO-TORO, J. P., A. SCOTTI DE CAROLIS AND V. G. LONGO. *Intensification of central catecholaminergic and serotonergic processes by the hypothalamic factors MIF and TRF and by angiotensin II.* PHARMAC. BIOCHEM. BEHAV. 3(2) 235–242, 1975. – The present work deals with the action of MIF (melanocyte stimulating hormone release-inhibiting factor), TRF (thyrotropin releasing factor), and angiotensin II on the behavioral effects of L-DOPA and of D,L-5-hydroxytryptophan (5-HTP) in mice. The influence of MIF and TRF on the antagonistic effect of L-DOPA of harmine tremors in rabbits was also studied. MIF and TRF, injected i.p., intensify the effects of L-DOPA in mice. The minimal dose of MIF required to induce a +3 response is 0.1 μg/kg; TRF is active at 500 μg/kg. When MIF or TRF are injected into the brain, potentiation of L-DOPA is obtained with exceedingly small quantities of MIF (0.1 pg); the effective dose of TRF is 1 μg. The behavioral effects of 5-HTP are potentiated by TRF only, at doses of 0.1 μg/kg, i.p. When TRF is administered intracerebrally, the active dose per mouse is 0.1 ng. Harmine (5 mg/kg i.v.) induces, in the rabbit, sustained whole body tremors; if L-DOPA (5 mg/kg) is administered i.v. at the peak of the harmine effect, the tremors subside. When the rabbit is pretreated with MIF, administered i.p. 1–2 hr before harmine, in doses devoid of an antitremor effect per se (10 μg/kg), the L-DOPA antagonism appears at lower doses. Also dopamine (5–10 mg/kg i.v.) proved effective in abating harmine tremors; previous treatment with MIF (50 μg/kg) potentiated the antagonistic effect of dopamine. According to the prevailing theories on the mechanism of neurotransmission, some hypotheses will be discussed to explain the observed potentiation: impaired uptake, impaired degradation, interference with the turnover of the bioamines, supersensitivity of the receptors.

Angiotensin II  MSH release inhibiting factor  Thyrotropin releasing factor
Catecholaminergic processes  Serotonergic processes

IN THE last few years investigational work on central neurotransmission has shifted to synaptic mechanisms involving substances other than the classical neurotransmitters. Part of this new approach deals with the study of some polypeptides which belong to the class of the neuroendocrine transducers and have the task of translating to hormonal output the neurotransmitters' signals acting at synapses. Until now the attention of researchers was focused on the effects of the known biogenic amines on these polypeptides, paying little or no attention to the inverse relationships. Some data, however, seems to indicate that these polypeptides are potential regulatory participants in amine-dependent neurotransmission.

Behavioral effects of L-DOPA in mice have been recently shown to be potentiated by the polypeptides MIF (melanocyte stimulating hormone release-inhibiting factor) and TRF (thyrotropin releasing factor) [23,27]. MIF, which has been identified as prolyl-leucyl-glycine amide, was used with encouraging results in parkinsonian patients [14] while TRF (pyroglutamyl-histidyl-proline amide) was found to be clinically active as an antidepressant [30].

L-DOPA potentiation is one of the tests used in the laboratory for the evaluation of antidepressant drugs [8]. Potentiation thus obtained with tricyclic compounds has been attributed to an inhibition of the norepinephrine (NE) reuptake mechanism at the nerve terminals; this causes a high concentration of the mediator at the central synaptic sites, resulting in greater pharmacological effects. On the other hand, the pharmacological profile of tricyclic compounds includes potentiation of serotonin (5-HT) action on tissues, increase in brain 5-HT, and inhibition of tissue uptake [3]. Therefore, intensification of the central

effects of 5-HT has also been postulated as a possible determinant of the antidepressant activity [16]. Clinical data also suggests that both NE and 5-HT may be involved: patients suffering from a depressive illness improve following large doses of tryptophan combined with MAO inhibitors [5].

The present work deals with the action of MIF and TRF on the behavioral effects of L-DOPA in mice. The influence of these polypeptides on the behavioral effects of D,L-5-hydroxytryptophan (5-HTP) was also investigated. This test has been previously employed [6,31] to study the agonists and antagonists of 5-HT in vivo. Moreover, a previous study carried out in our laboratory [18] has indicated that impairment of the uptake mechanisms after destruction of the serotoninergic terminals by means of 5,6-dihydroxytryptamine (5,6-DHT) leads to a potentiation of the behavioral effects of 5-HTP.

The influence of angiotensin II on the behavioral effects of L-DOPA and of 5-HTP in mice has also been investigated. Several centrally mediated effects have been described by angiotensin II. Recently, Palaic and Khairallah [21] have demonstrated in vivo an inhibiting effect of this octapeptide on NE uptake both at the central and peripheral synapses.

Data of Plotnikoff *et al.* [24,25] indicates that MIF antagonizes the tremors induced by oxotremorine in normal as well as hypophysectomized mice, and reverses the sedative effects of deserpidine. Therefore we have studied the antitremor effects of MIF using another of the laboratory methods proposed for the screening of antiparkinsonian drugs, namely the antagonism of the tremors induced by harmine [10,40].

## METHOD

### *Experiments in Mice*

Swiss white male mice, weighing 20–30 g were used. The L-DOPA poteniation test was carried out in groups of 4 animals as described by Everett [8]. The animals were treated with pargyline 40 mg/kg orally, 8 hr before the administration of L-DOPA 100 mg/kg i.p. The animals were then placed in large plastic containers, and during the first hour after L-DOPA injection they were evaluated every 10 min by two experienced observers (one of whom was unaware of the treatment) for the presence of piloerection, salivation, and Straub tail phenomenon, as well as reactivity to external stimuli (evidenced by jumping, squeaking, running) and aggressive and stereotypic behavior. On the basis of behavioral observation, a global score of +1, +2, or +3 was assigned to each group of animals [8]. As there was no noticeable difference in the results obtained in animals injected with the polypeptides 2 or 4 hr before L-DOPA, only the results obtained when the polypeptides were administered 2 hr before are reported.

In the 5-HTP potentiation experiments, groups of 4 animals were injected with pargyline 20 mg/kg i.p.; 2 hr later the polypeptides were administered, and after 2 more hr 75 mg/kg of 5-HTP was injected (all these injections were made i.p.). Visual evaluation of the motor response presented some practical difficulties, therefore the tremors and the incoordinated activity induced by administration of 5-HTP were recorded by placing small plastic cages containing one animal over a Grass FT-10C force displacement transducer connected to a polygraph. The animals' reaction was then rated +1, +2, or +3 by evaluating the amplitude of the polygraph tracings.

In a separate series of experiments, the animals were injected with the polypeptides intracerebrally. The injections were done according to Haley and McCormick's [11] technique, the maximum amount of liquid injected was 10 $\mu$l. All mice were treated orally with pargyline; 6 hr later they were treated intracerebrally with the polypeptides, and after 2 additional hr were challenged with L-DOPA or 5-HTP.

Each experimental group had its own control, which received an equivalent volume of vehicle. In some experiments, pargyline pretreatment was omitted and the polypeptides injected 2 hr before L-DOPA, or 5-HTP. The solutions of polypeptides, L-DOPA and 5-HTP were prepared immediately before use.

### *Experiments in Rabbits*

Adult rabbits of both sexes were used to study the influence of MIF or TRF on the antagonistic effect of L-DOPA and of dopamine on harmine tremors, according to a method developed in this laboratory [10]. Rabbit's tremors were registered by placing the animal in a large cage (70 × 100 cm) with the bottom suspended over a pneumatic system connected to a transducer.

## RESULTS

### *Mice*

*L-DOPA potentiation test.* MIF, TRF and angiotensin II, injected parenterally, proved active in potentiating the effects of L-DOPA in mice. The results are charted graphically in Fig. 1. By comparing the minimal doses required to produce a +3 response in mice treated with L-DOPA, the compounds were ranked in the following order of efficacy: MIF (active at 0.1 $\mu$g/kg), angiotensin II (active at 10 $\mu$g/kg), and TRF (active at 500–1000 $\mu$g/kg). When angiotensin was administered in doses larger than 500 $\mu$g/kg, a decline in the L-DOPA potentiation was noticed. Enhancement of L-DOPA response was less prominent when pargyline pretreatment was omitted. In this case, much higher doses of the three polypeptides were needed in order to obtain a potentiation which, in the case of angiotensin II and TRF never reached a maximal score.

The same potentiation is obtained when MIF or TRF are directly injected into the brain. In the case of MIF, potentiation of L-DOPA is obtained with exceedingly small quantities of the drug (0.1 pg), while the effective dose of TRF was 1 $\mu$g (Fig. 2).

From these experiments it became evident that MIF was the most active compound in potentiating the behavioral effects of L-DOPA. In order to study in more detail the mechanism of this potentiation, two series of experiments were carried out. The first experiment was planned to ascertain if, in animals pretreated with MIF, a +3 response could be obtained with doses of L-DOPA lower than 100 mg/kg. Doses of L-DOPA of 1, 10, 30 and 100 mg/kg were administered to groups of 4 animals each, pretreated with pargyline. A +1 response was present only in the mice receiving the highest dose. In animals pretreated either with 0.5 ng or 100 ng of MIF, injected intracerebrally 2 hr before the L-DOPA challenge, a +3 potentiation could be only observed with the dose of 100 mg/kg, while the other doses of L-DOPA did not induce any appreciable alteration in

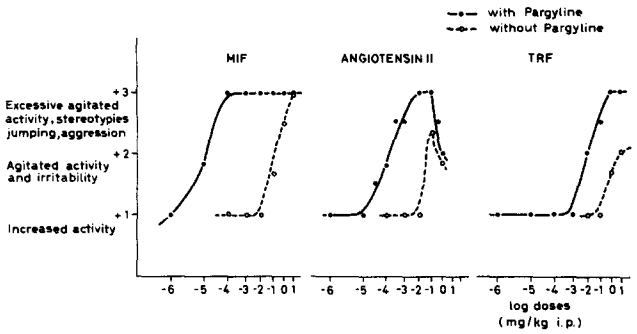


FIG. 1. Effect of MIF, TRF and angiotensin II on the behavioral effect of 100 mg/kg L-DOPA in mice. Groups of 4 animals were used for each dosage: see text for the details of administration. Potentiation of L-DOPA is more marked in mice pretreated with pargyline (40 mg/kg p.o.). In these animals MIF induces a +3 reaction at doses of 0.1 μg/kg, angiotensin II at 10 μg/kg, and TRF at 500 μg/kg. Saline-treated mice that received pargyline + L-DOPA were rated +1 (not shown on the graph) Ordinates: behavioral scale; abscissa: log dose in mg/kg.

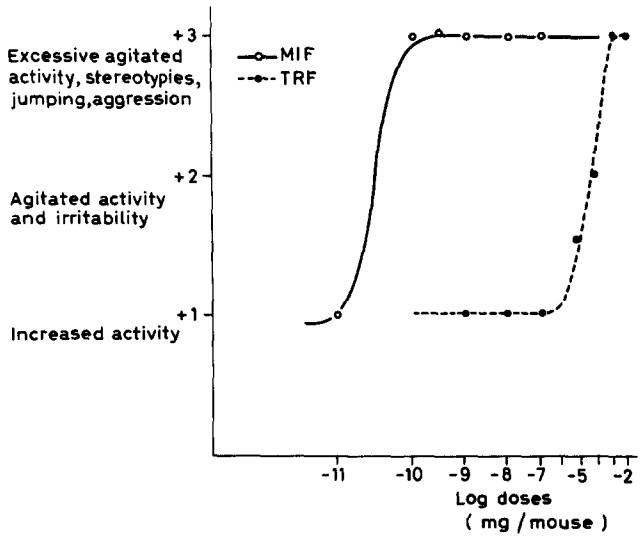


FIG. 2. Effect of MIF and TRF injected intracerebrally on the behavioral effects of 100 mg/kg of L-DOPA in pargyline-treated mice. A +3 response is obtained with 0.1 pg of MIF and with 1 μg of TRF.

behavior (Fig. 3). The second experiment was devised to rule out the peripheral effects of L-DOPA and was carried out by injecting increasing doses of L-DOPA directly into the brain. Due to the low solubility of L-DOPA, and to the limitations inherent to the route of administration, the maximum amount injected was 100 μg/mouse. This dose did not cause any behavioral alteration in control, untreated mice. In animals pretreated with pargyline, exophthalmus, increased spontaneous activity, and Straub tail phenomenon appeared about 1 hr after the intracerebral administration of 30, 50 or 100 μg of L-DOPA (score of +1). The other doses of L-DOPA tested (1, 10 and 20 μg) were ineffective. In animals which received a combined treatment of pargyline and MIF (10 μg i.p.), 100 μg of L-DOPA induced a marked excitation, jumping,

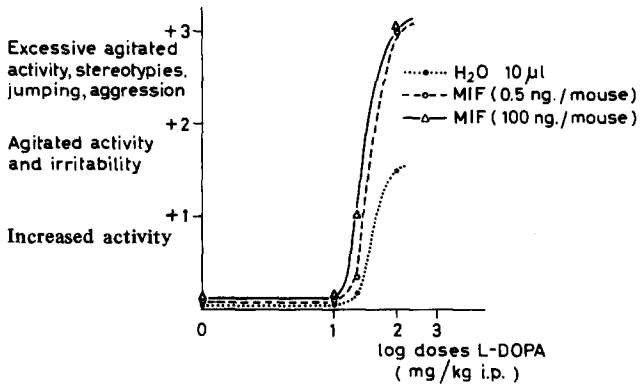


FIG. 3. Effects of increasing doses of L-DOPA in mice treated with MIF intracerebrally. Doses of 1, 10, 30 and 100 mg/kg were administered i.p. to groups of 4 animals. Mice pretreated intracerebrally with solvent alone exhibited a +1.5 response when injected with the highest dose of L-DOPA (100 mg/kg); the other doses proved ineffective. Mice pretreated intracerebrally with either 0.5 or 100 ng of MIF exhibited a +3 response when injected with the highest dose of L-DOPA, whereas the other doses of L-DOPA did not induce an appreciable effect.

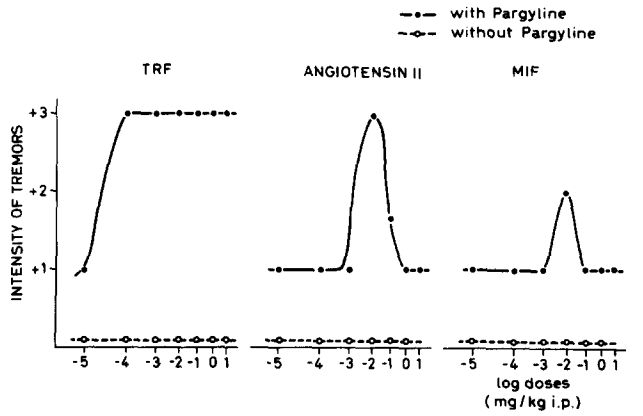


FIG. 4. Effects of MIF, TRF and angiotensin II on the behavioral effects induced by 5-HTP (75 mg/kg) in mice. The animal's reaction was graded according to the intensity of the tremors (see methods for details). Potentiation of 5-HTP is present only in mice pretreated with pargyline. In these animals, TRF induces a +3 rating at doses of 0.1 μg/kg; MIF and angiotensin II produce potentiation at a very narrow dosage range. Saline pretreated mice that received pargyline + 5-HTP had a +1 reaction (not shown on the graph). Ordinates: intensity of tremors; abscissa: log dose in mg/kg.

aggression, and stereotypies (score +3), ensuing about 20 min after treatment.

*5-HTP potentiation.* In contradistinction with the results obtained in the L-DOPA protentiation test, in which all of the three polypeptides were found active, the behavioral effects of 5-HTP were significantly potentiated only by TRF. The influence of each of the three polypeptides on the motor effects of 5-HTP is shown in Fig. 4. Angiotensin and MIF were found to produce a slight potentiation effect at a very narrow dosage range; on the other hand, potentiation by TRF was already noticeable after administration of 0.1 μg/kg. However, when pargyline pretreatment was

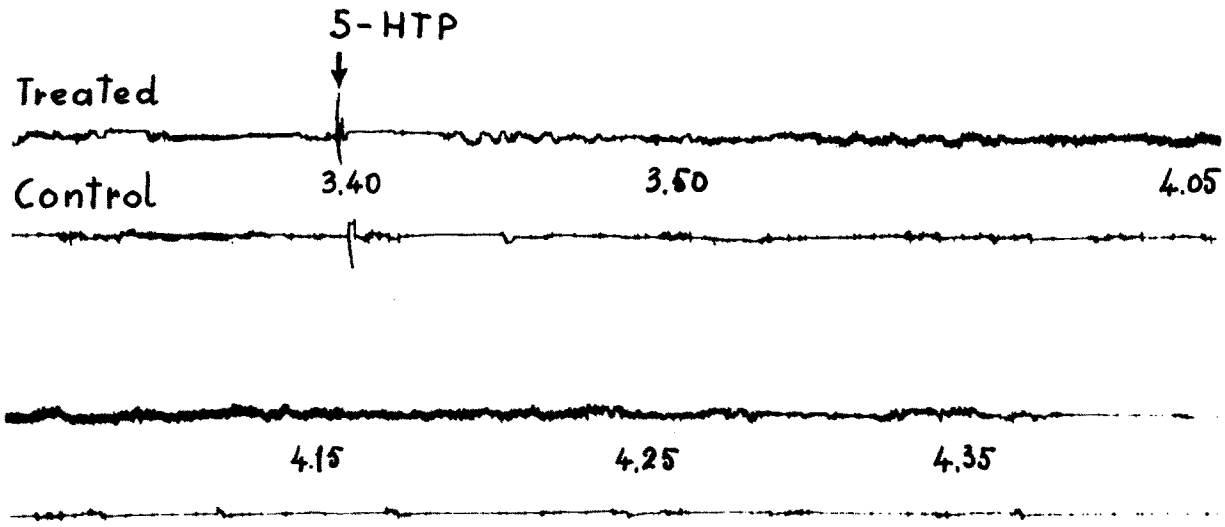


FIG. 5. Representative record of the tremors induced by 5-HTP in mice. The treated animal received 1 mg/kg of TRF 2 hr before 5-HTP (at the arrow). In the treated mouse, tremors appear earlier and are more intense than in the control. For details of technique, see text.

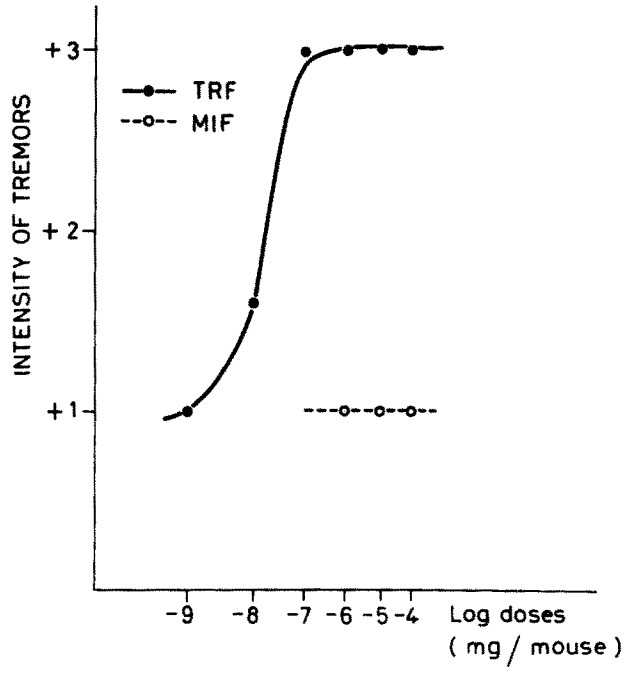


FIG. 6. Influence of TRF and MIF injected intracerebrally on the behavioral effects of 75 mg/kg of 5-HTP in pargyline-treated mice. A +3 response obtained with 0.1 ng of TRF; MIF, in doses up to 0.1 μg was without effect. Ordinates: intensity of tremors; abscissa: log doses in mg/mouse.

omitted, this effect of TRF disappeared. As shown in Fig. 5, obtained by recording the movements of the animals according to the technique described in methods, the tremors induced by 5-HTP in mice pretreated with TRF were more intense, appeared earlier and lasted longer than in the control animals. Imipramine (20 and 40 mg/kg), desipramine (5, 10 and 20 mg/kg) and chlorimipramine (1, 5 and 25 mg/kg) tested under the same experimental conditions as TRF, did not potentiate the effects of 5-HTP.

The potentiating effect of TRF was confirmed in the experiments in which the polypeptide was administered intracerebrally. The active dose per mouse in this case was 0.1 ng. MIF in doses up to 0.1 μg was without effect (Fig. 6).

*Rabbits*

*Effect of MIF and TRF on the tremors induced by harmine.* Within 30 sec from its administration, harmine (5 mg/kg i.v.) induces in the rabbit sustained whole body tremors accompanied by excitation, ataxia and mydriasis. The tremors last for 10–15 min, while the excitation persists for a longer time.

A total of 30 rabbits were used to assess the effects of MIF and TRF on the harmine-induced tremors. The drugs were injected i.p. in doses varying from 0.1 to 500 μg/kg 2 hr before the harmine challenge. Groups of 4 animals were used for each dose. Attenuation of the tremors and of the excitation was noticed only with the highest doses of MIF employed (200–500 μg/kg), while TRF was found to be inactive.

*Influence of MIF and TRF on the antagonistic effect of L-DOPA towards harmine tremors.* In a previous investigation [10] L-DOPA was found effective in blocking the tremors, the ataxia and the excitatory syndrome induced by harmine in the rabbit. In the present experiments, a total of 50 animals were used to ascertain the influence of pretreatment with MIF or TRF on this effect of L-DOPA. The design of the experiments was as follows: groups of 4 rabbits received an i.v. injection of 5 mg/kg of harmine. When the tremors induced by the drug were at their maximal intensity (usually after 2–3 min), L-DOPA was administered i.v., in order to establish the minimal dose which abolished the tremors. A curve was constructed

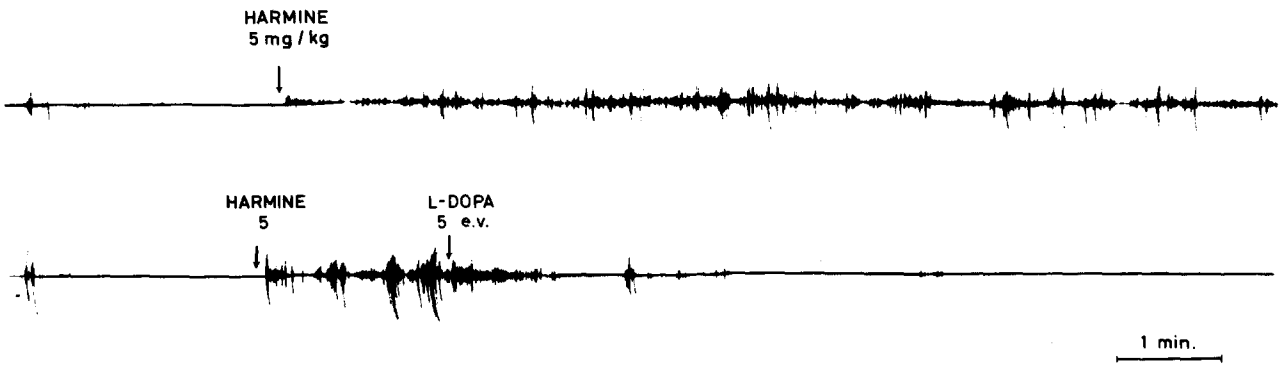


FIG. 7. Antagonistic effect of L-DOPA towards the tremors induced by harmine in rabbits. The graphs were obtained by placing the animal in a cage suspended over a pneumatic system connected to a transducer. Harmine (5 mg/kg i.v.) induces sustained tremors and excitation (upper tracing). These tremors are abated when L-DOPA (5 mg/kg i.v.) is administered when the harmine effect is at its maximum (lower tracing).

plotting on the abscissa the dosage of L-DOPA and on the ordinates the intensity of the tremors. A full antagonism was observed with 5 mg/kg of L-DOPA (Fig. 7); a dose of 2 mg/kg was still able to attenuate the tremors and the hyperreactivity, while 1 mg/kg was ineffective. When the rabbits were pretreated with MIF, administered i.p. 1–2 hr before harmine, in doses (10 μg/kg) which proved devoid of any antitremor activity per se, the L-DOPA antagonistic effect appeared at doses of 1 mg/kg. On the other hand, TRF was devoid of this potentiating effect on doses up to 100 μg/kg (Fig. 8).

*Influence of MIF on the antagonistic effect of dopamine towards harmine tremors.* According to Horst *et al.* [13] dopamine is able to prevent oxotremorine-induced tremors in mice. It was therefore decided to carry out a further series of experiments, in 30 additional rabbits, to study the effect of dopamine. While L-DOPA was able to reverse completely the syndrome induced by harmine, dopamine (5 and 10 mg/kg i.v.) proved able to abate the tremors only, without affecting the ataxia and excitation. Lower doses (1 and 2 mg/kg) were ineffective. In rabbits anesthetized with pentobarbital the cardiovascular effects of dopamine (1–10 mg/kg) were checked: only a slight and transitory increase in blood pressure and cardiac rate was noticed with the highest does.

When the rabbits were pretreated with MIF (50 μg/kg i.p.) 2 hr before harmine, 2 mg/kg of dopamine were able to abate the tremors and to annul ataxia.

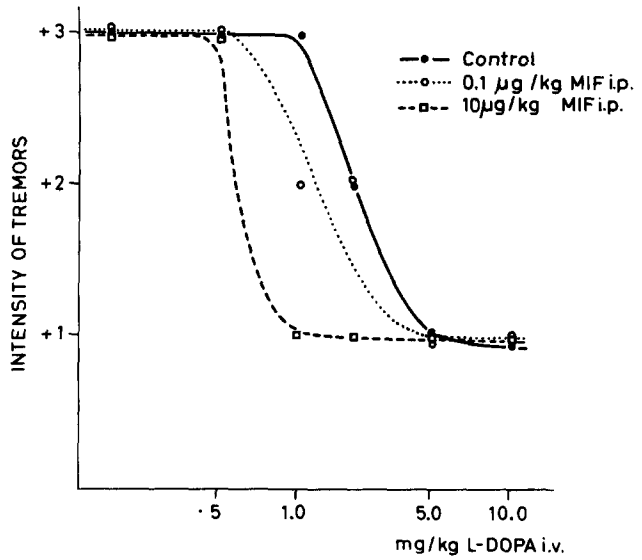


FIG. 8. Potentiation by MIF of the L-DOPA antagonistic effect towards harmine tremors. The control curve shows that L-DOPA antagonizes the harmine-induced tremors at 5 and 10 mg/kg. The dose of 2 mg/kg is only partially effective, 1 mg/kg has no effect. When MIF (10 μg/kg i.p.) is administered 1–2 hr before harmine, the L-DOPA antagonistic effect appears with 1 mg/kg. On the other hand the potentiating effects 0.1 μg/kg of MIF are hardly appreciable. Ordinates: intensity of tremors; abscissa: log doses of L-DOPA in mg/kg i.v.

## DISCUSSION

The results obtained in the present experiments with MIF and TRF are in accord with those of Plotnikoff *et al.* [23,27] who showed that these polypeptides potentiated L-DOPA effects in mice. In our studies TRF was found to be active at the same dosage range as these authors, whereas MIF intensified L-DOPA effects at one thousandth the dose reporter by Plotnikoff *et al.* [23].

Potentiation of L-DOPA by MIF was not limited to the behavioral symptoms in mice. MIF lowered the effective dose of L-DOPA in antagonizing the tremors induced by harmine in the rabbit. This finding agrees with the data by Plotnikoff *et al.* [25], who described after MIF a potentiation of the antagonistic effects of L-DOPA on oxotremorine tremors. The antagonistic effect of L-DOPA on the harmine tremors has been attributed to a restoration of the central dopaminergic functions impaired by harmine through a mechanism which is still obscure [10]. However, harmine tremors are also counteracted by the administration of dopamine, and MIF proved able to potentiate this effect of dopamine. Similar results have been obtained by Horst *et al.* [13], who reported that oxotremorine tremors and hypothermia in mice can be prevented by dopamine which has no influence on brain amine concentration. On the basis of these results, the possibility must be considered that harmine-induced tremors may also be influenced by peripherally circulating monoamines.

Only TRF was found to enhance the behavioral symptoms due to 5-HTP (cf also [28]). It seems therefore that MIF and TRF exhibit a certain selectivity on the behavioral effects of L-DOPA and 5-HTP respectively. How much this observation is relevant to the clinical effectiveness of these drugs is hard to assess at the present time, since the human trials are still in the preliminary phase.

MIF and TRF are also active in minute amounts when injected intracerebrally. This would indicate that the observed potentiation takes place at the central level. An additional demonstration of the central mechanism of action is given by the syndrome of excitation, jumping and stereotypies observed when L-DOPA was injected intracerebrally; also this syndrome was potentiated by MIF administered i.p.

Since our experiments were performed in intact mice, the observed effects of these polypeptides could be exerted through an influence on the hypophyseal-thyroid axis. It is known that thyroid hormones facilitate the actions of catecholamines at both peripheral and central synapses. Emlen *et al.* [7] showed that rats made hyperthyroid with thyroxine showed increased sensitivity to the activating effects on behavior of NE administered intraventricularly. In humans, enhancement of the antidepressant activity of imipramine has been reported in patients treated with L-triiodothyronine or with TSH (thyroid stimulating hormone) by Prange *et al.* [28]. Friedman *et al.* [9] examined the effects of MIF on brain DA metabolism. Doses of 0.5, 1 and 5 mg/kg increased DA synthesis in the striatum of normal but not of hypophysectomized rats. This effect was not found by other investigators [26]. In addition, it should be mentioned that both TRF and MIF are effective in the L-DOPA potentiation test carried out in hypophysectomized mice [23,28]. The possibility of a direct effect of the three polypeptides at the synaptic sites must therefore be considered. Within the limits of the prevailing theories on the mechanism of neurotransmission, several hypotheses can be advanced to explain the potentiation exhibited by these polypeptides: (1) impaired uptake; (2) impaired degradation; (3) interference with the rate of biosynthesis; (4) supersensitivity of the receptors.

*Impaired Uptake*

The patterns of L-DOPA potentiation obtained with MIF resemble those described in animals pretreated with tricyclic antidepressants or with intracerebral administration of 6-OHDA. In both cases the mechanism of this potentiation has been attributed to a loss of neurotransmitter inactivation due to an impaired reuptake by the nerve terminals [1, 8, 37]. Therefore a similar mechanism could be considered for MIF. There are some results that are difficult to explain by means of this hypothesis. If impairment of uptake is the mechanism of action of MIF, lower doses of L-DOPA, injected to animals pretreated with this drug, should be able to give rise to appreciable behavioral symptoms, but this was not observed in our experiments. The L-DOPA potentiation phenomenon seems to have the characteristic of an all-or-none effect, in the sense that it is present only when the dose of L-DOPA injected reaches a certain level. This level is 100 mg/kg when L-DOPA is administered parenterally, and 30 $\mu$g when injected intracerebrally. Whichever potentiating drug is administered, a lowering of this critical dose does not induce any symptom. It is likely that this dose of L-DOPA is essential for triggering some central reactions responsible for the excitation, the threshold of which can not be lowered by the so-called potentiating drugs.

Impaired uptake by serotonergic terminals may also be invoked for the potentiation of 5-HTP after TRF. This phenomenon was described in mice treated with 5,6-dihydroxytryptamine [18]. In this case, the increased response was attributed to an impaired uptake of the mediator due to destruction of serotonergic nerve terminals caused by 5,6-dihydroxytryptamine. Intensification of the central effects of 5-HTP has also been described for some drugs with antidepressant properties, such as Molindone [31], trazodone [17], imipramine and desipramine [16]. The above data are only in apparent contrast with the absence of potentiation found for imipramine, desipramine and chlorimipramine in the present experiments. In some trials carried out to confirm the data of Lapin and Oxenkrug [16], imipramine (10 mg/kg i.p.) was injected 2 hr before the administration of 5-HTP (75 mg/kg i.p.), omitting the treatment with pargyline. While mice injected with 5-HTP alone exhibited only negligible behavioral alterations, the animals pretreated with imipramine showed hyperirritability, exopthalomus, and increased activity. These behavioral patterns resembled those induced by L-DOPA and were different from those observed in pargyline + TRF-pretreated animals in which the tremorogenic component of 5-HTP was prevalent.

*Impaired Degradation*

Neither MIF nor TRF have any appreciable influence on brain amine content of mice [28]. It is therefore unlikely that these compounds have an inhibitory effect on brain MAO. However, other catabolic mechanisms relevant to bioamines inactivation should be considered. Impairment of O-methylation of dopamine has been suggested by Carman [4] as a possible extrahypophyseal mechanism of action of MIF. Reduced dopamine catabolism might explain the potentiation of the antiparkinsonian efficacy of L-DOPA and possibly the beneficial effects on parkinsonian signs exerted by MIF alone [14,38].

The potentiation of the antitremor effects of L-DOPA and dopamine observed in the present experiments in the rabbit might also be ascribed to an interference with destruction of dopamine.

*Interference with Biosynthesis and Release*

Another possibility of interaction which must be considered derives from the relationships existing between biogenic amines and endocrine functions [20].

The presence in the hypothalamus of cathecolamines and serotonin is well documented. This led several investigators to assume that monoamines might play a role in the regulation of the hypothalamic releasing factors [33,39]. It seems safe to assume that an inverse relationship might exist, since dopamine, serotonin, and histamine are normal constituents of some endocrine cells. The amine precursors L-DOPA and 5-HTP are taken up into many of these cells and are decarboxylated intracellularly, leading to accumulation and storage of the corresponding amine; these cells have been grouped by Pearse [22] under the name of APUD (amine precursor uptake and decarboxylation) cells. Both MIF and TRF are hypothalamic factors, whose effect on the endocrine system is well known. It is conceivable

that activation of endocrine cells to induce hormone release or inhibition is associated with changes in amine level turnover. Sandler *et al.* [32] have taken into consideration this possibility by checking the urinary metabolites of L-DOPA in rats treated with MIF or with TRF. Their results indicate that neither factor caused alteration in the total 24 hr metabolites output. On the other hand, from the findings of Keller *et al.* [15] it seems that TRF at high doses (10 mg/kg) activates the turnover of cerebral NE, since the drug, injected intraperitoneally to rats, increases the concentration of MOPEG and enhances the accumulation of $C^{14}$ NE after injection of $C^{14}$ tyrosine into the brain ventricles.

*Supersensitivity of the Receptors*

In addition to the possibility for a presynaptic mechanism, an effect of these polypeptides on the postsynaptic sites must be considered. There is now evidence that denervation induces two qualitatively different types of supersensitivity. The first type develops rapidly and can be attributed to a presynaptic alteration (impairment of uptake); the second type develops slowly and is probably dependent upon modifications of the receptor area [36]. Thoenen *et al.* [35] attributed the potentiation of the electrically-induced contraction of the cat spleen observed with angiotensin to a receptor's sensitization, since they were unable to find an increase in NE in the venous outflow from the spleen, which would instead indicate a block of uptake. An influence on the receptor, perhaps in the form of adenyl cyclase alterations, may play an important role in originating the supersensitivity. Adenyl cyclase has been shown to be influenced by several hormones or neurohormones [34]. In particular, it has been shown that some polypeptides such as vasopressin increase the accumulation of cyclic-AMP in tissues [12]. It is not inconceivable that simpler polypeptides could induce changes in the receptor similar to that observed in decentralization supersensitivity, but ensuing more rapidly.

The results obtained in the present experiments with angiotensin indicate that potentiation of the behavioral effects of L-DOPA is not specific for MIF and TRF. MIF activity was described for the C-terminal tripeptide chain of oxytocin (cf [23]) and for a pentapeptide isolated and identified as Pro-His-Phe-Arg-Gly-$NH_2$ by Nair *et al.* [19]. The disappearance of the potentiation observed with high doses of angiotensin may be due to a side effect, since behavioral depression has been described in mice receiving intracerebrally 1 $\mu$g of this drug [2].

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