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BRIEF COMMUNICATION

Action of Two Hypothalamic Factors (TRH, MIF) and of Angiotensin II on the Behavioral Effects of L-DOPA and 5-Hydroxytryptophan in Mice[1]

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HUIDOBRO-TORO, J. P., A. SCOTTI DE CAROLIS AND V. G. LONGO. *Action of two hypothalamic factors (TRH, MIF) and of angiotensin II on behavioral effects of L-DOPA and 5-hydroxytryptophan in mice.* PHARMAC. BIOCHEM. BEHAV. 2(1) 105–109, 1974. – Melanocyte stimulating hormone release-inhibiting factor (MIF), thyrotropin releasing hormone (TRH) and angiotensin II, injected i.p. to mice, potentiate the behavioral effect of L-DOPA and 5-hydroxytryptophan.

Angiotensin II L-Dopa 5-Hydroxytryptophan MIF TRH

ONE OF THE pharmacological tests used in the laboratory for the evaluation of antidepressant drugs is the L-DOPA potentiation in mice [6]. Potentiation thus obtained with tricyclic drugs has been attributed to an inhibition of the norepinephrine reuptake mechanism at the nerve terminals; this causes a high concentration of the mediator at the central synaptic site, with resulting greater pharmacological effects. Potentiation of L-DOPA has been recently described for the polypeptides MIF (Melanocyte stimulating hormone release-inhibiting factor, [13] and TRH (thyrotropin releasing hormone, [15]). This last compound was found to be clinically active as an antidepressant [17].

There is still much controversy on the biochemical etiology of the affective disorders, and both catecholamines and indoleamines have been involved. Results of Carlsson *et al.* [3] in the rat indicate that imipramine blocks *in vivo* the uptake mechanisms in central serotoninergic synapses. Clinical data also suggest both types of transmitters may be involved: patients suffering of a depressive illness improve following large doses of tryptophan combined with MAO inhibitors [4].

The present work deals with the action of MIF, TRH and angiotensin II the behavioral effects of L-DOPA in mice. Several centrally mediated effects have been described for angiotensin II. Recently, Palaic and Khairallah [12] have demonstrated *in vivo* an inhibiting effect of this polypeptide on norepinephrine uptake both at the central and peripheral synapses. The influence of these three polypeptides on the behavioral effects of D, L-5-hydroxytryptophan (5–HTP) was also tested. In mice pretreated with MAO inhibitors, this compound induces head twitchings and whole-body tremors, ensuing 5–7 min after intraperitoneal injection [1]. Previous results in this laboratory have 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 [11].

METHOD

All experiments were carried out on adult white male mice, weighing 20 to 30 g, bred at the Institute. The L-DOPA potentiation test was carried out as described by Florio [7]. Animals were treated with pargyline 40 mg/kg orally, 8 hr before the administration of L-DOPA. The polypeptides were dissolved in distilled water, and injected i.p. 1, 2 and 4 hr before 100 mg/kg L-DOPA i.p. The animals were then placed in large containers, and during the first hour after L-DOPA injection they were evaluated every ten minutes by two experienced observers 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 behav-

[1] Dr. John Biel, Abbott Labs., North Chicago kindly furnished samples of TRH and MIF.

ior. On the basis of behavioral observation, a global score of +1, +2 or +3 was assigned to each group of animals [6].

There was no noticeable difference in the results obtained in animals injected with the polypeptides 1, 2 or 4 hr before L-DOPA, therefore only the results obtained when the polypeptides were administered 2 hr before are reported.

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

Each experimental group had its own control, which received, instead of the polypeptides, distilled water. In some experiments, pargyline pretreatment was omitted and the polypeptides injected two hours before L-DOPA, or 5-HTP. Solutions of polypeptides, L-DOPA and 5-HTP were prepared immediately before use.

RESULTS

The results of the L-DOPA potentiation test are presented graphically in Fig. 1. By comparing the minimal doses required to induce a +3 response in mice treated with L-DOPA, we were able to rank the efficacy of the compounds. MIF was the most active (0.1 μg/kg), angiotensin II was the second (10 μg/kg), and TRH the least active (500–1000 μg/kg). When angiotensin was administered in doses larger than 500 μg/kg the L-DOPA potentiation was less prominent. Without pargyline pretreatment, the potentiation of L-DOPA by the polypeptides was less evident. In order to reach a score of +2, 1 mg/kg of MIF or 0.1 mg/kg of angiotensin II were needed; TRH, in doses up to 10 mg/kg had little or no effect.

The data obtained studying the influence of the three polypeptides on the motor effects of 5–HTP are presented in Fig. 2. In this case, TRH was the most active compound:

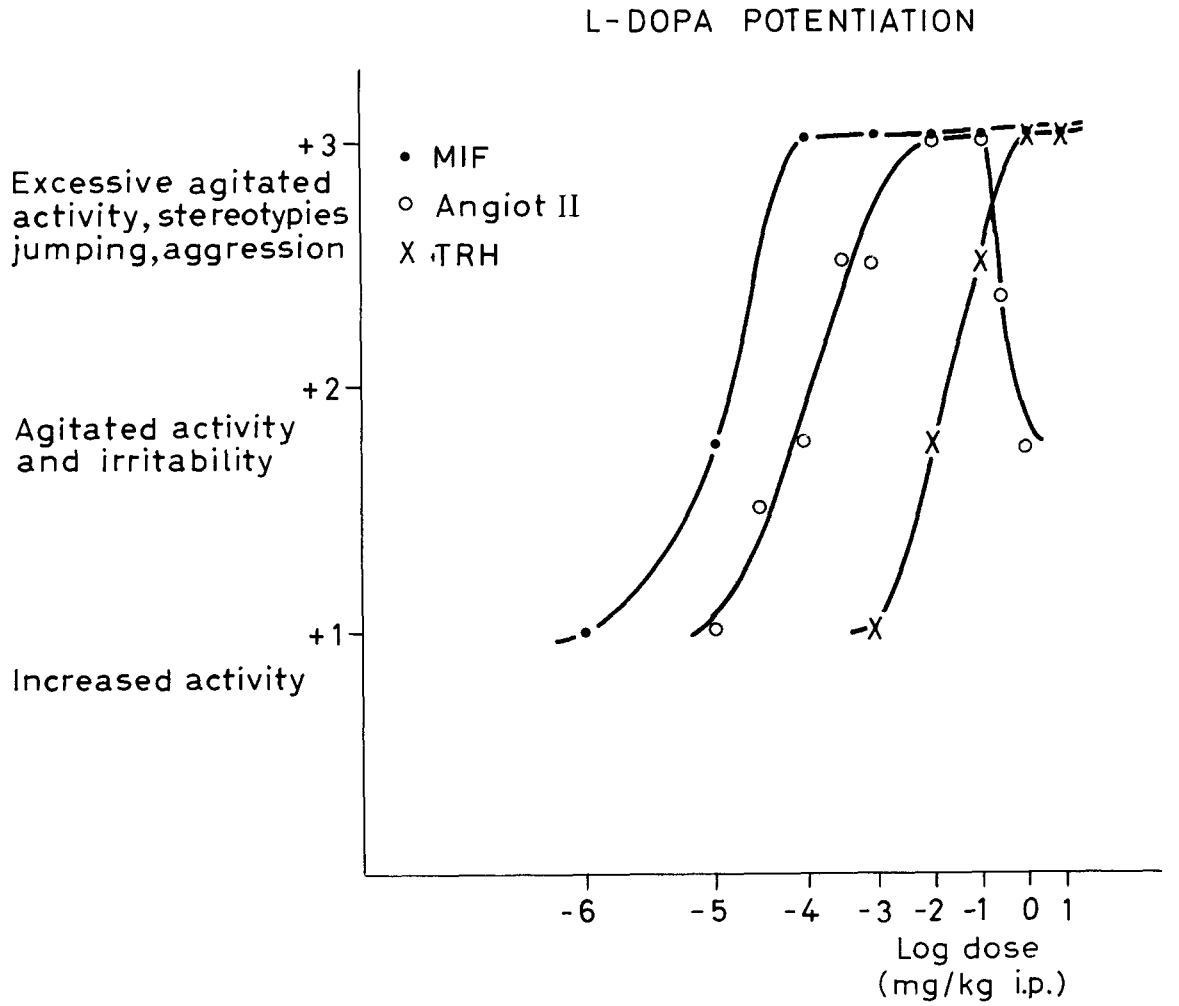

FIG. 1. Effect of MIF, TRH and angiotension II on the L–DOPA potentiation test. Groups of four mice were treated and rated as described in methods. Saline-treated mice that received pargyline and L-DOPA were rated +1. Ordinates: behavioral scale; abscissa: log dose in mg/kg.

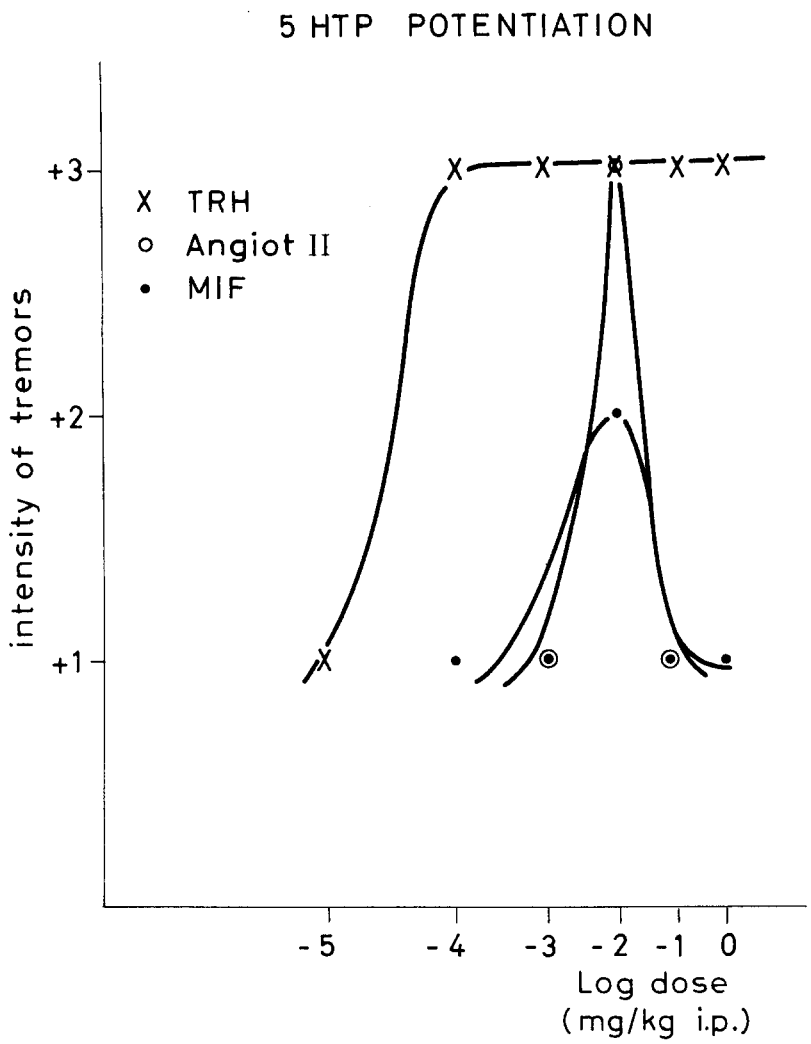

FIG. 2. Effect of MIF, TRH and angiotensin II on the behavioral effects induced by 5–HTP in pargyline-pretreated mice. The animal's reaction was evaluated as described in methods. Saline-treated mice that received pargyline and 5–HTP had a +1 reaction. Ordinates: intensity of tremors; abscissa: log doses in mg/kg.

0.1 μg/kg induced a score of +3. As seen in Fig. 3, the tremors induced by 5–HTP in mice pretreated with TRH are more intense, arise earlier and last longer than in the control animals. Angiotensin II and MIF (10 μg/kg) induced scores of +3 and +2 respectively, higher doses did not produce any potentiation. The potentiating effect of TRH disappeared when pargyline pretreatment was omitted.

DISCUSSION

The results for MIF and TRH are in accord with those of Plotnikoff *et al.* [13,15], who showed these polypeptides possess a potentiating effect on the behavioral response of mice to L-DOPA. We found TRH to be active in the same dosage range as these authors, whereas under our experimental conditions MIF was active at one thousandth the dose reported by Plotnikoff *et al.* [13]. Angiotensin II showed a potentiating activity which is between the two other compounds in efficacy. The disappearance of the potentiation observed with higher doses of this drug (0.5 mg/kg and up) may be due to a side effect, since behavioral depression has been described in mice receiving intraventricularly 1 μg of angiotensin II [2].

Only TRH showed a clear cut potentiation of the behavioral symptoms due to 5-HTP in mice; unlike the other two polypeptides, which showed a decline in the potentiation with increasing doses, TRH maintained its ability to potentiate 5-HTP at higher doses.

The patterns of 5-HTP potentiation observed with TRH strongly resemble that obtained in mice treated with 5, 6-DHT [11]. In this case, the increased response was attributed to an impaired uptake of the mediator due to destruction of nerve terminals caused by 5, 6–DHT.

In view of the evidence brought by Carlsson *et al.* [3] on an inhibitory effect of imipramine on serotonin uptake, we thought that 5-HTP potentiation could be a common property among the antidepressant drugs. However, imipramine (20 and 40 mg/kg) and desipramine (5, 10 and

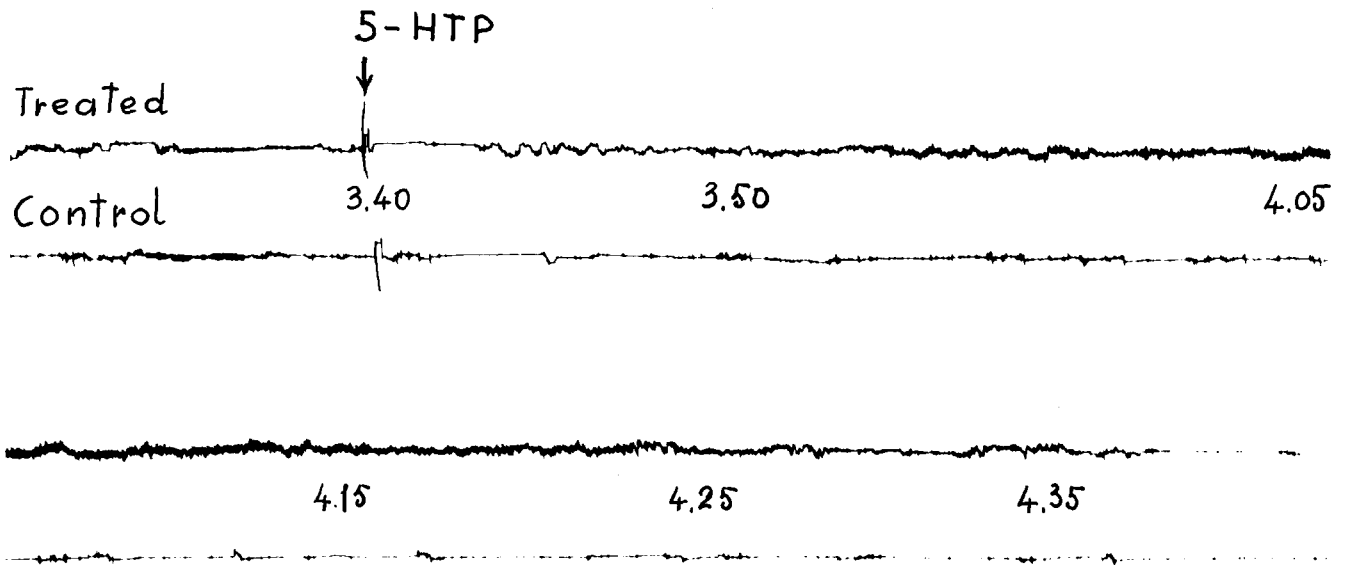

FIG. 3. Representative record of the tremors induced by 5–HTP in mice. The treated animal received 1 mg/kg of TRH two hours before 5–HTP (at the arrow). In the treated mouse, tremors appear earlier and are more intense than in the control. For details of technique, see text.

20 mg/kg), tested under the same experimental conditions as TRH, did not potentiate the effects of 5–HTP.

Since our experiments were performed in intact mice, the observed effects of TRH could be exerted through an influence on the hypophyseal-thyroid axis. In the last few years, it has become increasingly obvious that the function of thyroid gland is intimately related with indole metabolism [19], and 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.* [16]. Moreover, it is known that thyroid hormones facilitate the actions of catecholamines in both peripheral and central synapses. Emlen *et al.* [5] showed that rats made hyperthyroid with thyroxine showed increased sensitivity to the activating effects on behavior of NE administered intraventricularly. It should be mentioned, however, that both TRH and MIF are effective in the L-DOPA potentiation test carried out in hypophysectomized mice [13,15]. The possibility of a direct effect of the three polypeptides at the synaptic sites must therefore be considered. The mechanism of this interaction is not yet clarified. For angiotensin II, an inhibition of NE uptake by nerve terminals has been postulated by Palaic and Khairallah [12]. Thoenen *et al.* [18] attribute instead the potentiation of the electrically induced contraction of cat spleen by angiotensin to a receptor sensitization. The concept that polypeptides may play some role in synaptic transmission is now evolving in the minds of investigators engaged in this research [10]. The present results offer some leads which may be relevant to the identification of the role of such substances in central modulation.

An interesting fact which emerged from the present experiments is the selectivity of potentiation of the behavioral effects of L-DOPA and 5–HTP by MIF and TRH respectively. It is very suggestive that these factors are related to two distinct systems. TRH, through the thyroid gland, can influence the indoleamine metabolism, the other is related to the biosynthesis of melatonin and L-DOPA metabolism.

How much this observation is relevant to the clinical effectiveness of these drugs is hard to say at the present moment. TRH was found effective on unipolar depression [17], while MIF has been used with encouraging results in parkinsonian patients [9]. From the basis of published data and from unpublished results from this laboratory MIF has an antitremor effect on various experimental conditions. Plotnikoff *et al.* [14] reported that MIF antagonizes the tremors induced by oxotremorine in mice and potentiates the effects of L-DOPA in reducing the central effects of oxotremorine. In preliminary experiments we found that MIF antagonizes harmine-induced tremors in the rabbit and, at very low doses, potentiates the antagonistic effect of L-DOPA on these tremors [8]. From these observations it could derive that potentiation of serotoninergic system, as shown by enhancement of 5–HTP central effects, could be related to an antidepressant effect, while potentiation of catecholaminergic system would indicate antiparkinsonian properties.

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