MELANOCYTE STIMULATING ACTIVITIES OF THE o-NITROPHENYL SULFENYL DERIVATIVES OF ADRENOCORTICOTROPIN AND [5-GLUTAMINE] -α-MELANOTROPIN

by

J. Ramachandran

The Hormone Research Laboratory

University of California, San Francisco

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Summary. The o-nitrophenyl sulfenyl derivative of adrenocorticotropin in which the single tryptophan residue of the molecule is modified, is found to be fully active in stimulating amphibian melanophores. The melanophore stimulating activity of [5-glutamine] -α-melanotropin, a synthetic analog of α-melanotropin, is also found to be undiminished by chemical modification of the tryptophan residue.

Chemical modification of the single tryptophan residue in adrenocorticotropin (ACTH) by reaction with o-nitrophenyl sulfenyl chloride resulted in the loss of the lipolytic activity of the hormone on isolated rat fat cells (1). The o-nitrophenyl sulfenyl derivative of ACTH (NPS-ACTH) was found to be a potent inhibitor of the lipolytic action of ACTH on rat adipocytes. The effect of the chemical modification on another extra adrenal effect of ACTH, namely, the stimulation of amphibian melanophores, is presented in this communication. The preparation of the o-nitrophenyl sulfenyl derivative of [5-glutamine] -α-melanotropin (NPS-gln^5-α-MSH) (2), a synthetic analog of α-melanotropin (α-MSH), and its effect on frog melanophores are also presented.

Materials and Methods.

Highly purified ACTH from sheep pituitary glands was prepared in this laboratory (3). The preparation of NPS-ACTH has been described previously (1). [5-glutamine]-α-melanotropin (gln^5-α-MSH) was synthesized in this laboratory (2). o-Nitrophenyl sulfenyl chloride was purchased from Eastman Organic Chemicals.

Melanocyte stimulating activity was measured using isolated frog skins according to the methods of Shizume et al. (4) and Wright and Lerner (5). Rana pipiens, of both sexes, obtained from Dahl Biological Supplies, Emeryville, California, were used in this study. Frogs were sacrificed by decapitation and skins from each animal were prepared for reflectance studies as described by Shizume et al. (4). The reflectance of the skins was measured with a Photovolt photoelectric reflection meter (Model 610). All readings were taken with the skin immersed in 20 ml of solution in a 50 ml beaker. The original reading of the skin after soaking in Ringer's solution for 1 hr. was taken as its individual base line and the decrease in reflectance was measured from this. Readings were taken 1 hr. after the addition of the hormone. The decrease in reflectance is expressed as the percentage change of the initial value.

Preparation of NPS-gln^5-α-MSH.

Gln^5-α-MSH (20 mg) was dissolved in 1 cc of glacial acetic acid. o-Nitrophenyl sulfenyl chloride (20 mg) was added and the clear solution kept at room temperature for 4 hr. with occasional shaking. Anhydrous ether (10 cc) was added to precipitate the product. The precipitate was washed twice with ether by centrifugation. The product was dissolved in 1 cc of glacial acetic acid and precipitated again with ether. Finally the

precipitate was dissolved in 4 cc of 0.1 N acetic acid and lyophilized. NPS-gln^5-α-MSH was further purified by chromatography on a 25 x 1 cm column of carboxy methyl cellulose using a gradient of ammonium acetate as described for gln^5-α-MSH (2) and isolated in a yield of 12 mg. Electrophoresis on Whatman No. 3 paper in a pyridine acetate buffer, pH 3.7 (10 v/cm) for 4 hr. revealed a single yellow, ninhydrin positive, Ehrlich negative compact spot with a mobility of 0.54 x lysine. Gln^5-α-MSH was found to have a mobility of 0.6 x lysine under the same conditions. NPS-gln^5-α-MSH was found to be homogeneous by thin layer chromatography on silica gel in the solvent n-butanol-acetic acid-water (4:1:1 v/v). R_f 0.25; gln^5-α-MSH, R_f 0.14. The ultraviolet absorption spectrum of NPS-gln^5-α-MSH in 0.001 N HCl exhibited maxima at 282 mμ (ϵ = 16500) and 365 mμ (ϵ = 4000).

Results and Discussion.

The decrease in reflectance of isolated frog skins caused by ACTH, NPS-ACTH, gln^5-α-MSH and NPS-gln^5-α-MSH at different concentrations are given in Table I. It is apparent that both NPS-ACTH and NPS-gln^5-α-MSH are very potent melanocyte stimulating agents. In fact both the o-nitrophenyl sulfenyl derivatives appear to be more active than the parent molecules from which they are derived.

These results clearly show that the tryptophan residue of ACTH and α-MSH is not essential for the manisfestation of the melanocyte stimulating activity. In fact, modification of the tryptophan residue appears to have potentiated the ability to stimulate amphibian melanophores. Yajima and Kubo (6) found that substitution of D-tryptophan for L-tryptophan in the pentapeptide sequence his-phe-arg-trp-gly resulted in a 3-fold enhance-

Table I

MELANOCYTE STIMULATING ACTIVITIES OF ACTH, GLN^5-α-MSH AND THEIR o-NITROPHENYL SULFENYL DERIVATIVES

Hormone	Concentration ($\times 10^{-9}$M)	Per cent decrease in reflectance
None	-	3 ± 1 (6)*
ACTH:	0.570	36 ± 3 (10)
	1.710	59 ± 2 (10)
NPS-ACTH:	0.112	23 ± 2 (4)
	0.560	58 ± 6 (5)
	1.680	58 ± 5 (5)
Gln^5-α-MSH:	0.240	37 ± 3 (5)
	0.720	57 ± 4 (4)
NPS-gln^5-α-MSH:	0.132	44 ± 3 (4)
	0.395	66 ± 2 (4)

*Values are the mean ± S. E.; number of skins in parentheses.

ment of the melanocyte stimulating activity. NPS-ACTH, NPS-gln^5-α-MSH as well as the pentapeptide containing D-tryptophan are probably less susceptible to enzymic inactivation and this may account for the potentiation of the melanocyte stimulating activity observed with these derivatives.

The significance of the melanocyte stimulating activity of NPS-ACTH and its relationship to the action of NPS-ACTH on rat and rabbit adipose tissues are discussed in the accompanying communication.

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