Biochemical Pharmacology, Vol. 25, pp. 363–365. Pergamon Press, 1976. Printed in Great Britain.

COMMENTARY

NEUROPHARMACOLOGY OF HYPOTHALAMIC RELEASING FACTORS

NICHOLAS P. PLOTNIKOFF and ABBA J. KASTIN

Experimental Therapy Division, Abbott Laboratories, North Chicago, Ill. 60064, and
Endocrinology Section of the Medical Service, Veterans Administration Hospital, and Department of Medicine, Tulane University School of Medicine, New Orleans, La. 70146, U.S.A.

Early studies suggested that peptides from the pituitary gland might have affects on the central nervous system (CNS). Murphy and Miller [1] and Miller and Ogawa [2] showed that administration of corticotropin (ACTH) resulted in resistance to extinction of a conditioned avoidance response. De Wied [3] used this test involving electrical shock to demonstrate that melanocyte-stimulating hormone (MSH) can have the same effect as ACTH and that the active sequence is ACTH/MSH 4–10 [4]. Approaching the problem for the first time from a direct interest in MSH, several different behavioral tests in the rat and man as well as studies of electrical activity (EEG) in the brain of these species were initiated in 1966 by Kastin and colleagues. The results from these investigations are consistent with the hypothesis that MSH results in improved visual memory and sustained levels of attention [5, 6]. These studies differed in approach from the previous ones of Krivoy *et al.* [7, 8], who examined the spinal cord and electrical discharge, and from those of Ferrari *et al.* [9, 10], who tested stretching activity and yawning.

In an unrelated series of investigations, Wilson *et al.* [11] were investigating the interactions of tricyclic antidepressants and thyroid hormones. Their early findings suggested that the time of onset of antidepressant activity was shortened by the use of thyroid hormone [11] (T_3, tri-iodothyronine) as well as thyrotropin (TSH) [12], a pituitary hormone whose release is stimulated by TRH (thyrotropin-releasing hormone).

Thus, the stage was set for the next logical series of studies to determine whether the relatively newly identified and isolated hypothalamic releasing factors [13], MIF-I (melanocyte-stimulating hormone release inhibiting factor, Pro-Leu-Gly-NH_2) and TRH, had direct effects in the central nervous system. Later, other hypothalamic hormones like growth hormone release inhibiting hormone (GH-RIH) and gonadotropin-releasing hormone (GnRH) were also tested.

MSH-release inhibiting factor-I (MIF-I)

Since Cotzias *et al.* [14] reported that MSH appeared to aggravate the symptoms of Parkinsonism, and Kastin *et al.* [15, 16] demonstrated that MSH release was caused by phenothiazines, compounds which can cause extra-pyramidal effects, it seemed logical to determine whether MIF-I might reduce these effects. The animal models that we employed to identify potential anti-Parkinsonian activity included Dopa potentiation [17], oxotremorine antagonism [18], and reserpine reversal [19]. However, as a result of the work showing central actions of MSH, we looked for a direct effect of MIF-I on the brain in these same tests by using hypophysectomized animals. MIF-I was active in all these systems and the presence of the pituitary gland was not required. This was the first clear demonstration of extra-endocrine effects of any hypothalamic peptide, in this case MIF-I (independent of a change in MSH release from the pituitary). It has been confirmed by Huidobro-Toro *et al.* [20]. In subsequent studies, we found that there were no alterations in the activity of MIF-I on the brain in the Dopa test in adrenalectomized, ovariectomized, castrated, pinealectomized, spleenectomized, thyroidectomized, parathyroidectomized, nephrectomized or thymectomized animals [21]. This substantiated the extra-endocrine effects of MIF-I in the Dopa test [21].

The marked activity of MIF-I in potentiating Dopa was further demonstrated by its activity in potentiating the behavioral effects of apomorphine on mounting [22]. Yet, no change in the levels of biogenic amines in the brain was found [21] nor was there any alteration of dopamine turnover rates (as measured directly [23, 24] or indirectly by HVA [21]) in the striatum. However, using incorporation rates of tyrosine, Friedman *et al.* [25] observed increased synthesis of dopamine in the striatum. Perhaps it would be informative to measure turnover rates of labeled dopamine itself in different areas of the brain and also to determine whether differences in the strain of rat can be a varying factor. There are preliminary data which indicate that MIF-I may cross the blood-brain barrier and that it exerts a post-synaptic effect [23].

On the clinical side, early studies indicated that MIF-I appeared to reduce the symptoms of Parkinsonism [26–28]. Since two of our major tests (Dopa potentiation and reserpine reversal) are also animal models for clinical depression, MIF-I was also evaluated for potential antidepressant activity. Preliminary studies suggested that MIF-I may have slight effects in reducing symptoms of depression [29].

Thyrotropin-releasing hormone (TRH)

The original motivation for studying the effects of TRH in the brain was the observation that tri-iodothyronine (T_3) [11] and TSH [12] could potentiate the effects of imipramine in depressed patients. In animals we found that TRH potentiated Dopa centrally in

B.P. 25/4—A

intact, hypophysectomized and thyroidectomized animals [30, 31]. This finding established, as in the earlier case of another tripeptide MIF-I, that TRH was effective centrally and was not dependent upon the pituitary (TSH) or thyroid (T_3, T_4). It has been confirmed by Huidobro-Toro *et al.* [20]. In other studies [32], it was shown that TSH, T_3 and T_4 were also effective in the Dopa test, suggesting that in the intact animal there may be interactions between TRH, TSH, T_3 and T_4 in potentiating Dopa. In contrast to MIF-I, TRH was found ineffective in reducing symptoms induced by oxotremorine [33]. In addition, TRH but not MIF-I was found to potentiate the central effects of serotonin. These results emphasize the differences in the neuropharmacological profile between MIF-I and TRH.

The neurochemical mechanism of action of TRH has been ascribed, in part, to increases in brain norepinephrine turnover rates by some investigators [34] but not others [35, 36]. However, animals pretreated with large doses of Fla-63, a dopamine beta-hydroxylase inhibitor, and subsequently used in the Dopa test have still responded to TRH. This finding suggests that TRH may be acting through dopaminergic systems.

Initial clinical reports suggested that TRH may have antidepressant activity[37–39]. However, more recent studies have not confirmed the earlier reports [40–42]. Nevertheless, it would appear that certain patients exhibit pronounced improvement in certain components of behavior, like motivation and interest [43]. At the same time, a number of depressed patients appear to show a blunted or depressed TSH response to TRH [37, 38]. There is one intriguing preliminary report [44] that TRH appears to potentiate the antidepressant effects of imipramine in patients resistant to imipramine alone. Perhaps TRH may be found useful as an adjunct to tricyclic antidepressant. However, in another study [45], TRH administered once or twice a week with amitriptyline was not different from amitriptyline alone. Perhaps daily treatment with TRH together with the tricyclic antidepressant agents may be necessary.

Another new area of development is the finding that TRH can antagomize the sedative effects of alcohol or barbiturates in animals [46]. It remains highly speculative to consider whether TRH may eventually be found to have some use in the treatment of alcoholism.

Growth hormone release inhibiting hormone (GH-RIH)

In a neuropharmacological profile study, we observed that the tetradecapeptide GH-RIH potentiated Dopa centrally but did not potentiate serotonin or antagonize audiogenic seizures, oxotremorine or footshock-induced fighting in mice [47]. Clinically, there have been suggestions that GH-RIH may exhibit some activity as a mood elevator or tranquilizer.

Gonadotropin-releasing hormone (GnRH or LH-RH)

GnRH, like MIF-I, TRH and GH-RIH, is active in the Dopa potentiation test [33]. However, GnRH and GH-RIH require at least 2mg/kg for marked activity, whereas MIF-I and TRH are much more potent in this test (doses of 0·1 and 0·5 mg/kg respectively). GnRH slightly reduced spontaneous motor activity. A small tendency toward reduction in the frequency of audiogenic seizures in specially bred mice but no change in footshock-induced fighting behavior or the symptoms caused by oxotremorine was noted after administration of this decapeptide.*

Perspectives

The discovery of effects in the CNS of MSH, MIF-I, TRH, GH-RIH and GnRH all lead one to suspect that there are complex feedback systems between the hypothalamic releasing factors, pituitary hormones and the brain. Whether such effects hold promise as clinical and therapeutic agents in the treatment of psychiatric and neurological disorders will depend upon new developments. Already, there is a report [48] of an unusually active TRH analog that is at least five times more potent than MIF-I in reducing the symptoms of oxotremorine in rodents, although TRH itself is inactive in this test of potential anti-Parkinsonian agents. Clinically, MSH and its active core MSH 4-10 have been shown to improve the attention span of normal volunteers [6, 49, 50]. The clinical tests of MSH or MSH 4-10 in senile patients and mental retardates might provide useful information. One could even speculate about the possible use of TRH in the management of certain stages of alcoholism.

Probably the most dramatic developments await the systematic development of a series of chemically related peptides. It is quite possible that new analogs will have more specific clinical effects with therapeutic utility. However, in the meantime, studies of the hypothalamic peptides should provide a sound basis for future investigations.

* N. P. Plotnikoff, W. White, A. J. Kastin and A. V. Schally, manuscript submitted for publication.

REFERENCES

1. A. V. Murphy and R. E. Miller, *J. comp. physiol. Psychol.* **48**, 47 (1955).
2. R. E. Miller and N. Ogawa, *J. comp. physiol. Psychol.* **55**, 211 (1962).
3. D. De Wied, *Int. J. Neuropharmac.* **4**, 157 (1965).
4. D. De Wied, B. Bohus and H. M. Greven, *Endocrinology and Human Behaviour*, p. 188 Oxford University Press, London (1968).
5. A. J. Kastin, L. H. Miller, R. Nockton, C. A. Sandman, A. V. Schally and L. O. Stratton, *Progress in Brain Research*, Vol. 39, p. 461. Elsevier, Amsterdam (1973).
6. A. J. Kastin, C. A. Sandman, L. O. Stratton, A. V. Schally and L. H. Miller, *Progress in Brain Research* Vol. 42. Elsevier, Amsterdam (1975).
7. W. A. Krivoy and R. Guillemin, *Endocrinology* **69**, 170 (1961).
8. W. A. Krivoy, M. Lane, H. E. Childers and R. Guillemin, *Experientia* **18**, 521 (1962).
9. W. Ferrari, G. L. Gessa and L. Vargiu, *Experientia* **17**, 90 (1961).
10. G. L. Gessa, M. Pisano, L. Vargiu, F. Crabai and W. Ferrari, *Revue can. Biol.* **26**, 229 (1967).
11. I. C. Wilson, A. J. Prange, Jr., T. K. McClane, A. M. Rabon and M. A. Lipton, *New Engl. J. Med.* **282**, 1063 (1970).
12. A. J. Prange, Jr., I. C. Wilson, A. Knox, T. K. McClane and M. A. Lipton, *Am. J. Psychiat.* **127**, 191 (1970).

13. A. V. Schally, A. Arimura and A. J. Kastin, *Science, N.Y.* **179** 341 (1973).
14. G. C. Cotzias, M. A. van Woert and L. M. Schiffer, *New Engl. J. Med.* **276**, 374 (1967).
15. A. J. Kastin and A. V. Schally, *Endocrinology* **79**, 1018 (1966).
16. A. J. Kaston, A. V. Schally, S. Viosca and M. C. Miller, *Endocrinology* **84**, 20 (1969).
17. N. P. Plotnikoff, A. J. Kastin, M. S. Anderson and A. V. Schally, *Life Sci.* **10**, 1279 (1971).
18. N. P. Plotnikoff, A. J. Kastin, M. S. Anderson and A. V. Schally, *Proc. Soc. exp. Biol. Med.* **140**, 811 (1972).
19. N. P. Plotnikoff, A. J. Kastin, M. S. Anderson and A. V. Schally, *Neuroendocrinology* **11**, 67 (1973).
20. J. P. Huidobro-Toro, A. Scotti De Carolis and V. G. Longo, *Pharmac. Biochem. Behav.* **2**, 105 (1974).
21. N. P. Plotnikoff, F. N. Minard and A. J. Kastin, **14**, 271 (1974).
22. N. P. Plotnikoff and A. J. Kastin, *Archs. int. Pharmacodyn. Ther.* **211**, 211 (1974).
23. A. J. Kastin, N. P. Plotnikoff, C. A. Sandman, M. A. Spirtes, R. M. Kostrzewa, S. M. Paul, L. O. Stratton, L. H. Miller, F. Labrie, A. V. Schally and H. Goldman, *Anatomical Neuroendocrinology*, S. Karger, Basel, Switzerland (1975).
24. R. M. Kostrzewa, M. A. Spirtes and A. J. Kastin, *Pharmacologist* **16**, 320 (1974).
25. E. Friedman, J. Friedman and S. Gershon, *Science, N.Y.* **182**, 831 (1973).
26. A. J. Kastin and A. Barbeau, *Can. med. Ass. J.* **107**, 1079 (1972).
27. T. N. Chase, A. C. Woods, M. A. Lipton and C. E. Morris *Archs. Neurol., Chicago* **31**, 55 (1974).
28. P.-A. Fischer, E. Schneider, P. Jacobi and H. Maxion, *Eur. Neurol.* **12**, 360 (1975).
29. R. H. Ehrensing and A. J. Kastin, *Archs. gen. Psychiat.* **30**, 63 (1974).
30. N. P. Plotnikoff, A. J. Prange, G. R. Breese, M. S. Anderson and I. C. Wilson, *Science, N.Y.* **178**, 417 (1972).
31. N. P. Plotnikoff, A. J. Prange, G. R. Breese and I. C. Wilson, *Life Sci.* **14**, 1271 (1974).
32. N. P. Plotnikoff, A. J. Prange and G. R. Breese, *Pharmac. Biochem. Behav.*, in press.
33. A. J. Kastin, N. P. Plotnikoff, R. Hall and A. V. Schally, *Hypothalamic Hormones: Chemistry, Physiology, Pharmacology and Clinical Uses, Lond.* Academic Press, New York (1975).
34. H. H. Keller, G. Bartholini and A. Pletscher, *Nature, Lond.* **248**, 528 (1974).
35. T. G. Reigle, J. Avni, P. A. Platz, J. J. Schildkraut and N. P. Plotnikoff, *Paychpoharmacologia* **37**, 1 (1974).
36. G. R. Breese, B. R. Cooper, A. J. Prange, J. M. Cott and M. A. Lipton, *The Thyroid Axis, Drugs, and Behavior*, p. 115, Raven Press, New York (1974).
37. A. J. Kastin, R. H. Ehrensing, D. S. Schalch and M. S. Anderson, *Lancet* **2**, 740 (1972).
38. A. J. Prange, I. C. Wilson, P. P. Lara, L. B. Alltop and G. R. Breese, *Lancet* **2**, 999 (1972).
39. M. J. E. van der Vis-Melsen and J. D. Wiener, *Lancet* **2**, 1415 (1972).
40. R. H. Ehrensing, A. J. Kastin, D. S. Schalch, H. Friesen, R. Vargas and A. V. Schally, *Am. J. Psychiat.* **161**, 714 (1974).
41. C. Q. Mountjoy, J. S. Price, M. Weller, P. Hunter, R. Hall and J. H. Dewar, *Lancet* **1**, 958 (1974).
42. S. Takahashi, H. Kondo, M. Yishimura and Y. Ochi, *Folia psychiat. neurol. jap.* **27**, 305 (1973).
43. T. Itil, *J. Pharmac* **5**, (suppl. 1.) 86 (1974).
44. P. Kielholz, *Medical Tribune*, Germany (1974).
45. A. Coppen, S. Montgomery, M. Peet, J. Bailey, V. Marks and P. Woods, *Lancet* **2**, 433 (1974).
46. A. J. Prange, G. R. Breese, J. M. Cott, B. R. Martin, B. R. Cooper, I. C. Wilson and N. P. Plotnikoff, *Life Sci.* **14**, 447 (1974).
47. N. P. Plotnikoff, A. J. Kastin and A. V. Schally, *Pharmac. Biochem. Behav.* **2**, 693 (1974).
48. H. Sievertsson, S. Castensson, S. Bjorkman and B. Lindeke, *Hypothalamic Hormones: Chemistry, Physiology, Pharmacology and Clinical Uses*, Academic Press, New York (1975).
50. A. J. Kastin, L. H. Miller, D. Gonzalez-Barcena, W. D. Hawley, K. Dyster-Aas, A. V. Schally, M. L. Velasco-Parra and M. Velasco, *Physiol. Behav.* **7**, 893 (1971).
50. L. H. Miller, A. J. Kastin, C. Sandman, M. Fink and W. J. van Veen, *Pharmac. Biochem. Behav.* **2**, 663 (1974).