Purification of Hypothalamic Melanocyte-Stimulating Hormone (MSH)-Releasing Factor with Sephadex.* (30946)

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Evidence has been presented recently for the existence of a melanocyte stimulating hormone-releasing factor (MSH-RF) in crude acidic extracts of rat hypothalamic tissue(1). Such extracts were capable of depleting the pituitary of its MSH activity within 20 minutes of their intravenous (i.v.) administration into normal rats. Cerebral cortical extracts were inactive, and the effect could not be accounted for by the presence of other known pharmacologically active agents in the hypothalamic extract. However, to establish MSH-RF as an independent entity, its purification and separation from other releasing factors is essential. In the present work, purification of ovine MSH-RF has been obtained by gel filtration through Sephadex G-25.

Materials and methods. Extractions. An acetone powder prepared from a large number of freshly frozen stalk-median eminence (SME) fragments from ovine hypothalami was extracted with glacial acetic acid and the glacial acetic acid extract was lyophilized according to the procedure previously reported (2).

Gel filtration on Sephadex. A long column (150 × 4.5 cm) of Sephadex G-25 (fine grade) was employed. The lyophilized, glacial acetic acid extract was dissolved in 0.1 M ammonium acetate buffer at pH 5.0. The clear supernatant was applied to the column which had been equilibrated with the 0.1 M (pH 5.0) ammonium acetate buffer. This buffer was used for elution and 20 ml fractions were collected. Conditions were similar to those used previously(2).

Peptide concentration in the effluent from the column was followed by the Folin-Lowry technique(3).

Bioassay. Each fraction to be evaluated was evaporated and redissolved in 0.05 M acetic acid prior to i.v. injection in a volume of 0.3 ml into male rats weighing 180-200 g. Control rats were injected with 0.3 ml of diluent. The animals were anesthetized with ether and each fraction was injected into 2 test rats. Twenty minutes later the pituitaries from each group of rats were removed, weighed and pooled. They were suspended in distilled water, and frozen until assay. Two dose levels of control and experimental pituitaries were assayed for MSH activity in the toad, *Bufa arenarum* Hensel, by the *in vitro* technique already described(1). MSH activity of pituitaries from rats injected with the various fractions from the column was compared to that of pituitaries from animals injected with diluent. Potency and confidence limits were calculated according to accepted statistical methods(4).

Results. The MSH-releasing activity of the various fractions was estimated in 2 separate experiments from the same fractionation on Sephadex G-25 (Table I, Fig. 1). A significant depletion of pituitary MSH activity was observed with tubes 100-108 in the first experiment. Maximal activity was found in tube 108 which lowered the MSH activity to 31% of that found in the pituitaries of control animals. Tubes on either side of this active zone failed to alter significantly the concentration of hypophyseal MSH.

* Supported by U.S.P.H.S. grant AM-01236-09.

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‡ Supported by grant NB-04732-02 from Nat. Inst. Health.

TABLE I. MSH-Releasing Activity of Various Fractions from the Sephadex Column.

Tube No.	Pituitary MSH concentration (% control)* Assay 1	Assay 2	Mean
95	88 (66–112)†		88
100	80 (65– 95)		80
104	70 (48– 91)	67 (39– 92)	68
106		51 (35– 65)	51
108	31 (14– 43)	39 (25– 52)	35
110	82 (63–101)	60 (42– 75)	71
114	100 (83–120)	79 (61–100)	89
118	100 (73–126)	120 (101–151)	112
122	75 (51–101)	124 (99–165)	100

* $\frac{\text{MSH/mg injected rat's pituitary}}{\text{MSH/mg control rat's pituitary}} \times 100.$

† Mean (95% confidence limits).

The results of the second experiment were remarkably similar, and again the peak of activity was seen in tube 108. The peptide concentration in the dose used for tube 108 was 80 μg as measured by the Folin-Lowry reaction.

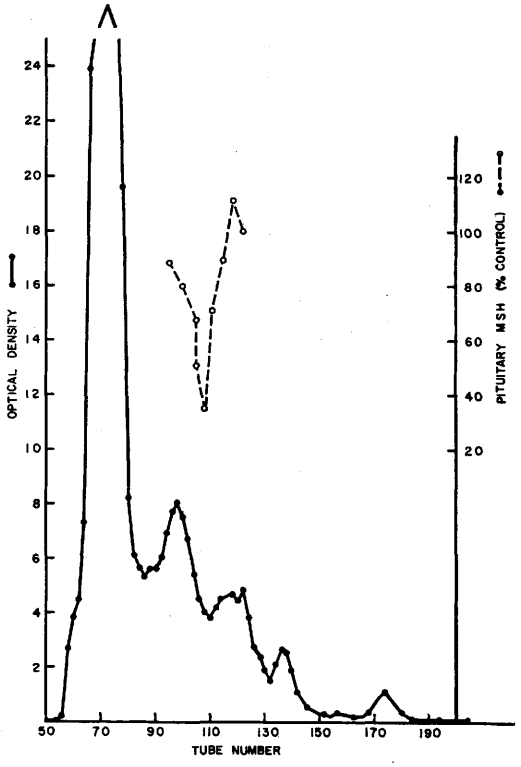

FIG. 1. MSH-releasing activity (pituitary MSH in % of control) and peptide concentration as indicated by the Folin-Lowry reaction (optical density) of fractions eluted from the Sephadex column.

Discussion. The *in vitro* assay for MSH upon which these results are based depends on the microscopic appearance of the melanocytes of toad skin incubated *in vitro* in the presence of pituitary extract. Since the melanocyte is sensitive to both adrenocorticotrophin (ACTH) and MSH, it is important to inquire if the observed changes were in the content of MSH. It has been previously shown that doses of rat hypothalamic extract, which caused a marked diminution in pituitary MSH activity by the present test, failed to alter pituitary ACTH content(1). Consequently, it is clear that in these earlier experiments a depletion of pituitary MSH was observed.

It is almost certain that we are also observing a depletion of MSH and not ACTH in the present experiments as well, since the active zone for depleting MSH activity is separated from the zone previously shown to have corticotrophin-releasing activity(5). The corticotrophin-releasing activity was eluted in a wide zone which ended at tube 100, whereas the peak activity in the present experiments was observed at tube 108.

The zone with MSH-releasing activity was also separated from that of most of the other hypothalamic-releasing factors so far examined. The growth hormone-releasing factor emerged from the column in a zone which overlapped that which contained the corticotrophin-releasing factor(6). The luteinizing hormone-releasing factor (LH-RF)(2) and prolactin-inhibiting factor (PIF)(7) emerged later than the MSH-RF as did vasopressin (2). The follicle stimulating hormone-releasing factor (FSH-RF) emerged in the same zone which had MSH-releasing activity(8).

Thus, preliminary extraction followed by gel filtration on Sephadex is sufficient to separate the MSH-RF from most other releasing factors. Presumably, the MSH-RF constitutes another chemically distinct polypeptide in what has now become a family of hypothalamic factors which influence hormonal secretion by the adenohypophysis. Further work will be necessary in an attempt to separate the MSH-RF from FSH-RF.

On the basis of its migration rate on Sephadex, MSH-RF, like other hypothalamic pitui-

tary stimulating hormones, seems to be a small polypeptide, with a molecular weight between 1500 and 2000.

The rapidly induced depletion of hypophyseal MSH produced by hypothalamic extracts has been interpreted to mean that a release of stored MSH has occurred and consequently the active agent has been termed the MSH-RF. Final proof that a release of MSH has taken place requires the demonstration of increased plasma levels of MSH after injection of hypothalamic extracts.

Kastin(9) has recently reported in an abstract that crude hypothalamic extracts inhibited the release of MSH by pituitaries incubated *in vitro*. He postulated the existence of an MSH-inhibiting factor (MIF) in the extract. Our results do not negate the possibility that hypothalamic extracts may contain a MIF in addition to the MSH-RF the purification of which is reported here.

Summary. Acetone powders prepared from ovine stalk-median eminence tissue were extracted with glacial acetic acid and the glacial acetic acid extract was lyophilized. An ammonium acetate extract of the resultant powder was filtered through a column of Sephadex G-25. The eluent was assayed for its ability to deplete the pituitary of MSH. Extracts were injected i.v. into normal male rats which were sacrificed 20 minutes later. The MSH activity in pituitaries from rats receiving extracts was compared to that found in pituitaries from diluent-injected controls. MSH activity was estimated in an *in vitro* assay by microscopic grading of the melanocytes. An active zone was eluted from the column. At the peak of this zone 65-70% of the MSH activity had disappeared from the pituitaries of the test rats. The MSH-releasing zone was separated from the zones which contained CRF, GH-RF, LH-RF, PIF and vasopressin, but overlapped the zone which contained FSH-RF. It was concluded that MSH-RF can be added to the family of hypothalamic polypeptides which influence adenohypophyseal secretion.

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Received January 3, 1966. P.S.E.B.M., 1966, v121.

Rat Liver Acid Phosphatase: Differences in Lysosomal and Cytoplasmic Forms. (30947)

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Acid phosphatase is primarily a lysosomal enzyme. In liver tissue approximately 70 to 80% of its activity is confined to these particles, the remainder being distributed throughout the cytoplasm(1). Since, however, acid phosphatase is composed of multiple molecular forms(2-4), it is possible that certain organelles may contain specific forms of the enzyme. Indeed, differences have been reported in the acid phosphatases of the lysosomal and supernatant fractions of the liver of the guinea pig(5), but this duality of acid phosphatase has not been clearly established in another species(6). It was felt that further