Topographic Distribution of the Melanocyte-Stimulating Hormone-Releasing Factor in Rat Hypothalamus.* (31125)

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Recently, it has been reported that acidic extracts of rat hypothalamus injected intravenously into recipient rats induce an acute decrease of the melanocyte-stimulating hormone (MSH) concentration in the pituitary. The active agent in the hypothalamus has been referred to as a melanocyte-stimulating hormone-releasing factor (MSH-RF). It has been shown to be effective at low doses and its effect was related to the dose injected.

Because of these characteristics of the extracts, attempts were made in the present study to determine the topographic distribution of the MSH-RF in the hypothalamus.

Methods. The hypothalami were removed from adult albino male rats sacrificed with ether and extracts from different portions were prepared in 0.1 N HCl according to the technique previously described(1). The extracts were injected intravenously into male rats which weighed 180-200 g. Twenty minutes later the animals were killed with ether and their hypophyses immediately removed, weighed, suspended in an adequate volume of distilled water and kept frozen until the MSH activity was determined.

In order to obtain the different portions of the hypothalamus, serial frozen sections, 30 μ thick, were cut in either the frontal, sagittal or horizontal planes. Extracts were prepared from the sections located between the planes shown in Fig. 1 and 2. Each plane was established by careful examination of the specimens with a magnifying glass. Each extract was injected into one recipient animal and the MSH activity of its pituitary estimated in relation to the value of simultaneously assayed pituitaries from animals injected with cerebral cortex extract taken as 100%. Duplicate assays were performed for each hypothalamic region.

Extracts were also prepared from the neural lobe of the hypophysis. Extracts obtained from pools of several glands were injected into 2 recipient animals from which the hypophyses were pooled and assayed for MSH activity. Pituitary MSH activity was measured by an *in vitro* technique(1) using skin of toads and grading melanin dispersion in the melanocytes.

Results. In Table I are given the values of the pituitary MSH activity found in animals injected with extracts from different portions of hypothalamus as compared to that in animals injected with cerebral cortex extract taken as controls. The study of the extracts from frontal sections showed that the area situated rostral to the optic chiasma (F1) and that corresponding to the mammillary bodies (F6) did not have any effect on the pituitary MSH activity. The maximum pituitary MSH depleting activity was found in the zone F3 which corresponds to the anterior hypothalamus and included the paraventricular nucleus. The activity decreased in the extracts prepared from sections caudal and rostral to zone F3.

Extracts prepared from horizontal sections showed the highest pituitary MSH-depleting activity in the area H3 which included the paraventricular nucleus. Activity was also found in the region immediately overlying that area. The region of the median eminence and arcuate nucleus named as H1 was next in activity to the H3 area. The H1 and H3 areas were separated by a zone in which less activity was found. When the extracts obtained from sections taken in the sagittal plane were injected, the greatest decrease in pituitary MSH activity was found with the zone S3 which corresponds to the periventricular and the median eminence structures. There was less decrease by injecting the in-

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TABLE I. Effect of Extracts from Various Portions of Hypothalamus on Pituitary MSH Activity.

	MSH activity per mg pituitary in injected rats / MSH activity per mg pituitary in control rats × 100					
Frontal sections	F 1	F 2	F 3	F 4	F 5	F 6
Assay 1	101.0	67.4	47.8	58.7	74.1	98.3
" 2	110.2	90.0	25.0	50.5	60.7	105.0
Mean	105.6	78.7	36.4	54.6	67.4	101.6
Horizontal sections	H 1	H 2	H 3	H 4		
Assay 1	46.8	55.3	31.8	49.0		
" 2	37.1	51.3	20.9	40.7		
Mean	41.9	53.3	26.3	44.8		
Sagittal sections	S 1	S 2	S 3			
Assay 1	102.0	55.2	40.3			
" 2	106.0	59.3	25.9			
Mean	104.0	57.2	33.1			

termediate hypothalamic area S2 and no changes were obtained with the area S1 which includes the supraoptic nucleus.

From the results obtained in previous work (1) in which different doses of rat hypothalamus were injected, the following regression was obtained:

$$\text{Eq. 1} \qquad X = \frac{y - 113.47}{-46.94}.$$

In this equation X = log dose of hypothalamic extract, and y is the response based on pituitary MSH activity. The potency of each hypothalamic area was estimated from this equation using the mean value for pituitary MSH activity of the two bioassays presented (Table I).

These values were converted into a percentage of the total activity of the hypothalamus by the following equation:

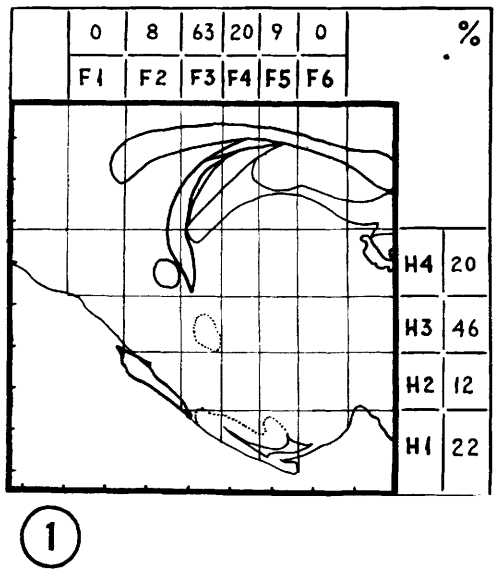
$$\text{Eq. 2} \qquad \%\ \text{activity} = \frac{X}{X + X_1 + X_2} \times 100.$$

This represents the ratio between the value for an area calculated by equation 1 and the several values of the different areas in which the hypothalamus was divided. In this case X is the potency of a given region in percent of the total activity of one rat's hypothalamus. X_1 and X_2 would be the potency of the other regions in that plane of sectioning. The values in each plane of section are shown in Fig. 1 and 2. They total 100% except for the sagittal sections. These latter values total 50% since only one-half of the hypothalamus was tested.

As can be seen in Fig. 3, the extracts of neural lobe when injected into recipient animals, could also induce a decrease in pituitary MSH activity. The animals injected with extracts containing the equivalent of 1 neural lobe had only 50% of the pituitary MSH activity observed in control animals. To establish to what extent the oxytocin and vasopressin contained in the neural lobe might be responsible for the results obtained, synthetic oxytocin and arginine-vasopressin in doses of 1 IU were injected. As shown in Fig. 3 neither of these hormones nor synthetic lysine-vasopressin modified MSH activity. On the other hand, a slight decrease was observed with vasopressins obtained from natural sources.§

Discussion. The results of this study confirm those obtained in previous work(1) which showed the capacity of rat hypothalamic extracts to decrease pituitary MSH activity. It has also been shown that this effect is not due to a decrease in ACTH concentra-

§ Synthetic arginine-vasopressin, lysine-vasopressin and oxytocin (Syntocinon) were a gift of Sandoz, Basle. Lysine-vasopressin NIH was kindly provided by the Endocrinology Study Section of the NIH and Pitressin was supplied through the courtesy of Parke, Davis and Co.



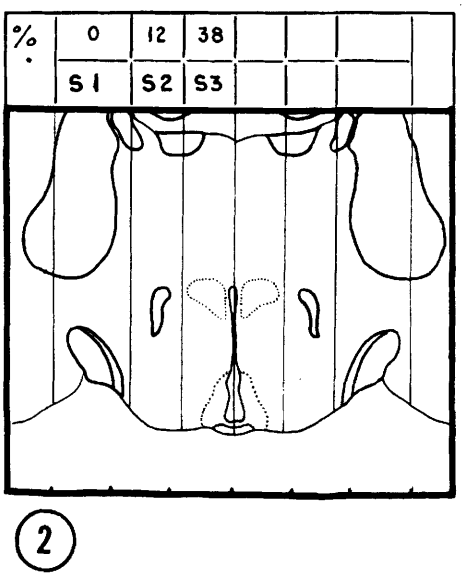


FIG. 1 and 2. Mid-sagittal and frontal sections of rat hypothalamus. Vertical and horizontal lines are the limits of the areas from which extracts were prepared. The figures in % are the MSH-RF potency of each area in percent of the summed activity of the different areas in the corresponding plane.

tion in the pituitary. The active agent in the hypothalamic extracts has been referred to as a MSH-RF.

The present results show that the portions of greatest activity in depleting pituitary MSH concentration included the paraventricular nucleus in each one of the 3 planes in which the hypothalamus was sectioned. It can therefore be inferred that the greatest content of MSH-RF in the hypothalamus of the rat is in the area of the paraventricular nucleus. This is in contrast to the complete lack of activity in the area which included the supraoptic nucleus. The median eminence appears as the second hypothalamic area of high activity being separated from the paraventricular region by an area with lower activity. The presence of activity in the zone dorsal to the paraventricular nucleus might be caused by aberrant cells extending up to the columnae formices. Indeed, Gomori-positive elements have been shown to extend up to that area.

The extracts of hypophyseal neural lobe were also capable of depleting pituitary MSH concentration, though their activity was lower than that of the hypothalamus. Neither oxytocin nor vasopressin was responsible for this action since the synthetic hormones were not able to reproduce the effects of the extracts even though they were given in higher concentration than those known to be contained in the dose of posterior pituitary injected. The depletion of pituitary MSH concentration induced by arginine-vasopressin and lysine-vasopressin obtained from natural sources indicates the presence of MSH-RF in these products probably because contaminating

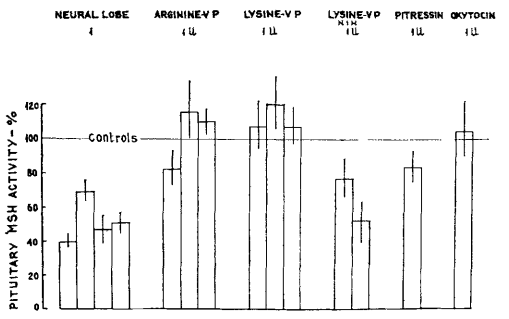


FIG. 3. Effect of extracts from neural lobe of the hypophysis or of neurohypophyseal hormones on pituitary MSH activity. Each column is the potency of the pooled pituitaries from 2 recipient rats as compared to that of control animals. Vertical lines are 95% confidence limits. Each recipient animal was injected with 1 neural lobe or 1 IU of neurohypophyseal hormone. Arginine-vasopressin, lysine-vasopressin and oxytocin are synthetic products. Lysine-vasopressin NIH and pitressin are neurohypophyseal hormones obtained from natural sources.

MSH-RF was not removed by the purification procedures.

The results of the present study give support to the idea that MSH-RF could be produced by cells having their soma in the paraventricular nucleus and their axons ending in the median eminence and neural lobe of the hypophysis. From these areas the neurosecretory material could reach the intermediate lobe of the hypophysis through the vessels which irrigate it. The vascularization of the intermedia lobe, described by Duvernoy(2), starting from the capillaries that initially run through the neural lobe and the median eminence gives anatomical support to this point of view.

Summary. The topographic distribution of MSH-RF in the hypothalamus was studied in the rat. MSH-RF was determined by measuring the capacity of hypothalamic extracts to deplete pituitary MSH concentration in recipient animals. The activity of extracts prepared from sections of hypothalamus taken in 3 different planes showed the highest concentration of the releasing factor in the area of the paraventricular nucleus. The median eminence was the second most important active zone. Extracts from neural lobe of the hypophysis were also active though to a lesser degree than those from hypothalamus and their effect did not depend on either oxytocin or vasopressin. The possibility that MSH-RF could be produced by neurons having their soma in the paraventricular nucleus and axons ending in the median eminence and neural lobe of the hypophysis is discussed.

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The Species Specificity of Cholestyramine in Its Effect on Synthesis Of Liver Lipids and Level of Serum Cholesterol. (31126)

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Cholestyramine, an insoluble quarternary ammonium anion exchange resin, has a strong affinity for bile acids *in vitro* and is not absorbed from the gastrointestinal tract(1). When given orally cholestyramine causes an increased fecal elimination of bile acids(2,3). The fecal excretion of neutral sterols is also enhanced by cholestyramine(2,3), probably because of a reduced concentration of bile acids in the intestinal lumen(4).

This increased fecal loss of steroidal substances during cholestyramine administration has been noted in all species studied. The loss is reflected by a decrease in plasma cholesterol levels in some species, notably the chicken(2), dog(2) and man(3,5); in other species, such as the rat(6), mouse* and pig (7) the feeding of cholestyramine does not lower plasma cholesterol levels. In the rat and pig the increased fecal loss of steroids caused by cholestyramine is accompanied by a large compensatory increase in *de novo* cholesterol biosynthesis(6,7). These observations suggest that the rat, mouse and pig can increase sterol synthesis sufficiently to maintain constant tissue sterol levels when cholestyramine is administered; species such as the chicken and man presumably cannot compensate to the same degree.

Data supporting this hypothesis were obtained by studying the effects of dietary cholestyramine on plasma and liver cholesterol levels and on the *in vitro* synthesis of lipids by homogenates of livers obtained from rats and chickens. The results showed that cholestyramine had relatively little effect on hepatic

* Gallo, D. G., Sheffner, A. L., unpublished observations.