Vol. 88, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
June 13, 1979 Pages 1153-1157

THE STEROIDOGENIC ACTIVITY OF BIOTINYLCORTICOTROPIN-AVIDIN COMPLEXES

Frances M. Finn, Joon Ja Kim and Klaus Hofmann

Protein Research Laboratory
University of Pittsburgh
School of Medicine
Pittsburgh, Pennsylvania 15261

Received May 7, 1979

The steroidogenic activity of complexes of [biocytin^{25}]-corticotropin$_{1-25}$ amide (biotinylcorticotropin) with avidin (1:1), (4:1) and (1:10) was compared to that of biotinylcorticotropin using isolated rat adrenal cells. Parallel log-dose responses and maximal stimulation were observed with all these materials. The 1:1 complex is approximately 25% as active as biotinylcorticotropin (ED_{50} 22.5 and 5.6 nM respectively). The 4:1 complex is more active than the 1:1 complex (ED_{50} 9.0 nM). The presence of an excess of avidin (1:10 complex) does not interfere with the ability of biotinylcorticotropin to stimulate steroidogenesis (ED_{50} 18.0 nM). It is concluded that biotinylcorticotropin attached to avidin binds specifically to receptors on the rat adrenal cell and elicits its biological response. These results indicate that biotinylcorticotropin can be noninvasively labeled with ^{125}I-avidin.

Recently we described the synthesis of [biocytin^{25}]-corticotropin$_{1-25}$ amide (biotinylcorticotropin) (Fig. 1) and have shown that its ability to stimulate corticoid production in bovine adrenal cortical cells is identical to that of corticotropin$_{1-24}$ (1,2). The biotinylated hormone was attached to Sepharose-immobilized avidin to form affinity columns whose utility for isolation of solubilized corticotropin receptors is being explored. Progress in the comprehension of corticotropin-receptor interactions has been slow largely because convenient techniques for labeling corticotropins to high specific activity without interference with their biological activity are not readily available. Some time ago (3) we synthesized [^{14}C-Phe^{7}] corticotropin$_{1-20}$ amide and used this material (sp. act. 0.13 mCi/mmole) for binding and displacement studies of corticotropin analogs and fragments on bovine adrenal cortical plasma membranes. However, the specific activity of the labeled peptide

0006-291X/79/111153-05$01.00/0

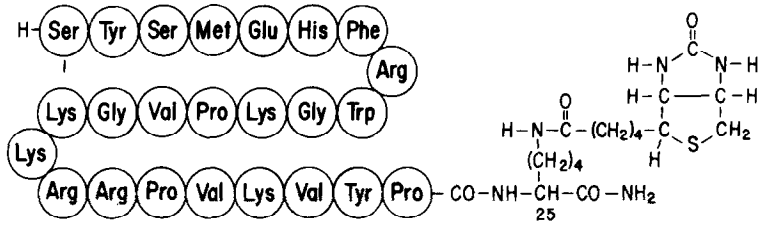

Fig. 1: The structure of [biocytin^{25}]-corticotropin$_{1-25}$ amide

was too low for exploration of high affinity corticotropin binding sites whose existence was postulated (4). Biologically fully active tritium labeled corticotropin$_{1-39}$ and corticotropin$_{1-24}$ of high specific activity have been described (5,6,) but are difficult to prepare. In this connection it occured to us that attachment of ^{125}I-labeled avidin (7) to biotinylcorticotropin may provide a convenient, noninvasive route to labeled corticotropin$_{1-24}$. Since the attachment of the bulky avidin molecule (mol. wt. 68,000) to corticotropin$_{1-24}$ may interfere with its binding to receptors we have determined the steroidogenic activity of a number of biotinylcorticotropin-avidin complexes using isolated rat adrenal cells.

Experimental. [Biocytin^{25}]-corticotropin$_{1-25}$amide (Biotinylcorticotropin) was prepared in our laboratory. Chromatographically purified collagenase (type VI) and avidin were from Sigma. Glassware used in the preparation or assay of adrenal cells was siliconized (Siliclad). Water was distilled from potassium permanganate. Rat adrenal cells were prepared essentially as described by Moyle et al. (8) and tested for viability by trypan blue exclusion (9). Incubations were carried out as described by Finn et al., (10). Fluorescence was measured with an Aminco fluorocolorimeter 45 minutes following addition of the H_2SO_4/ethanol reagent (7/3,v/v). Formation of emulsions during the dichloromethane extractions was minimized by addition of 0.2 ml of saturated ammonium acetate. Maximum response corresponds approximately to a 4 fold stimulation above base level. Cell suspensions contained 1.5 to 2.0x 10^5 cells/ml and the results presented were normalized to a concentration of 1x10^6 cells/ml.

Results and Discussion

The avidin molecule is composed of four identical subunits, each containing a single binding site for biotin. Because of this

tetrameric arrangement it is possible to obtain complexes in which the ratio of biotinylhormone/avidin may vary from 1:1 to 4:1. The crosslinking ability of complexes containing more than one hormone molecule can thus be assessed. Using the highly sensitive rat adrenal assay of Sayers (11) we have compared the steroidogenic activity of biotinylcorticotropin (standard) to that of hormone-avidin complexes in which the ratio of hormone to avidin was 1:1, 4:1 and 1:10 respectively. All these materials elicit maximum steroidogenesis and the log dose response curves are parallel (Fig. 2). The 1:1 complex is approximately 25% as active as biotinyl corticotropin (ED_{50} 22.5 and 5.6 nM respectively). The 4:1 complex is more active than the 1:1 complex (ED_{50} 9.0 nM). The presence

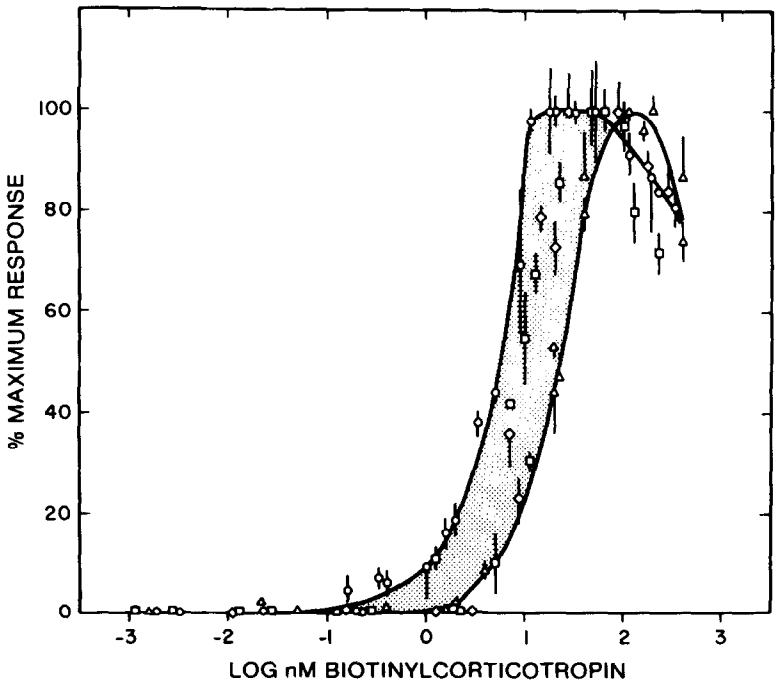

Fig. 2: The steroidogenic activity of biotinylcorticotropin and of biotinylcorticotropin-avidin complexes on rat adrenal cells. ○ Biotinylcorticotropin: △ biotinylcorticotropin:avidin 1:1; □ biotinylcorticotropin:avidin 4:1; ◇ biotinylcorticotropin:avidin 1:10. Each point is the average of triplicate assays and the curves represent a composite of assays on several cell preparations. Vertical bars represent ± S. E. M.

of an excess of avidin (1:10 complex) does not interfere with the ability of biotinylcorticotropin to stimulate steroidogenesis (ED_{50} 18.0 nM). We have reported (1) that the ability of biotinylcorticotropin to stimulate steroidogenesis in bovine adrenal cortical cells is identical to that of corticotropin $_{1-24}$; this result has been confirmed with rat adrenal cells (data not shown). The results presented in this communication show conclusively that biotinylcorticotropin-avidin complexes exhibit the ability to stimulate steroidogenesis in isolated rat adrenal cells. Although the ED_{50} of the 1:1 complex is higher than that of the free hormone the conclusion is inescapable that corticotropin attached to avidin binds to the specific receptors on the cell and elicits its biological response. The lower activity of the complexes vis a vis biotinylcorticotropin argues against the possibility that the hormone-avidin complex dissociates and that the observed activity is that of the liberated hormone. In addition the strong biotinylcorticotropin-avidin bond ($K_D 10^{-15}$ M) is not likely to dissociate under the conditions of the cell assays. As concerns destruction of avidin due to proteolysis it is well known that this unusual protein resists the action of proteolytic enzymes and passes unaltered through the alimentary canal of rats.

Based on the reported results it would appear that the attachment of ^{125}I-labeled avidin to biotinylcorticotropin offers a promising approach to the labeling of this hormone. Recent experiments with biotinylinsulin-^{125}I-avidin complexes (7) support this conclusion.

Acknowledgment Supported by Grant AM 01128 from the USPHS

References

1. Hofmann, K., and Kiso, Y. (1976) Proc. Natl. Acad. Sci., USA 73, 3516-3518.

2. Hofmann, K., Finn, F. M., and Kiso, Y. (1978) J. Amer. Chem. Soc. 100, 3585-3590.
3. Moroder, L., and Hofmann, K. (1970) J. Med. Chem. 13, 839-843.
4. Lefkowitz, R. J., Roth, J., Pricer, W., and Pastan, I. (1970) Proc. Natl. Acad. Sci. USA 65, 745-752.
5. Brundish, D. E., and Wade, R. (1973) J. Chem Soc. Perkin Trans. 1, 2875-2879.
6. Brundish, D. E., and Wade, R. (1977) Biochem. J. 165, 169-171.
7. Abstracts 61st meeting of the Endocrine Soc.
8. Moyle, W. R., Kong, Y. C., and Ramachandran, J. (1973) J. Biol. Chem. 248, 2409-2417.
9. Medzihradsky, F., and Marks, M. J. (1975) Biochem. Med. 13, 164-177.
10. Finn, F. M., Johns, P. A., Nishi, N., and Hofmann, K. (1976) J. Biol. Chem. 251, 3576-3585.
11. Sayers, G., Swallow, R. L., and Giordano, N. D. (1971) Endocrinology 88, 1063-1068.