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Receptors for Insulin and CCK in the Acinar Pancreas: Relationship to Hormone Action

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I. Introduction

The pancreatic acinar cell has been a model for studying the various ultrastructural events involved in the synthesis, packaging, and release of secretory proteins. Secretory proteins are synthesized on membrane-bound ribosomes, sequestered within the lumen of the endoplasmic reticulum (ER), transported through the Golgi, packaged into granules, moved to the cell membrane, and then released by exocytosis (Case, 1978; Palade, 1975; Scheele, 1980). Subsequently, this model has been used (with some cell-specific modifications) to explain the secretory processes of a large number of endocrine, exocrine, and blood cells.

In contrast, the biochemical events involved in the control of pancreatic secretion are less well understood. *In vivo*, the major regulation of the exocrine pancreas is via polypeptide hormones and cholinergic neurons. Two recent developments for studying acini *in vitro* have contributed to an understanding of the regulation of pancreatic acinar cell function. First, hormone-sensitive prepara-

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tions of pancreatic acini have been developed. Second, biologically active, radiolabeled hormones have been prepared. This article will survey the recent studies carried out in our laboratory and other laboratories to probe the receptors and mechanism of action of two major polypeptide hormone regulators of the exocrine pancreas, cholecystokinin (CCK), and insulin.

II. Isolated Pancreatic Acini for the Study of Exocrine Function

Amsterdam and Jamieson (1972) were the first to devise a procedure for preparing isolated pancreatic acinar cells which employed digestion of the pancreas with collagenase and chymotrypsin, chelation of divalent cations with EDTA, and mechanical shearing. Similar preparations have now been used for studies of acinar cell hormone receptors, ion fluxes, and cyclic nucleotide levels (Christophe *et al.*, 1976a,b; Gardner *et al.*, 1975; Kondo and Schulz, 1976; Williams, 1977; Williams *et al.*, 1976). The ability of secretagogues to induce $^{45}Ca^{2+}$ efflux in isolated cells suggests that both hormone receptors and the initial steps in stimulus–secretion coupling are intact. In most investigations, however, the measurement of enzyme secretion (a distal event) by isolated acinar cells has been difficult. For example, amylase release from the perfused rat pancreas is increased 8- to 20-fold by both acetylcholine and CCK (Kanno, 1972), whereas it is increased only 2-fold or less from isolated rat acinar cells (Kondo and Schulz, 1976).

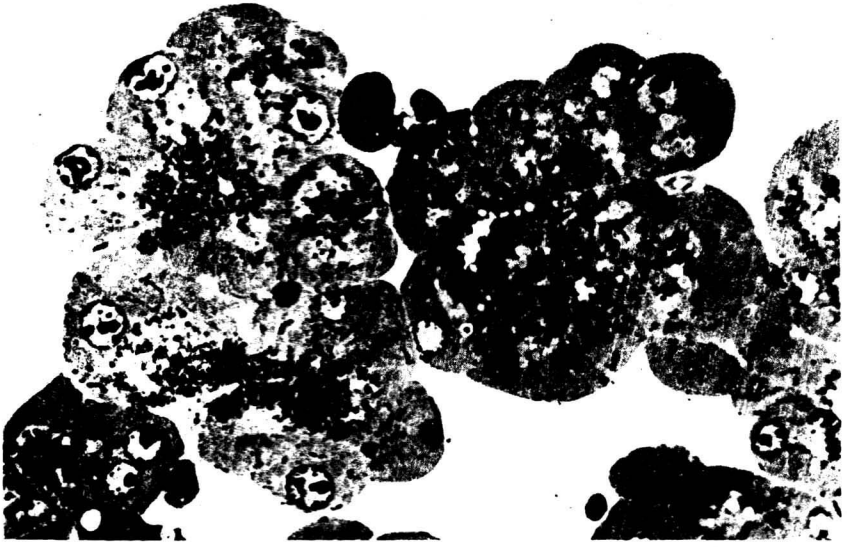

FIG. 1. Light micrograph of isolated mouse pancreatic acini.

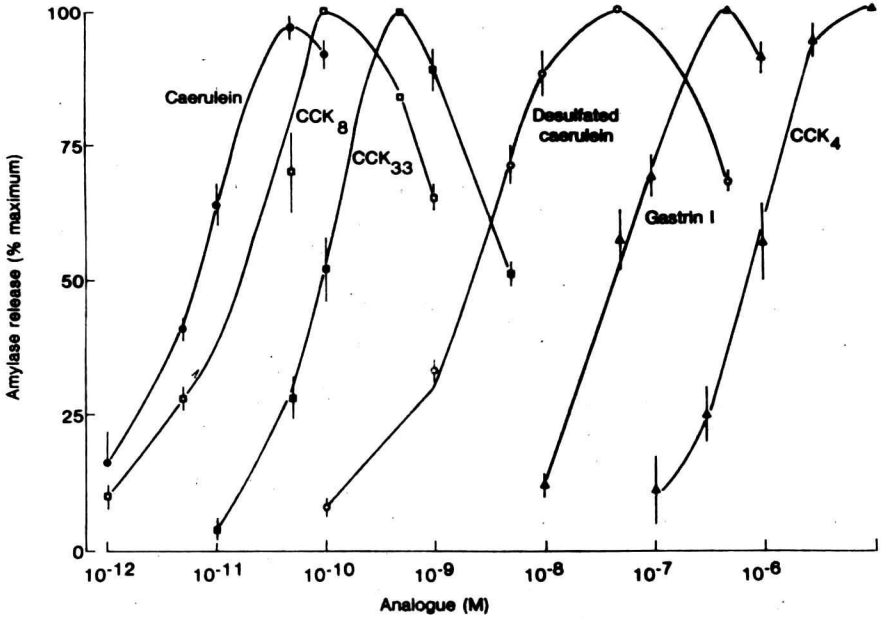

FIG. 2. Dose–response relationship for amylase release from isolated rat pancreatic acini induced by CCK and its analogs. In each case, basal release was subtracted and secretagogue-induced release was calculated as percentage of maximal release. All analogs induced a similar maximal release. (From Williams *et al.*, 1981.)

When the dissociation procedure is modified to produce isolated acini, a considerably improved secretory response is observed (Peikin *et al.*, 1978; Schultz *et al.*, 1980; Williams *et al.*, 1978). Isolated acini are prepared in a manner similar to that for isolated cells but without the calcium chelation step necessary to break junctional complexes (Amsterdam and Jamieson, 1974). They consist of groups of acinar (and occasionally centroacinar) cells arranged around an intact lumen (Fig. 1). Ultrastructural evaluation reveals that the tight junctions connecting adjacent acinar cells are maintained along with the microvilli and their underlying microfilament network located at the apical border of the acinar cell (Schultz *et al.*, 1980; Williams *et al.*, 1978). Since isolated acini can be studied as a homogeneous suspension and have their basolateral plasma membrane exposed to the incubation medium, they possess all the advantages of the isolated cells. Moreover, it is possible to measure and correlate enzyme release with other cellular functions, including occupancy of membrane receptors (Williams, 1980). Isolated pancreatic acini have proven especially useful in evaluating the complex dose–response curves for secretagogues such as the neurotransmitter ACh and the hormone CCK (Fig. 2). Isolated acini have also been utilized to study secretagogue inhibitors, including atropine for the cho-

linergic agents and dibutyryl cyclic GMP for CCK (Peitkin *et al.*, 1979; Williams *et al.*, 1978).

III. Cholecystokinin

A. Background

Cholecystokinin (CCK) which was originally isolated from the porcine intestine based on its ability to stimulate both gallbladder contraction and pancreatic secretion, is a straight chain 33 amino acid peptide (CCK_{33}) with an amidated C-terminus (Jorpes and Mutt, 1973; Mutt and Jorpes, 1971). A prominent feature of CCK is the presence of a sulfated tyrosine at position 27 (7 residues from the C-terminus) (Table I). The C-terminal octapeptide (CCK_8) possesses a high degree of biological activity and is, in fact, more potent than CCK_{33} (Mutt and Jorpes, 1968; Ondetti *et al.*, 1970a,b). The unsulfated octapeptide, however, has only 1/150 of the activity of the sulfated form (Gardner *et al.*, 1975; Ondetti *et al.*, 1970a; Williams *et al.*, 1981). The C-terminal tetrapeptide appears to contain all the biological activity of CCK although it is 30,000-fold weaker than the octapeptide whereas the C-terminal tripeptide or the deamidated tetrapeptide has no activity (Morley *et al.*, 1965; Rajh *et al.*, 1980; Sankaran *et al.*, 1981a). CCK is also structurally similar to the frog skin decapeptide, caerulein, which acts like CCK_8 (Anastasi *et al.*, 1968).

Another notable feature of the C-terminal portion of CCK is its homology to the similar portion of the gastrin molecule. The C-terminal pentapeptides of both molecules are identical, and both CCK and some forms of gastrin contain a sulfated tyrosine, although not in the identical position (Table I). Gastrin, however, has only weak effects on acinar cells (Fig. 2). Separate CCK and gastrin-like molecules are only found in higher animals, such as reptiles, birds, and mammals (Larsson and Rehfeld, 1977), but not lower animals. The basic biological activity of CCK is contained in the C-terminal tetrapeptide amide (which is shared with gastrin) whereas the additional amino acids are essential to increase specificity for pancreas and gallbladder.

B. Effects on Acinar Cells

Cholecystokinin has a number of actions on pancreatic acinar cells that have been demonstrated both *in vivo* and *in vitro* (Table II). Injection of CCK *in vivo* leads to the release of zymogen granule contents into the acinar lumen. In some species, especially rodents, CCK also stimulates the production of a Cl^--rich pancreatic juice such that the secreted enzymes pass via the pancreatic ducts to the intestine (Case, 1978). In some other species, such as the cat, fluid secretion

TABLE I

AMINO ACID SEQUENCE OF CHOLECYSTOKININ AND GASTRIN

Cholecystokinin$_{33}$	Lys -Ala -Pro -Ser -Gly -Arg -Val - Ser -Met -Ile -Lys -Asn -Leu -Glu -Ser - SO_3H Leu -Asp -Pro -Ser -His -Arg -Ile -Ser -Asp -Arg -Asp -Tyr -Met -Gly -Trp -Met -Asp -Phe -NH_2
Gastrin$_{17}$	Glp -Gly -Pro -Trp -Met -Glu -Glu -Glu -Glu -Glu -Ala -Tyr[a] -Gly -Trp -Met -Asp -Phe -NH_2

[a]Gastrin exists in two forms, I (nonsulfated tyrosine) and II (sulfated tyrosine). Glp, pyroglutamic acid.

TABLE II
EFFECTS OF CHOLECYSTOKININ ON PANCREATIC ACINAR CELLS

1. Zymogen synthesis and secretion
2. Secretion of Cl^--rich pancreatic juice
3. Pancreatic hypertrophy and hyperplasia
4. Increased glucose and amino acid utilization and oxygen consumption

is bicarbonate rich and requires stimulation by the hormone secretin. CCK *in vivo* also accelerates the synthesis of digestive enzymes, indicating coordinated stimulation of zymogen synthesis and secretion (Case, 1979; Webster *et al.*, 1977). More detailed studies suggest that CCK may regulate the synthesis of specific pancreatic zymogens (Dagorn and Mongeau, 1977). Chronic stimulation with either CCK or its analogs for 5–15 days also induces pancreatic hypertrophy and hyperplasia due to an increase in structural proteins and nucleic acids as well as zymogen (Mainz *et al.*, 1973; Solomon *et al.*, 1978).

In vitro studies of the effects of CCK and the mechanisms involved have largely been carried out with isolated pancreatic acini. Secretion by isolated pancreatic acini is usually quantitated by measuring either the amount of a specific, easily measured digestive enzyme such as amylase (Fig. 2) or by pulse labeling the newly synthesized zymogen with radioactive amino acid. Enzyme secretion is an event that is clearly separated from enzyme synthesis, as secretion can take place even when protein synthesis is inhibited (Jamieson and Palade, 1971; Otsuki and Williams, 1982b). The predominant view is that all pancreatic zymogens are secreted in parallel by exocytosis and in proportion to their pancreatic content, implying a single control process (Case, 1978; Palade, 1975). An alternative view is that the secretion of digestive enzymes is controlled individually and that enzymes leave the acinar cell by a nonexocytotic mechanism (Rothman, 1975, 1980).

Although it has not been possible in isolated acinar cells to directly measure fluid production, a direct effect of CCK on this function is indicated by the actions of either CCK or its analogs to increase both radiosodium uptake (Putney *et al.*, 1980) and the turnover of the transport enzyme Na^+–K^+-ATPase (S. R. Hootman and J. A. Williams, unpublished data).

In addition to its effects on secretion, the synthetic and metabolic effects of CCK have also been studied *in vitro*. Recently, studies with isolated pancreatic acini have shown a direct effect of low concentrations of CCK on stimulation of acinar protein synthesis whereas higher concentrations of hormone bring about inhibition (Fig. 3) (Korc *et al.*, 1981a). CCK stimulates the oxidation of glucose, alanine, and leucine by fragments of mouse pancreas (Danielsson and Sehlin, 1974). Oxygen consumption is increased (Dickman and Morrill, 1957), presumably reflecting increased energy turnover as both secretion and synthesis require

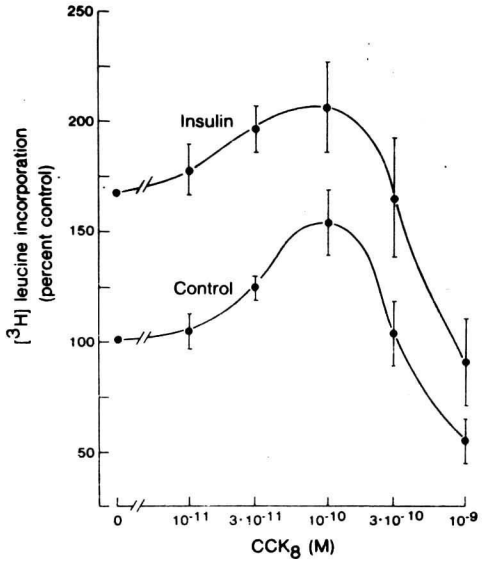

FIG. 3. Effect of CCK_8 and insulin on [^{3}H]leucine incorporation in isolated pancreatic acini from diabetic rats. Insulin was added at 0.17 μM. Each value is the mean ± SE from four experiments. (From Korc *et al.*, 1981a.)

ATP. CCK also stimulates glucose transport by isolated pancreatic acini as determined by using both 2-deoxyglucose (Fig. 4) and 3-*O*-methylglucose. In contrast, CCK inhibits the uptake of the nonmetabolized amino acid α-aminoisobutyric acid (AIB); this inhibition is thought to be due to a reduction in the electrochemical gradient for Na^+ (Iwamoto and Williams, 1980). Of interest is the difference in shape of the various dose–response curves for CCK acting on isolated pancreatic acini. The curves for enzyme secretion and protein synthesis are biphasic, whereas those for stimulation of glucose transport and the inhibition of AIB uptake are monophasic and require higher concentrations of CCK.

Since CCK binds initially to receptors localized on the basolateral plasma membrane and then rapidly initiates zymogen release at the luminal membrane, it has long been apparent that that action of CCK must be mediated by a second messenger. Although early work focused on a possible role for cyclic AMP, it is now clear that this nucleotide is not involved. Under physiological conditions, CCK does not increase pancreatic cyclic AMP content and neither exogenous derivatives of cyclic AMP nor phosphodiesterase inhibitors mimic the action of CCK (Case, 1978). CCK does bring about an increase in cyclic GMP, but the rise in cyclic GMP appears secondary to a rise in intracellular Ca^{2+} (Christophe *et al.*, 1976b). In contrast to the results with cyclic AMP and cyclic GMP, considerable evidence exists for the role of cytoplasmic Ca^{2+} as the intracellular

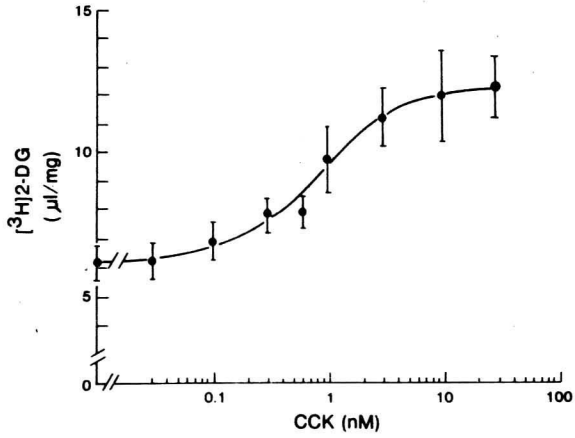

FIG. 4. Effect of CCK on uptake of [^{3}H]2-deoxyglucose (2-DG) by isolated mouse pancreatic acini. Each value is the mean ± SE from four experiments. (From Sankaran *et al.*, 1982.)

mediator of CCK (Case, 1978; Schulz, 1980; Williams, 1980). The major points are that (1) CCK increases the movement of Ca^{2+} into and out of acinar cells, (2) removal of extracellular Ca^{2+} either reduces or abolishes the action of CCK, and (3) the action of CCK can be mimicked by artificial introduction of Ca^{2+} into acinar cells by means of calcium ionophores, particularly A23187. A recent report using Ca^{2+}-sensitive microelectrodes has determined that the concentration of ionized Ca^{2+} in unstimulated pancreatic cells is 3×10^{-7} *M* and that Ca^{2+} increases upon stimulation with acetylcholine (O'Doherty and Stark, 1982). Since CCK is known to act similarly to acetylcholine (although via distinct receptors), it seems likely that CCK will have a similar effect. Some controversy exists over the source of Ca^{2+} as both entry from the extracellular fluid and release from intracellular stores has been proposed (Schulz, 1980; Williams, 1980). It seems clear, however, that release from intracellular stores is the predominant event since enzyme secretion can take place for several minutes in the complete absence of extracellular Ca^{2+}, even in the presence of extracellular chelators such as EGTA (Scheele and Haymovits, 1980; Williams, 1980). It is not completely clear which organelle(s) releases Ca^{2+} in response to stimulation with CCK; mitochondria, plasma membrane, and endoplasmic reticulum have been proposed (Chandler and Williams, 1978; Dormer and Williams, 1981; Schulz *et al.*, 1980). The nature of the signal from CCK receptors located on the plasma membrane to the intracellular Ca^{2+} stores is also a matter for further investigation.

Little is known about the mechanism by which the rise in cytoplasmic Ca^{2+} brings about the increase in amylase release. This process is energy dependent since inhibitors which lower cellular ATP levels block the action of Ca^{2+} on

secretion (Williams and Lee, 1974). Calmodulin, a calcium receptor protein, is present in pancreas and may be involved in secretion (Vandermeers *et al.*, 1977). In other cell types calmodulin, after binding Ca^{2+}, activates a number of Ca^{2+}-activated protein kinases. In support of such an effect in acini, it has recently been shown that CCK, as well as both the cholinergic analogs and Ca^{2+} ionophore A23187, alter the phosphorylation of at least five proteins (Burnham and Williams, 1982). In this study the secretagogues increased the phosphorylation of a M_r = 32,000 particulate protein and M_r = 16,000 and 23,000 soluble proteins. The agents also caused the dephosphorylation of M_r = 21,000 and 20,500 soluble proteins (Fig. 5). The time course of phosphorylation, its

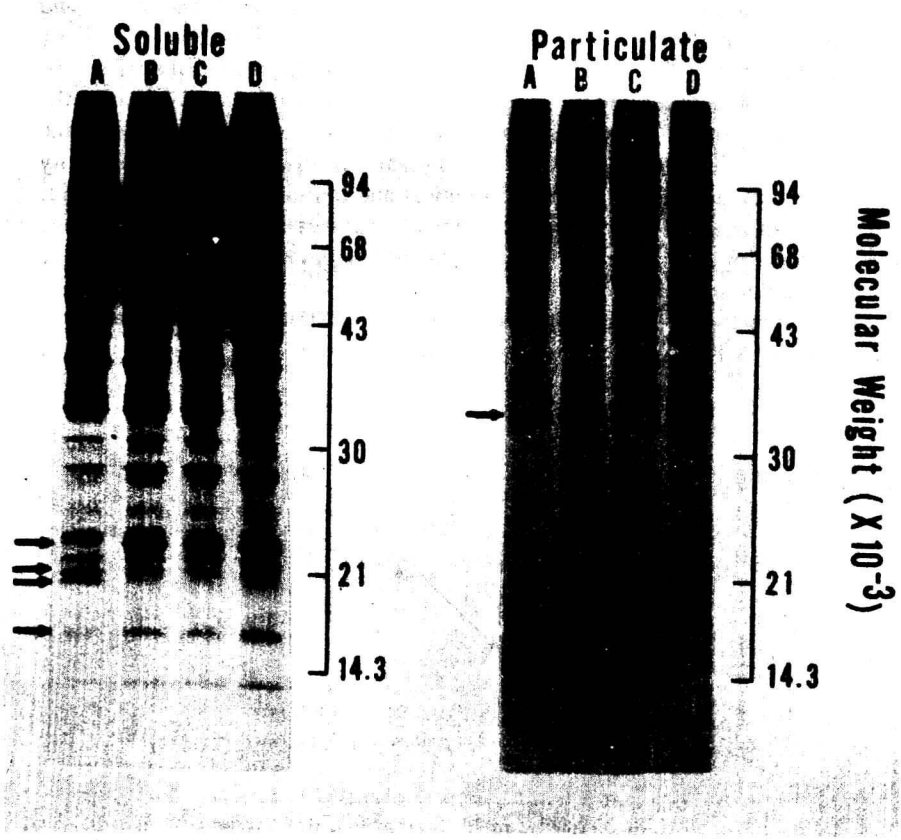

FIG. 5. Autoradiographs of soluble and particulate fractions from mouse acini prelabeled with ^{32}P for 1 hour and then incubated for 5 minutes with no additions (A), 3 μM carbachol (B), 3 μM ionophore A23187 (C), or 300 pM CCK_8 (D). (From Burnham and Williams, 1982.)