DIVERGENT EFFECTS OF o-NITROPHENYL SULFENYL ACTH ON RAT AND RABBIT FAT CELL ADENYL CYCLASES

by

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Summary. The o-nitrophenyl sulfenyl derivative of ACTH (NPS-ACTH) is inactive in stimulating adenyl cyclase in ghosts isolated from rat epididymal fat cells. NPS-ACTH inhibits the stimulation of adenyl cyclase by ACTH in rat fat cell ghosts. On the other hand, NPS-ACTH is found to be as active as ACTH in stimulating adenyl cyclase in ghosts isolated from rabbit fat cells.

In a previous communication (1), we described the preparation of the o-nitrophenyl sulfenyl derivative of ACTH (NPS-ACTH) in which the single tryptophan residue of the molecule was modified. NPS-ACTH was shown to inhibit selectively the ACTH-stimulated lipolysis in cells isolated from rat epididymal adipose tissue. The effects of NPS-ACTH on the adenyl cyclase in ghosts isolated from rat and rabbit adipose tissues are presented in this report. Adenyl cyclase activity was measured by a new, simple procedure developed in this laboratory (2).

Materials and Methods.

Merck aluminum oxide (neutral, activity I, for column chromatography) was purchased from Brinkmann Instruments, Inc., Westbury, N. Y.:

FractionV bovine serum albumin from Armour Pharmaceutical Company; ATP (disodium salt), cyclic 3', 5'-AMP, theophylline, creatine phosphate and creatine kinase from Mann Research Laboratories.

ATP-α-^{32}P was purchased from International Chemical and Nuclear Corporation, Irvine, California. ^{3}H-cyclic 3', 5'-AMP was obtained from Schwarz BioResearch, Inc. Highly purified ACTH from sheep pituitaries was prepared in this laboratory (3).

Fat cell ghosts were prepared from isolated fat cells according to the procedure of Birnbaumer and Rodbell (4). Fat cells were obtained from the epididymal fat pads of Sprague-Dawley rats (180-200 g) or from perirenal adipose tissue of New Zealand-White male rabbits (3.5-4 kg) by the procedure of Rodbell (5).

Assay of Adenyl Cyclase.

The composition of the incubation mixture was as follows: 3.2 mM ATP-α-^{32}P (20 to 40 cpm per pmole), 5 mM $MgCl_2$, 10 mM theophylline, 0.1% albumin, 30 mM tris-HCl buffer pH 7.4, 0.2 mg per ml of creatine kinase (40-50 units/mg), 10 mM creatine phosphate and 0.2 mM $KHCO_3$ (present in the fat cell ghost preparation). The final volume of the assay mixture was 50 µl containing 10 µl of adenyl cyclase preparation (30-60 µg protein) and 5 µl of hormone. Incubations were started by the addition of the ghosts and maintained for 15 minutes at 37°. At the end of this period 250 µl of a solution containing 3 mM cyclic 3', 5'-AMP and 0.05 µc of ^{3}H cyclic 3', 5'-AMP in 10 mM tris-HCl buffer pH 7.4 was added and the reaction terminated by placing the tubes in boiling water for 3 minutes.

Cyclic 3', 5'-AMP was separated from ATP and other nucleotides by a new, simple method developed in this laboratory (2). The tubes were allow-

Table I

EFFECT OF ACTH AND NPS-ACTH ON RAT FAT CELL ADENYL CYCLASE

Adenyl cyclase was assayed as described under "Materials and Methods." Each reaction tube contained 43 μg ghosts. Values are the mean ± standard deviation.

Hormone	Concentration	Cyclic 3', 5'-AMP accumulated nano moles/15 min/mg protein
None	-	0.29 ± 0.07
ACTH:	4×10^{-7}M	0.41 ± 0.17
	1.2×10^{-6}M	0.85 ± 0.09
	3.6×10^{-6}M	1.22 ± 0.05
NPS-ACTH:	3×10^{-6}M	0.37 ± 0.19
	9×10^{-6}M	0.40 ± 0.06
	2.7×10^{-5}M	0.35 ± 0.01

ed to cool and 250 µl from each tube was applied on a dry column of neutral aluminum oxide (0.5 x 6 cm; prepared by pouring dry alumina into Pasteur pipets plugged with glass wool). Each column was washed with 3 ml of 10 mM tris-HCl buffer pH 7.4. The effluent was collected in a scintillation vial, mixed with 15 cc of scintillant and counted for ^{32}P and ^{3}H in a three channel packard liquid scintillation counter. ATP, ADP, AMP and P_i are completely retained on the alumina under these conditions and cyclic 3', 5'-AMP is eluted in 90% yield. The purity of the cyclic 3', 5'-AMP isolated from hydrous alumina columns was established by chromatography on Dowex 50-H^+ and Dowex 1-Cl ion exchange resins, thin layer chromatography on silica gel in two solvents, paper electrophoresis, Ba-Zn precipitation (6) and crystallization to constant specific activity.

Blanks were prepared by incubating the tubes without the ghosts and adding the ghost preparation just prior to placing in boiling water. All assays were performed in triplicate. Each experiment was repeated twice. The results of a representative experiment are given.

Protein was determined by the method of Lowry et al. (7) using bovine serum albumin as standard.

Results.

The effect of ACTH and NPS-ACTH on the adenyl cyclase activity of ghosts isolated from rat epididymal fat cells is presented in Table I. Adenyl cyclase activity is increased four-fold over basal activity by ACTH at a concentration of 3.6×10^{-6}M. The stimulation of adenyl cyclase activity is directly proportional to the log of the concentration of ACTH in the range 4×10^{-7}M - 3.6×10^{-6}M. On the other hand, NPS-ACTH does not stimulate adenyl cyclase activity even at a concentration of 2.75×10^{-5}M. High-

Table II

EFFECT OF NPS-ACTH ON THE STIMULATION OF RAT FAT CELL ADENYL CYCLASE BY ACTH

Conditions of incubation were the same as described under "Materials and Methods." Each reaction tube contained 60 μg ghost protein. Values are the mean ± standard deviation.

Concentration of ACTH	Cyclic 3', 5'-AMP accumulated nano moles/15 min/mg protein	
	without NPS-ACTH	with NPS-ACTH (4.5×10^{-5}M)
0	0.28 ± 0.13	0.38 ± 0.23
5×10^{-7}M	0.62 ± 0.05	0.30 ± 0.16
2×10^{-6}M	1.06 ± 0.29	0.21 ± 0.19

er concentrations of NPS-ACTH (9×10^{-5}M) failed to show any significant stimulation over basal activity.

That NPS-ACTH can inhibit the action of ACTH on ghost adenyl cyclase derived from rat adipose tissue is seen from the results shown in Table II. NPS-ACTH appears to be an effective inhibitor of ACTH at an ACTH: inhibitor ratio of 1:20.

The stimulation of adenyl cyclase activity in rabbit fat cell ghosts by ACTH and NPS-ACTH is shown in Table III. It is seen that both ACTH and NPS-ACTH produce a 10-15 fold stimulation over basal activity. It is evident that NPS-ACTH is at least as active as ACTH in stimulating adenyl cyclase in rabbit fat cell ghosts. In fact, at low concentrations NPS-ACTH produces a greater stimulation than a comparable concentration of ACTH.

Table III

STIMULATION OF RABBIT FAT CELL ADENYL CYCLASE BY ACTH AND NPS-ACTH

Adenyl cyclase in rabbit fat cell ghosts was assayed as described under "Materials and Methods." Each reaction tube contained 30 μg ghost protein. Values are the mean ± standard deviation.

Hormone	Concentration	Cyclic 3', 5'-AMP accumulated nano moles/15 min/mg protein
None		0.32 ± 0.03
ACTH:	1.4×10^{-7}M	0.50 ± 0.23
	3.5×10^{-7}M	2.71 ± 0.44
	8.75×10^{-7}M	4.38 ± 0.60
NPS-ACTH:	1.44×10^{-7}M	1.45 ± 0.16
	3.6×10^{-7}M	3.10 ± 0.56
	9.0×10^{-7}M	3.95 ± 0.52

Discussion.

The lipolytic action of ACTH is known to be mediated by cyclic 3', 5'-AMP formed by hormonal stimulation of adenyl cyclase activity (8). It is apparent that NPS-ACTH is unable to stimulate adenyl cyclase activity in ghosts isolated from rat fat cells. The results shown in Table II suggest that NPS-ACTH inhibits the lipolytic action of ACTH by blocking the ACTH-induced stimulation of adenyl cyclase. These results further imply that modification of the tryptophan residue in ACTH does not affect the ability of the hormone to bind to its receptor.

The stimulation of adenyl cyclase activity in rabbit fat cell ghosts by NPS-ACTH shows that there are striking differences between rabbit and rat adipose tissues. It is highly unlikely that the activity is due to the formation of ACTH from NPS-ACTH in rabbit fat cell ghosts in view of the fact that NPS-ACTH produces a 4-fold stimulation of adenyl cyclase activity at a concentration at which ACTH itself produces insignificant stimulation. The striking difference in the action of NPS-ACTH on rat fat cell ghosts as compared to rabbit fat cell ghosts is probably due to differences in the structure of the hormonal receptors in the two tissues.

Differences in the responsiveness of rat and rabbit adipose tissues to pituitary hormones are well known (9, 10). ACTH is found to be equally potent as a lipolytic agent in both rat and rabbit adipose tissues. The melanotropins (α-MSH and β-MSH) are practically inactive in rat adipose tissue but are the most potent lipolytic agents in rabbit adipose tissue. Several synthetic peptides corresponding to various segments of the N-terminal decapeptide sequence of ACTH (which contains the amino acid sequence common to ACTH, α-MSH and β-MSH) were found to be moderately active

lipolytic agents in rabbit adipose tissue but completely inactive in rat adipose tissue (9). In all these cases, the lipolytic activity on rabbit adipose tissue was found to correlate well with the melanocyte-stimulating activity.

The correlation between lipolytic activity in the rabbit and the melanocyte-stimulating activity in amphibians appears to hold in the case of NPS-ACTH also. NPS-ACTH was found to be at least as active as ACTH in stimulating amphibian melanophores (11).

It seems likely that the hormonal receptors in rabbit fat cells are similar if not identical to the receptors on amphibian melanophores and quite different from the receptors in rat adipose tissue. The receptors in rat fat cells may be classified as ACTH type receptors and appear to have stringent structural requirements for favorable interaction with the hormone. The rabbit fat cell receptors appear to be MSH type receptors which are less selective. NPS-ACTH is seen to be a useful tool in distinguishing ACTH receptors from MSH receptors.

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