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

12. PERSONAL AUTHOR(S)
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The major objectives ot this proposal were to investigate the biochemical properties of somatostatin (SRIF) receptors. We were able to solublize the receptor in an active form and have shown using immunoprecipitation procedures that the receptor is coupled to subtypes of G_i and G_o. The receptor contains sialic acid residues involved in promoting agonist binding. We have developed antibodies against the receptor. The antibodies selectively recognize the receptor by immunoblotting and can specifically immunoprecipitate the receptor from AtT-20 cells. The antibodies are being used to further investiate the physical properties of the receptor and to clone cDNA encoding the receptor from an expression library generated from AtT-20 cells. We have shown that subtypes of SRIF receptors exist and have developed selective agonists at each receptor subtype. The receptors have different distributions in brain, have distinct pharmacological and biochemical characteristics, can be differentially regulated and mediate distinct physiological actions of SRIF in brain and peripheral tissues. Attempts are underway to identify the physical basis for the functional differences in these receptor subtypes.

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FINAL REPORT ON CONTRACT ONR N00014-88-K-0048

Principle Investigator: Dr. Terry Reisine

Contract Title: Inhibition of ACTH release by peptide hormones: Molecular mecnanisms and possible role as anti-stress factors.

Contract Period: July 1, 1988 to Oct. 31, 1990.

Research Objective: The major goal of the proposal was to investigate the mechanisms by which somatostatin (SRIF) inhibits the release of adrenocorticotropin (ACTH) in order to access whether it may be useful as a non-steriodal anti-stress factor. We proposed to investigate the structure of the SRIF receptor, and to determine whether subtypes of SRIF receptor exist.

Overall, these specific aims have been completed during the granting period.

Progress Report:

1)Physical Properties of SRIF receptors: To characterize the properties of SRIF receptors, we developed procedures to solubilize the receptor in an active form using the detergent CHAPS (He et al., 1990). The soluble receptor exhibited the same pharmacological specifity as the membrane bound receptor and maintained its coupling with GTP binding regulatory (G) proteins. Solubilization of the receptor has allowed us to identify properties of the receptor that could not be analyzed using previous procedures. We were able to identify the G proteins coupled to the receptor (He et al. 1990; Law et al., in press). This was accomplished by employing antibodies against different subtypes of the alpha subunits of G proteins (G_a) to immunoprecipitate SRIF receptor/G_a complexes. The results of these studies have shown that SRIF receptors are coupled to G_{ia1}, G_{ia3} and G_{oa} but not to G_{ia2}. Furthermore, we were able to characterize the carbohydrate structure of the receptor by lectin affinity chromatography and through the use of glycolytic enzymes (Rens-Domiano and Reisine, in press). The results of these studies have shown that sialic acid residues are associated with SRIF receptors and are necessary for the binding of agonists to the receptor. In fact, preliminary studies suggest that sialic acid residues may be localized near the ligand binding site of the receptor to promote agonist binding. This characteristic is unique among all neurotransmitter and hormone receptors. With the solublized receptor we have now been able to generate antibodies against the receptor (Theveniau et al., in press). The antibodies specifically recognize the receptor by immunoblotting and selectively immunoprecipitate the receptor. We are now using the antibodies to screen an AtT-20 cell cDNA library for clones expressing the receptor. These studies may allow us to clone the gene encoding the SRIF receptor.

2.Subtypes of SRIF receptors. Recently we have demonstrated that subtypes of SRIF receptors exist in mammalian cells (Raynor and Reisine, 1989; Martin et al., 1991, Raynor et al., in press; Raynor and Reisine, in press). One receptor has high affinity for SRIF and a potent SRIF agonist MK 678 and is refered to as the $SRIF_1$ receptor. The other receptor ($SRIF_2$ receptor) has high affinity for a different SRIF agonist CGP 23996 but has no affinity for MK 678. We have now identified a series of structural analogs of MK 678 or CGP 23996 that are highly selective at each receptor subtype. The receptors have different distributions in brain and are coupled to different cellular effector systems. The $SRIF_1$ receptor is coupled to K^+ channels, the $SRIF_2$ receptor is coupled to adenylyl cyclase and both receptor subtypes are coupled to Ca^{++} channels. The receptor subtypes can also be differentially regulated. In previous studies we were able to solublize the $SRIF_1$ receptor (He et al., 1990). Under those same conditions, the $SRIF_2$ receptor remains membrane bound. Presently, we are developing procedures to solubilze both receptor so as to physically separate them so as to characterize their properties separately. The receptor subtypes appear to mediate distinct physiological actions an behaviors of SRIF. We have

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been able to inject MK 678 into the nucleus accumbens and stimulate locomotor activity (Raynor et al., 1990). SRIF has a similar effect but CGP 23996 has no effect, indicating that the locomotor enhancing effects of SRIF are selectively mediated by $SRIF_1$ receptors. We have now begun studies to investigate the cognitive effects of SRIF. We can deplete brain SRIF levels with the drug cysteamine and induce cogntive impairments, as measured in the eight arm radial maze test. The cognitive impairments can be completely reversed by icv injection of MK 678. We are presently testing CGP 23996 in the same behavioral paradigm to determine whether the cognitive enhancing effects of SRIF are also selectively mediated by $SRIF_1$ receptors.

Inventions: None

Personnel: Henry Wang, Karen Raynor, Susan Law.
Women or minorities-2, Non-citizens-1.

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Awards/Fellowships: None.

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