Pharmacology Biochemistry & Behavior, Vol. 15, pp. 983–985, 1981. Printed in the U.S.A.

Effects of MIF-1 and Three Related Peptides on Reserpine-Induced Hypothermia in Mice

ABBA J. KASTIN, LYNDA C. HONOUR AND DAVID H. COY

VA Medical Center and Tulane University School of Medicine, New Orleans, LA 70146

Received 9 October 1981

KASTIN, A. J., L. C. HONOUR AND D. H. COY. *Effects of MIF-1 and three related peptides on reserpine-induced hypothermia in mice.* PHARMAC. BIOCHEM. BEHAV. 15(6)983–985, 1981.—MIF-1, Tyr-MIF-1, pGlu-Leu-Gly-NH_2, and cyclo- Leu-Gly were tested for 3 hr (60, 120, and 180 min) at 3 doses (0.1, 1.0, and 10.0 mg/kg IP) in mice pretreated 18 hr earlier with reserpine. Reversal of hypothermia was significant only with MIF-1 ($p<0.05$) at 120 and 180 min but not with the 2 analogs (pGlu-Leu-Gly-NH_2 and cyclo-Leu-Gly) reported to be more active in other tests than MIF-1 or with Tyr-MIF-1, a recently described brain peptide. Thus, the results demonstrate that the relative potency of related peptides can differ depending upon the experimental situation.

Temperature Peptides Rauwolfia Depression Ptosis Sedation

THE concept of the extra-pituitary or extra-endocrine effects of hypothalamic peptides was introduced with the demonstration in hypophysectomized animals that Pro-Leu-Gly-NH_2 (MIF-1) potentiated the behavioral effects of dopa [11]. It was soon extended to reversal by MIF-1 of the tremors induced by oxotremorine [12] and of the sedation induced by deserpidine [10]. More recently, MIF-1 has also been shown to share some properties of naloxone [7, 8, 14, 16], even on body temperature [18].

In some of these tests, analogs of MIF-1 were subsequently reported to be more potent than MIF-1 itself. The first potent MIF-1 analog was described by Castensson *et al.* [2] for the oxotremorine test. Substitution of pyro-Glu for the N-terminal Pro resulted in pGlu-Leu-Gly-NH_2, a compound found to be twice as potent as MIF-1 in reversing the tremors induced by oxotremorine [2]. The most potent analog in the dopa-potentiation test (Pro-N-Me-D-Leu-Gly-NH_2) was slightly less potent than the parent MIF-1 [15]; because of this and restrictions from the supplier, it was not used in the present study. The stable MIF-1 analog cyclo-Leu-Gly has been reported to be more potent than MIF-1 in its actions on morphine dependence, although the effects are opposite in different labs [14,16]. Tyr-Pro-Leu-Gly-NH_2 (Tyr-MIF-1), or a closely related compound, has been described in the pineal gland [5] and brain [6] of the rat but its effects on a neuropharmacological test have not been previously reported.

Antagonism of the sedative actions of the rauwolfia alkaloid deserpidine by MIF-1 in mice and monkeys required the additional use of pargyline and dopa [10]. The present study investigated the effects of MIF-1, pGlu-Leu-Gly-NH_2, cyclo-Leu-Gly, and Tyr-MIF-1 without pargyline or dopa in mice pretreated with the rauwolfia alkaloid reserpine. The actions of these peptides in reversing the hypothermia and ptosis as well as sedation induced by pretreatment with reserpine [1,13] were measured.

METHOD

Male albino mice were obtained from Harlan Sprague Dawley (Madison, Wisconsin) and used at body weights of 20–25 g. They had free access to food and water before testing in a sound-attenuated laboratory.

Reserpine was added to corn oil (Mazola); the mixture was warmed for 2 min at 50°C, stirred for 15 min, and injected IP at a dose of 2.5 mg/kg. Eighteen hr later, the 4 peptides were dissolved in diluent (0.9% NaCl acidified to 0.01 M with acetic acid) and injected intraperitoneally (IP) at doses of 0.1, 1.0, and 10.0 mg/kg. Equal numbers of mice received the peptides and diluent at each dose during every experimental day. This resulted in a total of about 30 mice in each of the 15 groups (3 doses of 5 substances) except that the groups receiving cyclo-Leu-Gly contained only about half that number of animals because of a limited supply of peptide.

Immediately before (time 0) as well as 60, 120, and 180 min after injection of peptide or diluent in coded solutions, 3 measurements were made. The first was that of ptosis, graded by the method of Rubin *et al.* [13] on a scale of 1–4 where "1" indicates fully open eyes and "4" totally closed eyes. Spontaneous motor activity was measured for 15 sec on an activity meter (Stoelting model 31407) that recorded movement across tuned electromagnetic resonant circuits. Temperature was then measured by a probe inserted to a depth of 2 cm from the anus (Yellow Spring telethermometer); the differences (°C) from time 0 were obtained for each mouse.

Results were analyzed by analysis of variance. This was followed by Duncan's Multiple Range Test.

RESULTS

The administration of reserpine resulted in a significant decrease in body temperature in mice from 36.4 ± 0.4°C to

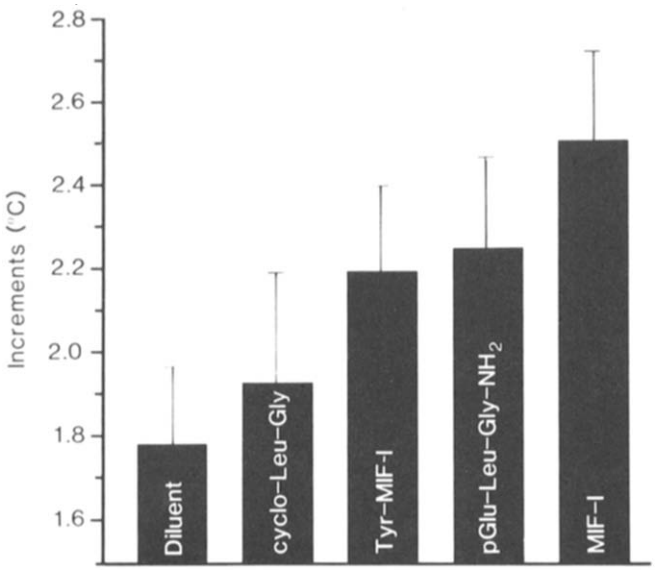

FIG. 1. Mean (±SEM) increase in colonic temperature (°C) 3 hr after peripheral injection of 3 doses (0.1, 1, and 10 mg/kg) of MIF-1, related peptides, or the diluent control in mice pretreated 18 hr earlier with reserpine.

30.3 ± 0.1°C ($p<0.01$). Overall, only MIF-1 reversed this hypothermia and caused a significantly ($p<0.05$) greater increase in body temperature than did the diluent at 120 and 180 min, but not at 60 min. The increments from time 0 for diluent and MIF-1 at 60 min were 1.72°C and 1.89°C (NS), at 120 min were 1.78°C and 2.43°C ($p<0.05$) and at 180 min (Fig. 1) were 1.78°C and 2.51°C ($p<0.05$). This change with time was greater with MIF-1 than with the other peptides and was reflected by the significant peptide by time interaction, $F(8,770)=1.97$, $p<0.05$. The main effect of time was also significant, $F(2,770)=14.38$, $p<0.001$.

Of the peptides tested, only MIF-1 tended to show a stronger effect with the increasing doses of 0.1, 1.0, and 10 mg/kg. In a preliminary experiment, however, the reversal of hypothermia after a dose of 20 mg/kg MIF-1 was not reliably greater than after diluent. The only other peptide tending to cause a greater increase in colonic temperature at the dose of 10 mg/kg was Tyr-MIF-1 ($p=0.1$ at 180 min), but the effect of 0.1 mg/kg tended to be greater than that of 1.0 mg/kg for this tetrapeptide. The most active dose of pGlu-Leu-Gly-NH_2 was 0.1 mg/kg. The effect of 10 mg/kg of cyclo-Leu-Gly tended to be less than that of the 1 mg/kg and 0.1 mg/kg doses of this dipeptide. No statistically significant differences existed among the groups of diluent. Although the peptide by dose interaction was not significant, the peptide by dose by time interaction was reliable, $F(16,770)=1.84$, $p<0.05$, providing some support for these differences in dose-response patterns.

The effects of each of the peptides on the ptosis and sedation induced by reserpine were not statistically significant, although in a preliminary experiment, the large dose of 20 mg/kg of MIF-1 did reliably reduce the ptosis at 120 min and 180 min. For ptosis, but not activity, the changes induced by diluent tended to be the least, followed by cyclo-Leu-Gly. These measures, however, seemed to be very susceptible to reversal by even moderately loud noise in the laboratory.

DISCUSSION

The significant reversal of reserpine-induced hypothermia by MIF-1 at 3 hr (Fig. 1) as well as 2 hr provides another example of the actions of this peptide after peripheral administration. Although this effect did not require the addition of a monoamine oxidase inhibitor and dopa [10], it is possible that these agents might be necessary for doses of MIF-1 between 0.1–10 mg/kg to affect the ptosis and sedation induced by reserpine. The variability in these 2 measurements, however, was very large.

Two analogs of MIF-1 that have been reported to be more potent than MIF-1 in other test situations [2, 14, 16] were not found to be more potent than MIF-1 in increasing body temperature after pretreatment with reserpine. Both pGlu-Leu-Gly-NH_2 and cyclo-Leu-Gly failed to cause a significantly greater effect than diluent at each time and each dose. This further emphasizes the caution that must be exerted in extrapolating from the effect of a peptide in one situation to that in another.

Reversal of reserpine-induced hypothermia by Tyr-MIF-1 was not statistically significant ($p=0.1$). This does not preclude the possibility that Tyr-MIF-1 will be found to share some actions with MIF-1, and already there are some indications of this [9]. In rat brain, moreover, levels of Tyr-MIF-1-like immunoreactivity were found to show diurnal rhythmicity and an increase after pinealectomy [6].

Dose-response relationships also differ according to experimental situations. The inverted U-shaped dose-response curve was first reported for MIF-1 in 1971 when 0.1 mg/kg and 1.0 mg/kg of the tripeptide were found to exert effects not seen with higher doses [11]. A middle dose (1.0 mg/kg) of MIF-1 was also found to potentiate the action of amphetamine on body temperature whereas a smaller and a larger dose did not [17]. Similar effects have been found in mental depression [3,4]. By contrast, in the present study and some others [7], the effect tended to increase as the dose of MIF-1 was raised from 0.1 mg/kg to 10 mg/kg.

Reversal of the effects of reserpine has been used as an animal model of depression [1]. Although tricyclic antidepressants are active in this system, so are other compounds not effective in depression [1]. Clinical trials with MIF-1, however, have shown some improvement in the symptoms of mental depression [3,4].

MIF-1 has no effect by itself on body temperature [17,18]. In combination with β-endorphin [18], morphine [18], d-amphetamine [17], chlorpromazine [17], and reserpine (this study), however, it does significantly affect temperature in laboratory animals. Clinical trials have not yet been performed with MIF-1 or its analogs on temperature.

ACKNOWLEDGEMENTS

The authors thank Dr. James Zadina for review of the manuscript, Maureen Reardon for preparation of the manuscript, and Nancy Kunen for the art work. The study was supported in part by Mrs. Louise Dunagan Kramer and the Veterans Administration.

REFERENCES

1. Askew, B. M. A simple screening procedure for imipramine-like antidepressant agents. *Life Sci.* **10:** 725–730, 1963.
2. Castensson, S., H. Sievertsson, H., Bj. Lindeke and C. Y. Sum. Studies on the inhibition of oxotremorine induced tremor by a melanocyte-stimulating hormone release-inhibiting factor, thyrotropin releasing hormone and related peptides. *FEBS Lett.* **44:** 101–105, 1974.
3. Ehrensing, R. H. and A. J. Kastin. Melanocyte-stimulating hormone-release inhibiting hormone as an anti-depressant: a pilot study. *Archs gen. Psychiat.* **30:** 63–65, 1974.
4. Ehrensing, R. H. and A. J. Kastin. Dose-related biphasic effect of prolyl-leucyl-glycinamide (MIF-1) in depression. *Am. J. Psychiat.* **135:** 562–566, 1978.
5. Kastin, A. J., S. P. Lawrence and D. H. Coy. Radioimmunoassay of MIF-1-like material in rat pineal. *Pharmac. Biochem. Behav.* **13:** 901–905, 1980.
6. Kastin, A. J., S. P. Lawrence and D. H. Coy. Radioimmunoassayable [Tyr^1]-MIF-1-like activity in rat brain is increased by pinealectomy. *Brain Res. Bull.*, in press.
7. Kastin, A. J., C. Nissen, J. E. Zadina, A. V. Schally and R. H. Ehrensing. Naloxone-like actions of MIF-1 do not require the presence of the pituitary. *Pharmac. Biochem. Behav.* **13:** 907–912, 1980.
8. Kastin, A. J., R. D. Olson, R. H. Ehrensing, M. C. Berzas, A. V. Schally and D. H. Coy. Differential actions of MIF-1 as an opiate antagonist. *Pharmac. Biochem. Behav.* **11:** 721–723, 1979.
9. Olson, R. D., R. C. Fernandez, A. J. Kastin, G. A. Olson, S. W. Delatte, T. K. von Almen, D. G. Erickson, D. C. Hastings and D. H. Coy. Low doses of naloxone and MIF-1 peptides increase fluid consumption in rats. *Pharmac. Biochem. Behav.* **15:** 921–924, 1981.
10. Plotnikoff, N. P., A. J. Kastin, M. S. Anderson and A. V. Schally. Deserpidine antagonism by a tripeptide, L-prolyl-L-leucylglycinamide. *Neuroendocrinology* **11:** 67–71, 1973.
11. Plotnikoff, N. P., A. J. Kastin, M. S. Anderson and A. V. Schally. DOPA potentiation by a hypothalamic factor, MSH release-inhibiting hormone (MIF). *Life Sci.* **10:** 1279–1283, 1971.
12. Plotnikoff, N. P., A. J. Kastin, M. S. Anderson and A. V. Schally. Oxotremorine antagonism by a hypothalamic hormone, melanocyte-stimulating hormone release-inhibiting factor, MIF. *Proc. Soc. exp. Biol. Med.* **140:** 811–814, 1972.
13. Rubin, B., M. H. Malone, M. H. Waugh and J. C. Burke. Bioassay of rauwolfia roots and alkaloids. *J. Pharmac. exp. Ther.* **120:** 125–136, 1957.
14. van Ree, J. M. and D. de Wied. Prolyl-leucyl-glycinamide (PLG) facilitates morphine dependence. *Life Sci.* **19:** 1331–1340, 1976.
15. Voith, K. Synthetic MIF analogues. Part II: dopa potentiation and fluphenazine antagonism. *Arzneim.-Forsch./Drug Res.* **27:** 2290–2293, 1977.
16. Walter, R., R. F. Ritzmann, H. N. Bhargava and L. B. Flexner. Prolyl-leucyl-glycinamide, cyclo (leucylglycine), and derivatives block development of physical dependence on morphine in mice. *Proc. natn. Acad. Sci. U.S.A.* **76:** 518–520, 1979.
17. Yehuda, S. and A. J. Kastin. Interaction of MIF-1 or α-MSH with D-amphetamine or chlorpromazine on thermoregulation and motor activity of rats maintained at different ambient temperatures. *Peptides* **1:** 243–248, 1980.
18. Yehuda, S., A. J. Kastin and D. H. Coy. Antagonistic actions of MIF-1 on the hypothermia and hypomotility induced by beta-endorphin or morphine. *Int. J. Neurosci.* **11:** 317–320, 1980.