RELEASE OF PITUITARY MELANOCYTE-STIMULATING HORMONE BY THE OXYTOCIN FRAGMENT, H-CYS-TYR-ILE-GLN-ASN-OH

Maria E. Celis, S. Taleisnik and Roderich Walter*

Instituto de Investigacion Medica, Mercedes y Martin Ferreyra, Cordoba, Argentina and *The Department of Physiology, The Mount Sinai Medical and Graduate Schools of the City University of New York, New York, New York 10029 U.S.A.

Received July 14, 1971

Summary: The effect of the pentapeptide H-Cys-Tyr-Ile-Gln-Asn-OH to act like melanocyte-stimulating hormone-releasing factor (MSH-RF) was studied. This peptide decreases at ng amounts the MSH content of the rat pituitary and increases plasma MSH concentration. This agent also stimulates the release of MSH in animals with median eminence lesion, indicating a direct action of the pentapeptide on the gland.

Freshly prepared extracts of median eminence (ME) of rats were shown to evoke the release of pituitary melanocyte-stimulating hormone (MSH) from the pituitary when injected into recipient rats (1,2). The active principle present in the extract has been referred to as MSH-releasing factor, although its chemical identity remains to be determined. Recently we have demonstrated that the incubation of a mitochondrial fraction prepared from extracts of paraventricular nuclei---the site of biosynthesis of oxytocin (3-5)---likewise gives rise to a factor which possesses the capacity to release pituitary MSH (6). Since this mitochondrial fraction degrades oxytocin we considered the possibility that the factor responsible for the release of MSH may be a metabolite of oxytocin. We proposed earlier (7,8) that the C-terminal fragment of oxytocin, Pro-Leu-Gly-NH_2, is the natural MSH-release-inhibiting hormone---a suggestion which was confirmed by Mair *et al.* (9).

Preliminary investigation of several synthetic oxytocin intermediates has shown that the pentapeptide H-Cys-Tyr-Ile-Gln-Asn-OH, a pentapeptide sequence found in the 20-membered ring component of oxytocin (10), exhibits MSH-releasing activity. Thus the pentapeptide was studied in more detail, and the data are presented in this communication.

MATERIALS AND METHODS

N-Tos-S-Bz-Cys-Tyr-Ile-Gln-Asn-OH (9 mg, ca. 10 μmoles), kindly supplied by Dr. L.A. Branda (11), was dissolved in liq. ammonia and deprotected by reduction with sodium (12). The mixture was lyophilized and the resulting powder subjected to gel filtration on a Sephadex G-15 column (96 x 1.2 cm) in the solvent system 50% AcOH:0,1N HCl:0,1M dithiothreitol (100:0.4:0.2, v/v/v). Fractions (2.32 ml each) were collected at a flow rate of 3.53 ml/hr and the eluate was monitored continually spectrophotometrically at 280 nm. The free pentapeptide, present in fractions 25-27, was homogeneous upon thin-layer chromatography in the solvent system BuOH:AcOH:H_2O (4:1:1). The preparation of the free pentapeptide was carried out by Dr. S. Hase and these data along with related work will be published in detail elsewhere.

The capacity of the free pentapeptide to induce the release of MSH in rats was studied using two parameters, viz. the decrease of pituitary MSH content and the increase in plasma MSH activity. The capacity of the pentapeptide to induce a decrease of pituitary MSH content was studied in male rats weighing 180 to 200g. The animals, anesthetized with ether, were injected intravenously with the pentapeptide and then killed 20 min later by placing them in a small container saturated with ether. The hypophyses were immediately removed and homogenized in bidistilled water (5 mg pituitary tissue/ml water) and kept frozen at -20°C until further use. The glands from two animals were pooled and the MSH content was measured by an in vitro assay (1).

In order to study the effect of the pentapeptide on MSH levels in plasma the animals were prepared the day before the experiment. A plastic cannula was implanted in the external jugular vein under ether anesthesia in order to be used for the iv administration of the pentapeptide without disturbing the animal. Ten minutes after the injection of the pentapeptide the animals were killed by decapitation and the blood was collected from their trunks into centrifuge tubes containing heparin. Aliquots of blood (0.75 ml each) were diluted with bidistilled water to 1 ml, and 8 to 9 pieces of toad skin were

then added. One hr later the stage of the melanocytes was read according to the Hogben index (13).

For the assay procedure the free pentapeptide was dissolved in aqueous 50% acetic acid which had been carefully deairated by prolonged flushing with nitrogen gas. Just prior to use this stock solution was diluted with deairated saline in order to obtain the desired amount of pentapeptide in a volume of 0.20 ml. Synthetic oxytocin (prepared by Miss P.L. Hoffman) possessing an avian vasodepressor activity of 500 U/mg (14, 15) was dissolved in distilled water and diluted with saline solution as described for the pentapeptide. Rats were hypophysectomized by removing the gland by a parapharingeal approach. The operation was performed 24 hrs prior to the injection of the animal with pentapeptide. Rats with median eminence lesions were prepared in a manner similar to the procedure described by Taleisnik et al. (16) by passing a d.c. of 1 mA for 30 sec through a platinum electrode which had been implanted with the aid of a stereotaxic instrument. Only animals which showed a water intake of 100 ml or more per day were used.

RESULTS AND DISCUSSION

The injection of 20, 50 or 100 ng of pentapeptide into rats provoked a significant decrease in pituitary MSH content (Table 1). No such decrease was observed when oxytocin (80 and 120 ng) was injected.

The injection of 40 ng pentapeptide into rats with ME lesions induced a highly significant decrease in pituitary MSH content as compared to that observed in animals injected with saline solution ($P<.001$) (Table 1). These data demonstrate that the pentapeptide induces the release of pituitary MSH by a direct action on the intermediate lobe rather than through the central nervous system.

The effect of the pentapeptide on plasma MSH activity was also studied. The injection of 40 ng pentapeptide into intact rats induced a significant increase in plasma MSH activity within a 10-min period, as indicated by the high melanocyte index (Fig. 1). The injection of an equal volume of saline

Table 1. Effect of various agents on the pituitary MSH content of intact rats and rats with median eminence lesions

	Treatment	no. of exp.	Decrease of pituitary MSH content*
Intact rats	100ng pentapeptide	1	54.1 (58.9 - 48.6)
	50ng pentapeptide	2	45.6 (52.5 - 36.1)
	20ng pentapeptide	1	68.1 (79.1 - 49.9)
	120ng oxytocin	2	1.6 (6.9 - 4.2)
	80ng oxytocin	2	5.2 (11.1 - 0.2)
Rats with ME lesions	40ng pentpeptide	4	58.5 (65.3 - 52.7)
	Saline solution(0.20ml)	4	5.2 (15.3 - 6.2)

* Potency and confidence limits were calculated according to accepted statistical methods (17).

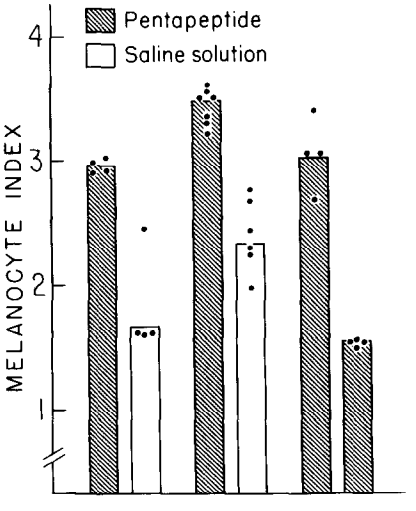

Fig. 1 Effect of H-Cys-Tyr-Ile-Gln-Asn-OH on plasma MSH activity in intact rats (first set of bars), in rats with median eminence lesions (middle set of bars) and in hypophysectomized rats (last bar; the penultimate bar represents intact animal which served as control). The animals were injected with a constant amount of 40 ng of pentapeptide. Each point represents one experiment, and the bars are the mean values.

solution into control animals did not provoke a detectable change in plasma MSH levels.

Analogous studies were performed with rats with ME lesions; in such animals it is supposed that the neural connections to the hypophysis were destroyed and any changes in pituitary MSH content are a result of a direct action of agents on the gland. The pentapeptide was injected ten days after the lesions were made. Significantly higher melanocyte indexes were found in animals injected with the pentapeptide than in those animals injected with saline solution ($P<.001$). It is noteworthy that the melanocyte index of rats possessing ME lesions was higher than that of intact rats when injected with saline solution. In contrast to the intact and ME-lesioned rats, no enhancement of MSH activity was detected in the plasma of the hypophysectomized rats upon injection of pentapeptide (Fig. 1). This suggests that the high melanocyte index observed in plasma after the injection of the pentapeptide depends on the presence of the pituitary gland. Next we studied the effect of increasing amounts of pentapeptide on plasma MSH activity. As shown in Fig. 2 a dose-dependent relationship was observed in two independent experiments.

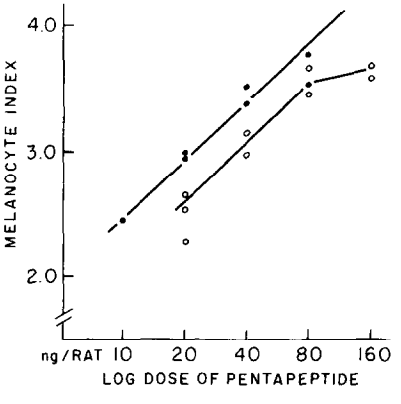

Fig. 2 Dose-response relationship between amount of H-Cys-Tyr-Ile-Gln-Asn-OH injected and MSH detected in blood. Each point represents the melanocyte index for a 0.75 ml plasma aliquot from one rat. White and black circles correspond to two different experiments.

The above results suggest that the pentapeptide H-Cys-Tyr-Ile-Gln-Asn-OH at ng concentrations induces the liberation of pituitary MSH as shown by the decrease in MSH content of the gland and the increase in plasma activity. This effect of the pentapeptide is also found in animals with ME lesions, indicating that the release of MSH is due to a direct action on the gland and not through an action involving the central nervous system. There exists a dose-response relationship between plasma MSH activity and the pentapeptide. Oxytocin per se has no detectable effect on pituitary MSH content. The possibility exists that the MSH-releasing activity observed after incubating oxytocin with the mitochondrial fraction obtained from paraventricular nuclei was due to the formation of the pentapeptide, H-Cys-Tyr-Ile-Gln-Asn-OH, or a peptide of related peptide sequence. The relationship of the peptides to the natural MSH-releasing factor found in the ME extracts of rat is under investigation.

ACKNOWLEDGMENT

This work was supported in part by the United States Public Health Service Grant AM-13567 and by the Consejo Nacional de Investigaciones Científicas y Tecnicas of Argentina.

REFERENCES

1. Taleisnik, S., and Orias, R., Amer. J. Physiol. 208, 293 (1965).
2. Taleisnik, S., and Tomatis, M.E., Endocrinology, 81, 819 (1967).
3. Scharrer, E., and Scharrer, B., Rec. Prog. Hormone Res. 10, 183 (1954).
4. Brooks, C., Ishikawa, T., Koizumi, K., and Lu, H., J. Physiol., 182, 217 (1966).
5. Sokol, H.W., and Valtin, H., Nature, 214, 314 (1967).
6. Celis, M.E., and Taleisnik, S., in preparation.
7. Celis, M.E., Taleisnik, S., Schwartz, I.L., and Walter, R., Biophys. J., 11, 98a (1971).
8. Celis, M.E., Taleisnik, S., and Walter, R., Proc. Natl. Acad. Sci. U.S., in press.
9. Mair, R.M.G., Kastin, A.J., and Schally, A.V., Biochem. Biophys. Res. Commun., 43, 1376 (1971).
10. Du Vigneaud, V., Science, 123, 967 (1956).
11. Branda, L.A., and du Vigneaud, V., J. Med. Chem., 9, 169 (1966).
12. Sifferd, R., and du Vigneaud, V., J. Biol. Chem., 108, 753 (1935).
13. Hogben, L., and Slome, D., Proc. Roy. Soc. London, Ser. B, 108, 10 (1931).
14. Chan, W.Y., and du Vigneaud, V., Endocrinology, 71,977 (1962).
15. Guttman, St., Helv. Chim. Acta, 49, 83 (1966).
16. Taleisnik, S., de Olmos, J., Orias, R., and Tomatis, M.E., J. Endocr., 39, 485 (1967).
17. Bliss, C.I., The Statistics of Bioassay, Academic Press, New York, 1952.