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Inhibition of ACTH release by peptide hormones: Molecular mechanisms and possible role as anti-stre

12. PERSONAL AUTHOR(S): Terry Reisine

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The major objective of this proposal was to investigate whether somatostatin (SRIF) may be a non-steroid inhibitor of ACTH release from the anterior pituitary. The goals of the proposal were to; 1)investigate the structure of the SRIF receptor; 2) examine the effects of glucocorticoids on the expression of SRIF receptors in the pituitary; and 3)determine the physical differences between functionally distinct subtypes of SRIF receptors in the pituitary. In the past year, we showed that the pituitary and brain SRIF receptor is a glycoprotein of 55 to 60 kilodaltons. Furthermore, we were able to purify the SRIF receptor from brain by affinity chromatography. Presently we are attempting to sequence the receptor in order to attempt to clone the gene coding for this protein. In addition, we showed that glucocorticoids downregulate SRIF receptors in the anterior pituitary cell line AtT-20, which consists of a homogeneous population of ACTH secreting cells. Interestingly, some of the biological actions of SRIF on AtT-20 cells (inhibition of adenylate cyclase activity) are not affected by glucocorticoid treatment. Finally, we have observed that minor structural differences in the SRIF receptor appear to be responsible for the vastly different functional properties of the SRIF receptor subtypes in the pituitary. Attempts are under way to establish the basis of these minor structural differences.

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DISTRIBUTION STATEMENT A

ANNUAL REPORT ON CONTRACT ONR N00014-88-K-0048
R and T Code 4414807

PRINCIPAL INVESTIGATOR Terry Reisine

CONTRACT TITLE: Inhibition of ACTH release by peptide hormones: Molecular mechanisms and possible role as anti-stress factors.

Contract Period: Nov. 1, 1987 to Oct, 30, 1988.

INTRODUCTION AND RESEARCH OBJECTIVES: The major objective of the research proposal was the identification of non-steriodal factors which inhibit ACTH release and may be useful in the treatment of stress. Previously, it was reported that the hypothalamic peptide somatostatin (SRIF) could inhibit CRF stimulated ACTH release from a tumor cell line of the anterior pituitary, AtT-20, which consists of a homogeneous population of ACTH secreting cells. While administration of SRIF to non-stressed, humans failed to alter plasma ACTH or cortisol levels, patients with adrenal insufficiency did respond to SRIF with a lowering of ACTH release. This latter finding, may indicate that SRIF has some role in the suppression of ACTH secretion in humans but that under normal conditions, glucocorticoids downregulate or diminish the effectiveness of SRIF in regulating ACTH secretion. In fact, several studies have suggested that glucocorticoids downregulate SRIF receptors in the anterior pituitary. Furthermore, Lamberts et al (Endocrinol. 118:2188, 1986), showed that SRIF could inhibit CRF stimulated ACTH release from anterior pituitary cells in culture grown in the presence of glucocorticoid antagonists such as RU 486. Thus, a major aspect of the proposal was to determine the mechanisms by which SRIF may regulate ACTH release and whether this effect was in fact tonically suppressed by glucocorticoids.

Three aspects of the proposal which have progressed since the start of funding are; 1) the structural analysis of the SRIF receptor, 2) the effect of glucocorticoid treatment on SRIF receptor expression; and 3) the structural analysis of SRIF receptor subtypes.

PROGRESS REPORT;

1. Physical properties of SRIF receptors: Concerning the structural analysis of the SRIF receptor, we have employed several approaches to investigate the physical properties of SRIF receptors in AtT-20 cells, rat anterior pituitary and brain. Using photocrosslinking techniques, SRIF receptors can be covalently labeled with the stable SRIF analogue [125I] CGP 23996 (Thermos and Reisine, Molecular Pharmacology, 33:370-377; Thermos et al. in press). [125I] CGP 23996 binds to SRIF receptors in membranes of AtT-20 cells, anterior pituitary and brain in a saturable and specific manner (Mahy et al., in press). With the photoactivated crosslinking agent HSAB, it was possible to covalently link [125I] CGP 23996 to the SRIF receptor in AtT-20 and GH_3 cells as well as rat anterior pituitary and brain (Thermos and Reisine, Mol. Pharmacol. 33:370; Thermos et al. in press).The binding could be blocked completely by SRIF but not by peptides which do not directly interact with the SRIF receptor. The size of the SRIF receptor labeled in these tissues is between 55 to 60 kilodaltons. The size and charge of the labeled proteins as assessed by two-dimensional polyacrylamide gel electrophoresis (PAGE) in all of these tissues is similar. The SRIF receptor does not appear to contain disulfide bridges since reducing agents do not alter its migration in SDS-gels. The receptor

appears to be a glycoprotein since it is able to bind selectively to wheat germ agglutinin. The sugar moiety of the receptor may be responsible for heterogeneities in the structure and function of the receptor in various tissues.

To further examine the physical properties of the SRIF receptor, we solubilized and purified it from brain using affinity chromatography (He et al., in press). The purified receptor is 60 kilodaltons in size and can be selectively labeled by [125I] CGP 23996. The purified protein is essentially homogeneous since iodination of the eluate from the SRIF affinity column with Bolton-Hunter reagent revealed that 95% of the radioactivity is present in the 60 kilodalton protein. Presently, we are attempting to obtain partial sequence information of the purified SRIF receptor in order to develop procedures for cloning the gene coding for this protein.

2. Effect of glucocorticoids on SRIF receptor expression: Concerning the effects of glucocorticoids on SRIF receptors, treatment of AtT-20 cells with dexamethasone decreased [125I] CGP 23996 binding to AtT-20 cell membranes by over 50%. The effect was dependent on the concentration of dexamethasone used with significant decreases occurring with 1 nM of the steroid and maximal effects with 100 nM. The decrease in binding was also dependent on the time of dexamethasone treatment with significant decreases seen after only 4 hr of pretreatment. The steroid antagonist RU 486 blocked the effects of dexamethasone on AtT-20 cells. Treatment of AtT-20 cells with high concentrations of other steroids such as testosterone and estrogen did not alter [125I] CGP 23996 binding to the AtT-20 cells SRIF receptor. Interestingly, glucocorticoid treatment did not affect the ability of SRIF to inhibit adenylate cyclase activity in AtT-20 cell membranes. This finding may indicate that there are either "spare" SRIF receptors or subpopulations of receptors, some of which are not coupled to adenylate cyclase. Future studies will determine whether dexamethasone affects SRIF receptors in rat anterior pituitary cells in culture and alters other the functional characteristics of SRIF receptors such as their coupling to ACTH release.

3. Structural analysis of SRIF receptor subtypes: SRIF receptors in AtT-20 cells and the growth hormone secreting tumor cell line, GH_3, are functionally distinct. The receptors in AtT-20 cells have higher affinity for somatostatin-28 than somatostatin-14 and desensitize following agonist treatment (Thermos and Reisine, Mol. Pharmacol. 33:370; Mahy et al., 1988). The SRIF receptors in GH_3 cells have higher affinity for somatostatin-14 than somatostatin-28 and do not desensitize (Thermos and Reisine, Mol Pharmacol. 33:370). Using photocrosslinking techniques, we covalently labeled the SRIF receptors in both cell lines with [125I] CGP 23996. The size, charge and peptide maps of the receptors, as assessed by SDS-PAGE were identical. However, analysis of the peptide maps by two-dimensional PAGE revealed small variations in the properties of the receptor subtypes. Future studies will investigate whether these small structural variations in the SRIF receptor subtypes are due to variations in sugar composition or amino acid sequence.

EXPENDITURES

Supplies: expenditures normal

Equipment: ONR funds were used to purchase a gamma counter which was requested in the original proposal.

Travel: Travel funds were used by the PI to attend the Soc. for Neuroscience Meeting in Toronto.

Personnel: Henry Wang: Research Assistant (100% support).

Women or minorities- 0; Non-citizens - 1.

Publications

Abstracts:

Thermos, K., He. H.T., Margolis, N. and Reisine, T.: Biochemical properties of brain Somatostatin receptors. Soc. Neurosci. Abst 14:14, 1988.

Manuscripts:

Mahy, N., Woolkalis, M., Manning, D. and Reisine, T.: Characterization of somatostatin desensitization in the pituitary tumor cell line AtT-20. J. Pharmacol Expt. Therap. 247:390-396, 1988.

Reisine, T.: Phorbol esters and corticotropin releasing factor stimulate calcium influx in the anterior pituitary tumor cell line AtT-20 through different intracellular sites of action. J. Pharmacol. Expt. Therap. (in press).

He, H.T., Johnson, K., Thermos, K. and Reisine, T.: Purification of a putative brain somatostatin receptor. Proc. Natl. Acad. Sci. (in press).

Thermos, K., He, H.T., Wang, H., Margolis, N. and Reisine, T.: Biochemical properties of brain somatostatin receptors. Neuroscience (in press).

2ND YEAR WORK PLAN: The objectives of year 2 of funding are 1) to obtain partial sequence information of the purified SRIF receptor so as to begin cloning of the gene coding for this protein; 2) submit the studies on the glucocorticoid regulation of the SRIF receptor for publication and to determine whether such regulation occurs in normal anterior pituitary corticotrophs; and 3) to determine what are the structural differences in SRIF receptor subtypes in AtT-20 and GH_3 cells.

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