Substances	CRF-activity Versus 4 μg of CRF 91 [a]	MSH activity U/g
Natural		
Fraction 3	1.0 μg : 1.3	5—6× 10^{8} [6]
Fraction 4	1.0 μg : 1.3	
Fraction 5	1.0 μg : 0.8	
Synthetic		
N-ε-formyl-α-MSH [b]	4 μg : 0.7	2× 10^{10} [9]
Hexapeptide [c]	4 μg : 1.2	2× 10^{5} [6]
Heptapeptide	0.1 μg : 1.0	2.8× 10^{5} [6]
Decapeptide	0.1 μg : 1.0	—

[a] As explained in ref. [1] and [2], one determines the amount of unknown substance, giving a release of ACTH equal to that given by 4 μg of the standard CRF 91: if we take for instance fraction 3, we see that 1 μg of this fraction provokes a release of ACTH equal to that produced by 1.3 × 4 μg of CRF 91, and that 0.1 μg of the heptapeptide produces a release equal to that given by 1.0 × 4 μg of CRF 91, etc. In the latter example, we would say that the heptapeptide is about 40 times more active than CRF 91; but this assumption can be made only when the ratio $x/4$ μg CRF 91 is equal to 1.0.

[b] N-ε-formyl-α-MSH: Ac.Ser.Tyr.Ser.Met.Glu.His.Phe.Arg.Try.Gly.Lys.Pro.Val(NH_2)
|
N-ε-formyl.

[c] Hexapeptide: NH_2
|
H.Glu.His.Phe.Arg.Try.Gly.OH

Heptapeptide: NH_2
|
H.Met.Glu.His.Phe.Arg.Try.Gly.OH

Decapeptide: NH_2
|
H.Ser.Tyr.Ser.Met.Glu.His.Phe.Arg.Try.Gly.OH

of PORATH *et al.*[3]. The 3 first steps have already been described[1]; we describe here only the 4th step:

The material coming from the counter current distribution (25 mg) was dissolved in 1 ml of 0.1 *N* acid and put on a cellulose column of 0.6 × 25 cm; dinitrophenyl-ethanolamine was used as a tracer and the run was performed in 0.6 *M* pyridinium acetate; 0.2% phenol was added as a preservative and the temperature of the buffer was maintained at about 10–12°C with circulating water. The electrophoresis was run under 30 mA and 300 volts. Fractions of 2 ml were collected every 20 min and ninhydrin analysis was performed on 0.2 ml samples after acid hydrolysis (HCl 15 h in sealed tubes at 100°C).

In this manner several fractions were obtained numbered 1, 2, 3, 4, etc., the first one appearing after 12 h. The fraction 2 consists mainly of the tripeptide Leu. Arg. Leu; the CRF activity was found in peaks 3 and 4 (see Table). These 2 active fractions (3 and 4) represent approximately 10% of the material put on the column. Fractions 3 and 4 were also analyzed for their amino-acid composition: fraction 3 has a composition similar to that of the α-Melanophore Stimulating Hormone (α-MSH) given by HARRIS[4], with the exception that threonine and leucine were present.

The identity between α-MSH and CRF has been envisaged for a while by ourselves and by GUILLEMIN *et al.*[5]. This identity does not seem possible now for the following reasons:

- the presence of threonine and leucine in the molecule of the peptide(s) possessing CRF activity,
- the fact that the active fraction 3 has an MSH activity amounting only to 1–2.5% of that of pure α-MSH; this weak MSH activity may be either intrinsic to the material or may represent some contamination with α-MSH[6],
- the fact that highly purified natural α-MSH[7], as well as synthetic formyl-α-MSH, kindly supplied by Dr. K. HOFMANN, have no significant CRF activity.

In the course of these experiments we have studied several synthetic peptides related to the group of the Corticotropins and the Melanophore Stimulating Hormones, generously supplied by Drs. SCHWYZER and KAPPELER[8]. *We have found that several of these peptides, having a sequence in common with the Corticotropins and the MSH have a strong in vitro CRF activity, higher than that of the most active natural fractions so far obtained by us* (*Table*).

M. PRIVAT DE GARILHE, C. GROS, J. PORATH, and EVA-BRITA LINDNER

Laboratoire de Chimie biologique de la Faculté des Sciences, Paris (*France*) *and Biokemiska Institutionen, Uppsala* (*Sweden*), *June 1, 1960.*

Résumé

Une fraction peptidique ayant une forte activité d'hypophyso-stimuline à effet corticotrope ou «Corticotropin Releasing Factor» (CRF), *in vitro*, a été obtenue à partir de la poudre d'hypophyses postérieures de porc par les opérations suivantes:

1. Extraction de la poudre avec l'acide acétique dilué; 2. Précipitation fractionnée à l'acétone; 3. Distribution à contre courant; 4. Electrophorèse de zones.

La constitution en amino acides de cette fraction est très voisine de celle de l'α-MSH; cependant α-MSH et CRF ne sont pas identiques pour des raisons chimiques et physiologiques. Bien que l'α-MSH n'ait pas d'activité CRF significative, des peptides synthétiques ayant une séquence commune avec l'α-MSH possèdent cette activité.

[3] J. PORATH, E. B. LINDNER, and S. JERSTEDT, Nature *182*, 794 (1958).

[4] J. I. HARRIS, Biochem. J. *71*, 451 (1959).

[5] R. GUILLEMIN, A. V. SCHALLY, and R. N. ANDERSEN, Fed. Proc. *19*, part I, 239 (1960).

[6] We are indebted to Dr. I. I. GESCHWIND (Berkeley) who was kind enough to perform MSH assays on this substance.

[7] A. V. SCHALLY, R. GUILLEMIN *et al.*, personal communication.

[8] H. KAPPELER and R. SCHWYZER, Exper. *16*, 183 (1960).

[9] K. HOFMANN, personal communication.

Synthetic Peptides Related to the Corticotropins (ACTH) and the Melanophore Stimulating Hormones (MSH) Possessing Corticotropin Releasing Activity (CRF-Activity)

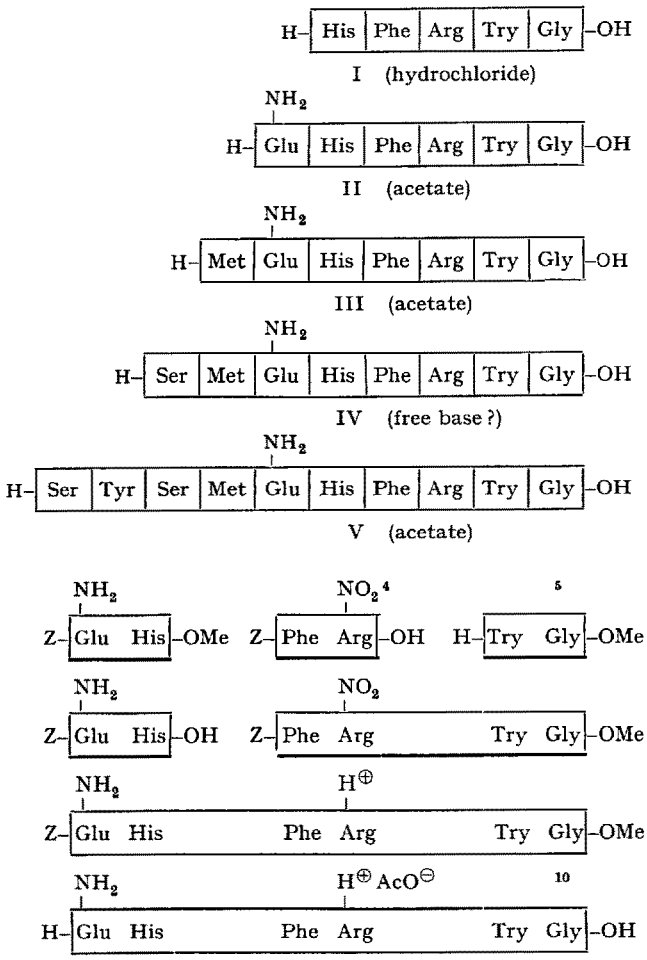
Our synthetic work in the field of β-MSH[1] required the synthesis of a number of peptides with amino-acid sequences common to ACTH, α-MSH, and β-MSH. Among these were the pentapeptide (I), the hexapeptide (II), and the heptapeptide (III). The latter compound contains the whole amino-acid sequence common to all three hormones of the pituitary mentioned above.

LI[2] and HARRIS[3] have put forward the hypothesis that the common sequence might have some special biological

[1] R. SCHWYZER, H. KAPPELER, B. ISELIN, W. RITTEL, and H. ZUBER, Helv. chim. Acta *42*, 1702 (1959).

[2] C. H. LI, Adv. Protein Chem. *12*, 288 (1957).

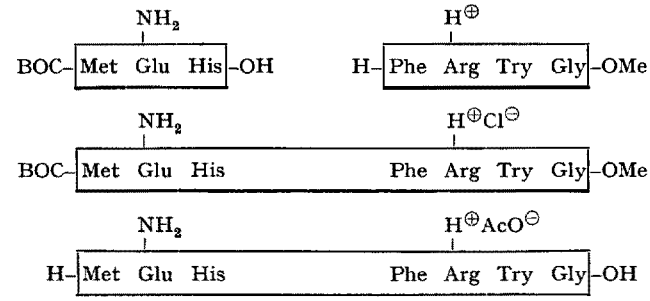
[3] J. I. HARRIS and P. ROOS, Biochem. J. *71*, 434 (1959).



II. amorphous, $[\alpha]_D^{26} = -23.6° \pm 1.8°$ ($c = 0.466$ in 1 *N* HCl); R_f 45[6] = 0.47; R_f 43[11] = 0.35 (Whatman No. 3); Amino-acid analysis[7]: Glu_1, His_1, Phe_1, Arg_1, Gly_1, $NH_{3\,1.2}$ (tryptophane was destroyed during hydrolysis). Leucineaminopeptidase completely hydrolyses II within a short period of time.

Fig. 1. *Synthesis and properties of the hexapeptide II*

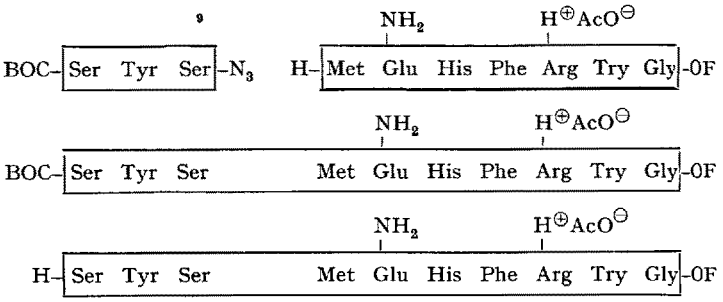
Z = carbobenzoxy. Strong underline denotes crystallinity of the compound. Condensations were performed with dicyclohexyl-carbodiimide[8].



III. Mp. 192°C (decomp.); $[\alpha]_D^{26} = -25.3° \pm 7°$ ($c = 1.108$ in dimethyl formamide); amino-acid analysis[12]: Met_1, Glu_1, His_1, Phe_1, Arg_1, Gly_1, $NH_{3\,1}$ (tryptophane was destroyed during hydrolysis).

Fig. 2. *Synthesis and Properties of the Heptapeptide III*

BOC- = *t*-Butyloxycarbonyl; condensation was performed with dicyclohexyl-carbodiimide[13].



V: amorphous, $R_f 45^{11} = 0.51$, $R_f 50^{10} = 0.47$, $R_f 54^{15} = 0.58$

Fig. 3. *Synthesis and properties of the decapeptide V*

significance, especially with respect to the melanophore stimulating properties of all three types of hormone. The peptides I, II, and III actually do elicit a very small MSH-activity which increases with the length of the peptide chain (I = 0.3×10^5, II = 2×10^5, and III = 2.8×10^5 U/g in the *in vitro* assay[11]), compared with α-MSH = 2×10^{10}, βb-MSH = 2×10^9 and αb-ACTH = 0.5×10^8 U/g). Hofmann *et al.* had already synthesized I as well as the octapeptide IV, the latter being active at a level of 7×10^5 U/g [12]. The heptapeptide III displays no ACTH-activity in the Sayers' assay[13] as found by Prof. Dr. W. Schuler in our biological laboratories.

Some of our synthetic peptides were kindly tested for CRF-activity at the Laboratoire de Chimie Biologique of the University of Paris with the *in vitro* assay of Saffran, Schally, and Benfey[14]. Dr. M. P. de Garilhe and Mlle Cl. Gros found that especially the heptapeptide (III) has a very high activity as compared with isolated, natural material. As the hexapeptide (II) was markedly less potent, we prepared the decapeptide (V): it proved to be active in the same range as III. These are the first synthetic peptides ever reported to have CRF-activity.

De Garilhe, Gros, Porath, and Lindner are simultaneously communicating the biological aspects of these

[4] Paper chromatography system No. 43 is: *t*-amylic alcohol/*iso*-propanol/water (100:40:55); system No. 45 is: *sec.*-butanol/3% solution of ammonia (100:44).

[5] S. Moore, D. H. Spackman, and W. H. Stein, Analyt. Chem. *30*, 1185, 1190 (1958). Our thanks are due to Prof. Dr. M. Brenner and Dr. R. Weber (Org.-chemische Anstalt der Universität Basel) for kindly doing the analyses.

[6] J. C. Sheehan and G. P. Hess, J. Amer. chem. Soc. *77*, 1067 (1955).

[7] Kindly furnished by Dr. B. Iselin of our Research Division.

[8] Paper chromatography system No. 50 is: *t*-amyl alcohol/isopropanol/triethylamine/veronal/water (100 ml:40 ml:0.8 ml:1.8 g: 50 ml); system No. 54 is: *sec.*-butanol/isopropanol/monochloroacetic acid/water (70 ml:10 ml:3 g:40 ml).

[9] We wish to thank Dr. C. H. Li, Hormone Research Institute, U. C., in Berkeley (California), for carrying out the assays.

[10] K. Hofmann, M. E. Woolner, G. Spühler, and E. T. Schwartz, J. Amer. chem. Soc. *80*, 1486 (1958). – K. Hofmann, Th. A. Thompson, and E. T. Schwartz, J. Amer. chem. Soc. *79*, 6087 (1957).

[11] M. A. Sayers, G. Sayers, and C. A. Woodbury, Endocrinology *42*, 379 (1948).

[12] M. Saffran, A. V. Schally, and B. G. Benfey, Endocrinology *57*, 399 (1957).

[13] M. P. de Garilhe, Cl. Gros, J. Porath, and E. B. Lindner, Exper. *16*, 414 (1960).

[14] K. Hofmann, W. Peckham, and A. Rheiner, J. Amer. chem. Soc. *78*, 238 (1956).

[15] H. Kappeler, detailed paper for Helv. chim. Acta in preparation.

findings[15]. We shall here only give a preliminary report on the syntheses of the compounds II, III, and V (Fig. 1–3); detailed papers are to appear elsewhere.

H. KAPPELER and R. SCHWYZER

Forschungslaboratorien der CIBA Aktiengesellschaft, Pharmazeutische Abteilung, Basel, June 1, 1960.

Zusammenfassung

Einige Peptide, strukturell ähnlich den gemeinsamen Aminosäuresequenzen der drei Hypophysenhormone ACTH, α- und β-MSH, wurden synthetisiert. Sie zeigen eine sehr schwache Wirkung auf die Melanophoren (V wurde nicht geprüft); das Heptapeptid (III) besitzt keine ACTH-Aktivität. Die drei Verbindungen II, III und V vergrössern die ACTH-Ausschüttung im *in vitro*-Versuch von SAFFRAN, SCHALLY und BENFEY[7] stark. Damit sind die ersten synthetischen Peptide mit dieser CRF-Aktivität gefunden.

Adrenaline-Noradrenaline Content of the Submaxillary Gland of the Cat

It has been suggested that some sympathicomimetic amines, e. g. tyramine, exert their effect by release of adrenaline-noradrenaline (BURN and RAND[1]). In the present experiments, the effect on the adrenaline-noradrenaline content of the submaxillary gland caused by infusion of tyramine has been studied.

Methods. Ten cats were used for the experiments. One series consisted of three normal cats. In a series of five cats, the effect of an intravenous infusion of tyramine was studied. In two cats, the superior cervical ganglion of one side was excised 3 weeks before the experiment.

In the acute experiment, the cats were given chloralose (80 mg/kg) after preliminary ether anesthesia. To facilitate a quick removal, the glands were dissected, leaving nerves, vessels, and secretory duct intact. The excised gland was immediately put into a cooled homogenizer containing 10 cm³ of ice – chilled 0.4 *N* perchloric acid. The homogenate was kept in a deep-freeze (– 20°C) until the estimation of the catechols was made one or two days later.

Estimation of adrenaline-noradrenaline[2]. The noradrenaline and adrenaline content of the homogenates was estimated according to the fluorometric method described by BERTLER, CARLSSON, and ROSENGREN[3]. The simplified extraction variant of the procedure was used. A slight error is admittedly introduced hereby, especially since the glands were not weighed, in order to reduce the time delay between removal and homogenization. The error introduced is, however, only of the order of 1%, even if it is assumed that the weight of an organ weighing about 1 g can be estimated by sight not better than within ± 0.5 g. The elution was made with 8 ml 1 *N* HCl and the estimation made on 2 ml of the eluate.

Further technical details are given below.

Results. The normal submaxillary gland was found to contain only small amounts of adrenaline in comparison with the amounts of noradrenaline. The two normal glands from a single animal were very similar in their noradrenaline content (Table), and therefore the effect of tyramine infusion or of ganglionectomy on the noradrenaline content could be studied on pairs of glands, using one gland as a control.

Infusion of tyramine was started after one gland had been removed and homogenized. A cannula was tied into the secretory duct of the remaining gland. Each animal was then given 150 mg of tyramine intravenously during approximately 1 h. At a constant rate of infusion, the secretory rate declines. In order to keep up a secretion the rate of infusion was increased intermittently. In spite of this, the gland can not be made to secrete for a long time. At the end of infusion, when the gland was removed, there was no secretion in any of the cases even though huge doses were given (up to 10 mg/min).

The infusion was found to lower the noradrenaline content of the gland to some 50% (Table).

Excision of the superior cervical ganglion caused a complete disappearence of noradrenaline, while adrenaline was found in seemingly normal quantities (Table).

Expressed in μg, the noradrenaline and, within brackets, the adrenaline content of the submaxillary gland of the cat.

Cat. No.	Procedure	Right gland	Left gland
1	Normal glands	0.71 (0.04)	0.71 (0.10)
2		0.78 (0)	0.74 (0.06)
3		0.69 (0)	0.66 (0)
		Mean value: 0.73 (0.01)	0.70 (0.05)
4	Right superior cervical ganglion excised 3	0 (0.06)	0.45 (0.07)
5	weeks earlier	0 (0.06)	1.24 (0.06)
		Mean value: 0 (0.06)	0.85 (0.07)
6	Left gland removed before and right gland	0.49 (0.06)	1.16 (0.04)
7	removed after infusion of tyramine	0.59 (0.02)	1.29 (0)
8		0.51 (0.02)	0.85 (0)
9		0.40 (0.07)	1.01 (0)
10		0.48 (0.05)	0.91 (0)
		Mean value: 0.49 (0.04)	1.04 (0.01)

[1] J. H. BURN and M. J. RAND, J. Physiol. *144*, 314 (1958).

[2] I am greatly indebted to Drs. Å. BERTLER, A. CARLSSON, and E. ROSENGREN for facilities to make the estimations.

[3] Å. BERTLER, A. CARLSSON, and E. ROSENGREN, Acta physiol. scand. *44*, 273 (1958).

[4] R. STRÖMBLAD, Acta physiol. scand. *36*, 154 (1956).

[5] B. C. R. STRÖMBLAD, Brit. J. Pharmacol., in press.

[6] N. EMMELIN and J. ENGSTRÖM, J. Physiol., in press.