Agonist-regulated Phosphorylation of the Pancreatic Cholecystokinin Receptor*

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The present study was undertaken to determine if the cholecystokinin (CCK) receptor may be phosphorylated, and to gain insight into its regulation. For this, the ATP pool of rat pancreatic acini was prelabeled with 32P, and the cells were stimulated with various secretagogues. CCK receptors from treated cells were enriched by sequential fractionation to produce plasmaemla, and subsequent solubilization and lectin-affinity chromatography. This protocol detected a phosphorylated M. = 85,000–95,000 plasma membrane glycoprotein with features similar to the CCK receptor. Phosphorylation of this protein occurred rapidly (less than 2 min) and in a concentration-dependent manner in response to CCK, and was inhibited by the CCK receptor antagonist L-364,718. Further evidence that this represented the CCK receptor included co-migration of phosphorylated and CCK radioligand affinity-labeled proteins on sodium dodecyl sulfate-polyacrylamide gels, both in native forms and after endoglycosidase F deglycosylation, and the specific adsorption of the phosphorylase to a CCK analogue affinity resin. Phosphorylation occurred predominantly on serine residues of the receptor protein. Phosphorylation of this protein was also enhanced in response to other secretagogues which, like CCK, stimulate a cascade leading to protein kinase C activation, and in response to direct activation of this enzyme by 12-O-tetradecanoylphorbol 13-acetate. Thus, the pancreatic CCK receptor is phosphorylated in a regulated manner, in response to both homologous and heterologous secretagogues, and to protein kinase C activation.

Plasma membrane receptors are uniquely situated to regulate cellular responsiveness to peptide hormones. Additionally, receptors themselves may be regulated, with receptor phosphorylation representing a principal mechanism for such regulation (1). Numerous examples of this common theme have been extensively studied (1).

Cholecystokinin (CCK)1 is a peptide hormone which represents the major physiologic stimulant of pancreatic exocrine secretion (2). Although numerous pancreatic acinar cell proteins demonstrate regulated phosphorylation in response to this hormone (3–6), to date there has been no demonstration of phosphorylation of the CCK receptor. However, due to the sparsity of such receptors on these cells, estimated as approximately 5000 molecules/cell (7), previous methods may not have been sensitive enough to detect receptor phosphorylation.

Recent work has provided insight into the biochemical nature of the pancreatic CCK receptor (8–12), and has contributed to the development of schemes for the enrichment and purification of this molecule (13, 14). Based on this knowledge, a protocol was developed for the rapid enrichment of this receptor while minimizing proteolysis and dephosphorylation. Using this approach, regulated phosphorylation of the pancreatic acinar cell CCK receptor was detected.

MATERIALS AND METHODS

Peptides and Reagents—Synthetic CCK-8 (CCK-26-33) was purchased from Peninsula Laboratories (Belmont, CA). The CCK analogues, D-Tyr-Gly-[Nle12,31, pNO2Phe33]CCK-26-33) and D-Tyr-Gly[Nle12,31]CCK-26-32]-phenethyl ester (OPE), were synthesized, iodinated oxidatively, and purified by reversed-phase high performance liquid chromatography to yield specific radioactivities of 2000 Ci/mmol, as we have described (10, 15). The nonpeptide CCK receptor antagonist, L-364,718 (16), was the kind gift of Dr. Roger Freidinger, Merck, Sharp, and Dohme Research Laboratories. Carbachol and 12-O-tetradecanoylphorbol 13-acetate (TPA) were from Sigma, and staurosporine was from Behring Diagnostics.

Protein Phosphorylation—Phosphorylation was performed in dispersed rat pancreatic acinar cells. Tissue was obtained from 125–150-g male Sprague-Dawley rats, and dissociated by sequential enzymatic and mechanical methods according to Schultz et al. (17). The pancreatic acini were washed and suspended in medium, containing 25 mM Hapes, pH 7.4, 104 mM NaCl, 5 mM KCl, 1.2 mM MgSO4, 2 mM CaCl2, 2.5 mM d-glucose, 0.2% bovine serum albumin, and 0.01% soybean trypsin inhibitor. The cellular ATP pool was then radiolabeled by the addition of 2 μCi of H3[32P]O4/ml (ICN Radiochemicals, Irvine, CA) to the medium with incubation for 90 min at 37 °C under 10% oxygen.

To determine the effects of various secretagogues, the prelabeled acini were incubated at 37 °C with different agents for specified times. The experimental incubations were terminated by the addition of iced medium, containing 10 mM NaF, 2 mM EDTA, 2 mM EGTA, 20 mM sodium pyrophosphate, 18.4 μg/ml vanadate, 1 mM phenylmethylsulfonyl fluoride, 0.01% soybean trypsin inhibitor, and 1 μg/ml leupeptin. Acini were then washed, resuspended in medium containing

1 The abbreviations used are: CCK, cholecystokinin; OPB, D-Tyr-Gly-[Nle12,31]CCK-26-32]-phenethyl ester; TPA, 12-O-tetradecanoylphorbol 13-acetate; Hapes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylenbis(oxyethylenenitrilo)tetraacetic acid; SDS, sodium dodecyl sulfate; UEA-I, Ulex europaeus aglutinin I; MBS, 4-(N-morpholino)ethanesulfonic acid; endo F, endo-β-N-acetylglucosaminidase.

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ing 40% sucrose, and disrupted by sonication using a Sonifier Cell Disruptor (Heat Systems, Ultrasonics, Plainview, NY) with a single burst at setting 7 for 8 s. The resulting homogenate was then overlaid with 20% sucrose and centrifuged at 225,000 \( \times g \) for 60 min at 4 °C. A fraction enriched for plasma membranes was collected at the interface between the sucrose layers and washed.

The plasma membrane fraction was then either directly fractionated by SDS-polyacrylamide gel electrophoresis and analyzed by autoradiography (as described), or further fractionated as described (13). The additional receptor enrichment involved solubilization of the membranes using 1% digitonin for 30 min at 4 °C, and removal of insoluble material by centrifugation at 200,000 \( \times g \) for 30 min. The supernatant was incubated with Ulex europaeus agglutinin I-agarose (UEA-I) (E-Y Laboratories, San Mateo, CA) using gentle agitation for 45 min at 4 °C. The resin was washed, and fucose-containing glycoproteins were eluted with buffer containing 300 mM L-fucose. In some experiments, this fraction was further enriched for the CCK receptor.

Eluate from the lectin column was further purified by binding it to a phenylglyoxal column of CCK, OPE, coupled to agarose (OPE-agarose) for 12 h at 4 °C in 50 mM MES buffer, pH 6.0. These conditions for the CCK analogue affinity chromatography have been previously established and validated. The specificity of this interaction was assessed by parallel incubations in the presence of 1 \( \mu M \) OPE or 0.1 \( \mu M \) L-364,718. Following incubations, the affinity resin was washed, resuspended in sample buffer containing 4% SDS, and proteins were separated by SDS-polyacrylamide gel electrophoresis.

Gels were loaded using a constant amount of receptor per lane. For this, the solubilized and partially purified CCK receptors were quantified in binding assays using the radioligand, \(^{125}\)I-\( \beta \)-Tyr-Gly-[Nle\(^{8},\) pNO\(_{2}\)-D-Tyr-\( \beta \)-Gly-(Nle\(^{8}\), pNO\(_{2}\)-OPE]. This ligand was particularly useful for CCK receptor quantitation, since it binds to both classes of pancreatic acinar cell CCK receptors with equal affinities (15). Therefore, quantitation could be performed using the LIGAND program (18), fixing the value for binding affinity and determining only \( B_{\infty} \). Binding of \(^{125}\)I-OP to solubilized CCK receptors was performed at 4 °C for 12 h. For separation of receptor-bound and unbound ligand, 20 \( \mu l \) 0.6% \( \gamma \)-globulin, 100 \( \mu l \) of 20% polyethylene glycol 8000, and 20 \( \mu l \) of 0.2 M KI were added to each sample, and these were processed by filtration on a Skatron cell harvester (Lier, Norway) using glass fiber filter mats. Under these conditions, nonspecific binding, determined in the presence of 1 \( \mu M \) OPE, represented less than 17% of total binding.

**Polyacrylamide Gel Electrophoresis and Phosphoprotein Quantitation**—Samples were prepared for SDS-polyacrylamide gel electrophoresis by addition of an equal volume of sample buffer containing 0.25 mM Tris, pH 6.8, 8% SDS, 20% glicerol, and 0.01% bromphenol blue. Proteins were resolved on 10% SDS-polyacrylamide gels (15.5 × 0.15 cm) as per Hennessey and Scarbrough (19). Labeled proteins were visualized by autoradiography using Kodak XAR-5 film with DuPont Quanta III intensifying screens after exposure at -70 °C. Apparent molecular weight values for labeled proteins were determined by interpolation on a plot of \( log M_{r} \) versus mobilities of standard proteins (myosin \( (M_{t}=200,000) \), \( \beta \)-galactosidase \( (M_{t}=116,000) \), phosphorylase \( b (M_{t}=92,500) \), bovine serum albumin \( (M_{t}=66,000) \), ovalbumin \( (M_{t}=45,000) \), and carbonic anhydrase \( (M_{t}=29,000) \).

Autoradiographs were digitized with a Microtek MSF-300 scanner (Torrance, CA), and protein phosphorylation of specific bands was quantitated by densitometric measurement using the Analyze program (20). Use of this computer program made it possible to determine total pixel intensity within relevant bands while correcting for local background pixel intensity.

**Affinity Labeling and Receptor Deglycosylation**—The possible identification of the phosphoprotein which was carried through the purification scheme as the CCK receptor was assessed by comparing it with affinity labeled CCK receptor, in both native and deglycosylated states. The receptor standard was prepared by affinity labeling rat pancreatic plasma membranes with \(^{125}\)I-\( \beta \)-Tyr-Gly-[Nle\(^{8},\) pNO\(_{2}\)-Phe\(^{39}\)]CCK-26-33, as we have described previously (10). For a core protein standard, the affinity labeled band was electroeluted from a SDS-polyacrylamide gel and deglycosylated enzymatically with endo-\( \beta \)-N-acetylglucosaminidase F (endo F), as described (9, 12). The labeled phosphoprotein band contained concentrations of CCK-8 for 30 min. On the left, a plasmalemmal fraction was directly applied, while on the right, the gel was further enriched for CCK receptor by lectin-affinity chromatography on UEA-I-agarose. The arrow marks the \( M_{t} = 85,000-95,000 \) region of the gel in which the receptor might be expected to migrate (8-12) (Fig. 1, left).

Following further purification with UEA-I lectin-affinity chromatography, a phosphoprotein of apparent \( M_{t} = 85,000-95,000 \) was apparent on the autoradiograph, particularly after stimulation with high concentrations of CCK-8 (Fig. 1, right). We previously demonstrated that the CCK receptor from this tissue should bind to this lectin and be eluted in the same fraction as this phosphoprotein (13).

Next, the time course of phosphorylation in response to CCK was studied. Equal amounts of receptor were loaded in each lane, as assessed in the \(^{125}\)I-OPE binding assay. Phosphorylation of the \( M_{t} = 85,000-95,000 \) band stimulated by 0.1 \( \mu M \) CCK-8 was rapid, being readily detectable after 1 min and maximal after 2 min of incubation (Fig. 2). This phosphorylation subsequently diminished, with the level of phosphorylation seen after 20 min still above the basal phosphorylation occurring in the absence of CCK.

**Homologous Effects on CCK Receptor Phosphorylation**—To be certain that the phosphorylation of the \( M_{t} = 85,000-95,000 \) band which was enhanced by CCK was mediated by the CCK receptor, the effect of the nonpeptide receptor antagonist, L-364,718, was studied. When increasing concentrations of this antagonist were added to the incubation 10 min prior to stimulating the acini with 10 nM CCK-8, phosphorylation of the \( M_{t} = 85,000-95,000 \) protein was inhibited in a concentration-dependent manner. Because the presence of L-364,718 interfered with the \(^{125}\)I-OPE binding assay which was routinely used to quantitate receptor loaded in each lane of a gel, equal recoveries of receptor were assumed for this experiment.

**Phosphoamino Acid Analysis**—Phosphoamino acid content of phosphorylated proteins, separated on SDS gels and visualized by autoradiography, was determined by the method of Nairn and Greengard (21), using thin layer chromatography.

**RESULTS**

**Methods Development**—Incubation of pancreatic acinar cells with Hf\(^{32}\)P\(_{i}\)O lead to radiolabeling of the cellular ATP pool, and incorporation of the radiolabel into numerous plasmalemmal proteins (Fig. 1, left). As might be expected, in this crude plasmalemmal preparation, no change in the pattern of phosphorylation was detectable in response to CCK, even focusing on the \( M_{t} = 85,000-95,000 \) region of the gel in which the receptor might be expected to migrate (8-12) (Fig. 1, left).

FIG. 1. Autoradiographs of SDS-polyacrylamide gels used to separate pancreatic acinar cell proteins phosphorylated in the presence of the noted concentrations of CCK-8 for 30 min. On the left, a plasmalemmal fraction was directly applied, while on the right, this was further enriched for CCK receptor by lectin-affinity chromatography on UEA-I-agarose. The arrow marks the \( M_{t} = 85,000-95,000 \) region of the gel in which the affinity labeled CCK receptor has been observed.

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"E. M. Hadac and L. J. Miller, unpublished data."
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**FIG. 2.** The time course of phosphorylation of the pancreatic $M_r = 85,000-95,000$ band in response to $10 \, \text{nM} \, \text{CCK-8}$ is shown with an autoradiograph and a graph of integrated pixel intensity (typical of five experiments).

**FIG. 3.** The concentration-response curve for CCK-8-stimulated phosphorylation of the pancreatic $M_r = 85,000-95,000$ band, with basal phosphorylation taken as $0\%$ and the response to $0.1 \, \mu \text{M} \, \text{CCK-8}$ taken as $100\%$ (typical of six experiments).

A mean $IC_{50}$ of $3 \, \text{nM} \, \text{L-}364,718$ was observed under these conditions in five experiments. This is consistent with the relative affinities of these ligands for the pancreatic CCK receptor ($15, 22$).

The CCK-induced phosphorylation of the $M_r = 85,000-95,000$ plasma membrane protein was concentration-dependent, with increasing amounts of agonist leading to increased phosphorylation, up to the highest concentration of CCK studied ($0.1 \, \mu \text{M}$) (Fig. 3). Under these conditions, enhanced incorporation of $^{32}\text{P}$ into the $M_r = 85,000-95,000$ protein was reproducibly observed using as little as $1.0 \, \text{nM} \, \text{CCK-8}$.

To further assess identity of the $M_r = 85,000-95,000$ phosphoprotein, the UEA-I lectin eluate was applied to OPE-agarose. The phosphoprotein bound to this affinity resin in a specific and saturable manner, with this protein not binding to the affinity resin in the presence of an excess of competing OPE unattached to agarose (Fig. 4).

As further evidence of its identity, the phosphorylated $M_r$, $85,000-95,000$ species co-migrated on an SDS-polyacrylamide gel with the band which had been photoaffinity labeled with $^{125}\text{I-D-Tyr-Gly-[Nle}^{33,34}, \text{pNO}_{2}-\text{Phe}^{35}] \text{CCK-26-33}$ (Fig. 5). Furthermore, deglycosylation of both the photoaffinity labeled and the phosphorylated $M_r = 85,000-95,000$ bands using endo $F$ generated similar core proteins of $M_r = 42,000$ (Fig. 5). These results provide strong evidence that the phosphorylated $M_r = 85,000-95,000$ band represents the same glycoprotein as that previously identified as the CCK receptor, based on affinity labeling studies ($9$).

Phosphoamino acid analysis of the $M_r = 85,000-95,000$ band phosphorylated in response to CCK demonstrated predominantly phosphoserine residues (Fig. 6). While minor amounts of phosphothreonine were sometimes observed, no phosphotyrosine was detected.

**FIG. 4.** Autoradiographs of SDS-polyacrylamide gels used to separate pancreatic acinar cell proteins phosphorylated in the presence of $10 \, \text{nM} \, \text{CCK-8}$ for $2 \, \text{min}$. On the left, the solubilized plasmalemmal fraction was enriched by UEA-I chromatography. The two lanes on the right represent phosphoproteins eluted from OPE-agarose after incubation with equal amounts of the UEA-I eluate, in the absence or presence of $1 \, \mu \text{M}$ competing OPE.

**FIG. 5.** Autoradiographs of SDS-polyacrylamide gels used to separate pancreatic proteins affinity labeled with $^{125}\text{I-D-Tyr-Gly-[Nle}^{33,34}, \text{pNO}_{2}-\text{Phe}^{35}] \text{CCK-26-33}$ (left) and phosphorylated in response to $0.1 \, \mu \text{M} \, \text{CCK-8}$ (right), before and after deglycosylation with endo $F$.

Heterologous Effects on CCK Receptor Phosphorylation—Treatment with carbachol enhanced phosphorylation of the $M_r = 85,000-95,000$ protein in a concentration-dependent manner (Fig. 7). Bombesin, another pancreatic ligand thought to act through coupling to a guanine nucleotide-binding pro-
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Phosphoserine $\rightarrow$
Phosphothreonine $\rightarrow$
Phosphotyrosine $\rightarrow$
Origin $\rightarrow$

Fig. 6. Autoradiograph of the thin layer plate used to separate phosphoamino acids present in the $M_r = 85,000-95,000$ protein phosphorylated in the presence of 0.1 $\mu\text{M}$ CCK-8.

Response to Carbachol

|                | Basal | Carbachol | CCK |
|----------------|-------|-----------|-----|
| Carbachol (log M) | -5    | -4        | -3  |

Band Phosphorylation (% of CCK control)

0 50 100

Fig. 7. The concentration-response curve for carbachol-stimulated phosphorylation of the pancreatic $M_r = 85,000-95,000$ band, with basal phosphorylation taken as 0% and the response to 10 nM CCK-8 taken as 100% (typical of three experiments).

TPA also enhanced phosphorylation of the $M_r = 85,000-95,000$ band in a concentration-dependent manner (Fig. 8). Enhanced phosphorylation was detectable after incubation of pancreatic acini with as little as 10 nM TPA, while higher concentrations of phorbol ester stimulated greater phosphorylation.

To confirm the identity of the $M_r = 85,000-95,000$ bands labeled in response to carbachol and TPA as the CCK receptor, the same preparation of acini were stimulated with CCK and these agents and were subjected to the same purification procedure, including binding of the solubilized phosphoproteins to OPE-agarose resin. For each of the three secretagogues, the labeled band specifically bound to the OPE-agarose affinity resin and was deglycosylated by endo F to yield a species with $M_r = 42,000$ (Fig. 9). Furthermore, to show that these heterologous agents were not acting through the CCK receptor, preincubation of the acini with L-364,718 had no effect on the intensity of labeling of the receptor band observed in response to TPA and carbachol, while it reduced the CCK-stimulated labeling to its basal level (data not shown).

When acini were preincubated for 30 min in buffer containing 10 $\mu\text{M}$ staurosporine, an inhibitor of protein kinase C, prior to stimulation with CCK, TPA, and carbachol, differential effects were observed. There was complete inhibition of the phosphorylation of the CCK receptor which was stimulated by carbachol and TPA, while there was only partial inhibition of the phosphorylation response stimulated by CCK (Fig. 10). Consistent with action via protein kinase C, the phosphoamino acid in the $M_r = 85,000-95,000$ protein observed in response to TPA and carbachol was, like that
Pancreatic acinar cell CCK receptor is shown to be enhanced thought to act through a mechanism similar to CCK, and to this work, phosphorylation of the CCK receptor in response to CCK observed here is consistent with activation of a serine/threonine kinase pathway. The sparsity of CCK receptors on this target cell necessitated the development of a protocol for the rapid enrichment for this molecule, while minimizing proteolysis and dephosphorylation. Simple fractionation to enrich for plasma membrane proteins was clearly inadequate for this purpose, even focusing carefully on the appropriate region for migration of the receptor on a SDS-polyacrylamide gel which was used to separate plasma membrane proteins. This was due to the endogenous phosphorylation of numerous acinar cell proteins masking any possible phosphorylation of such a minor membrane protein.

The addition of a lectin-affinity step after solubilization of membrane proteins provided adequate enrichment to begin to see receptor phosphorylation. The key for this was the use of UEA-I lectin, known to bind to fucose residues, which we recently demonstrated to be capable of providing greater than 300-fold enrichment for receptor, beyond an already partially purified fraction (13). This step was adequate to remove the vast excess of nonreceptor phosphoproteins from the $M_r = 85,000-95,000$ region of the gel. Although not tested, other lectins with more widespread recognition sites would be predicted to be less useful.

The ability to inhibit the CCK-enhanced phosphorylation of this band with the nonpeptide CCK receptor antagonist, L-364,718, suggests that this process is mediated by CCK binding to its receptor. Furthermore, several lines of evidence identify the $M_r = 85,000-95,000$ phosphoprotein as representing the rat pancreatic plasmalemmal glycoprotein which has been affinity labeled by CCK receptor probes (10-12). These molecules behave similarly to each other in the fractionation scheme, the purification protocol, and when separated by SDS-polyacrylamide gel electrophoresis, both in their native states and after endoglycosidase F deglycosylation (9, 13). Finally, this phosphoprotein binds specifically to a CCK-ligand-affinity resin, and its binding is competed for by a CCK receptor agonist, OPE.

The specific binding of the phosphoprotein to the CCK-ligand-affinity resin actually provides strong and important complementary evidence that the $M_r = 85,000-95,000$ protein represents the CCK-binding subunit of the receptor. Previous affinity labeling studies have identified two candidates to represent the pancreatic acinar cell CCK receptor, including an $M_r = 80,000$ glycoprotein with a $M_r = 65,000$ protein core (23, 24), and a $M_r = 85,000-95,000$ glycoprotein with a $M_r = 42,000$ protein core (8, 9). However, affinity labeling can identify near neighbor proteins as well as subunits associated with the hormone-binding subunit, depending on the site of cross-linking utilized. Based on the observations that the $M_r = 80,000$ protein was labeled using CCK-33-based probes, while the $M_r = 85,000-95,000$ protein was labeled by probes with sites of cross-linking which span and are even included within the theoretical receptor-binding domain, the latter was predicted to be the binding subunit (10-12). While affinity labeled proteins cannot have their ligand-binding properties further studied, phosphorylated proteins are amenable to this approach. In this work, we demonstrated that the $M_r = 85,000-95,000$ phosphoprotein bound a CCK analogue, directly proving its identity as a hormone-binding subunit.

The use of the intact cell system to initially study CCK receptor phosphorylation, rather than a broken cell system is a critical first step toward identifying the protein kinase mediating this event. The demonstration of phosphorylation on serine residues helps provide focus on one group of protein kinases. Among the candidates to mediate this reaction, CCK has been shown to activate protein kinase C (25), cyclic AMP-dependent protein kinase (5), and to stimulate cyclic GMP which could activate cyclic GMP-dependent protein kinase (26). By analogy to the $\beta$-adrenergic receptor, there may be an agonist-specific kinase for the CCK receptor which is similar to $\beta$-adrenergic receptor kinase (27). Finally, similar to a number of other peptide receptors, the CCK receptor may have an endogenous ligand-regulated protein kinase activity.

As an initial effort to study one of these pathways, we have focused on protein kinase C, since this enzyme has been shown to be activated by agonists and to phosphorylate other receptors which are coupled to guanine nucleotide-binding proteins (1), like the CCK receptor (28). Direct stimulation of this enzyme with the phorbol ester TPA enhanced phosphorylation of the same pancreatic acinar cell $M_r = 85,000-95,000$ protein. The identity of this band as the CCK receptor was also confirmed by specific ligand affinity chromatography and by endo F deglycosylation. Phosphorylation of the CCK receptor was observed with relatively low concentrations of TPA which may be specific for a protein kinase C effect (29), and it was inhibited by the protein kinase C inhibitor staurosporine.

Additionally, heterologous phosphorylation of the CCK receptor was observed by stimulating the acinar cells with carbachol and bombesin, other secretagogues known to stimulate protein kinase C. Again the identity of the $M_r = 85,000-95,000$ band as the CCK receptor was confirmed by affinity chromatography and by deglycosylation. Like TPA, the carbachol-stimulated phosphorylation of this protein was inhibited by staurosporine. Agents like vasoactive intestinal pep-
tide which act through another pathway (adenylate cyclase) had no effect on phosphorylation of this molecule.

The treatment of acini with CCK was associated with a time course of phosphorylation of the $M_r = 85,000-95,000$ receptor band characterized by rapid enhancement of phosphorylation over basal, reaching a peak within 1–2 min. The enhanced phosphorylation of this protein, however, was quite transient. Since gels were loaded according to the amount of receptor at each time point, it is unlikely that this decrease in the labeling of the band represented a disappearance of the receptor from the plasmalemma by endocytosis, unless both plasmalemma and a potentially sequestered compartment co-migrate on the sucrose gradient centrifugation step used. Furthermore, it is improbable that changes in the specific activity of ATP pool over such a short time period could account for this diminished labeling. It is most probable that the decrease in labeling observed was due to either CCK receptor dephosphorylation by a protein phosphatase or dilution of its phosphorylated plasmalemmal pool by a new population of unphosphorylated receptor.

The function of the phosphorylation event being studied in this work is unclear at the present time. In analogous receptor systems, such as the $\beta$-adrenergic receptor, serine phosphorylation has been a mechanism for receptor desensitization (30). Desensitization has also been shown to exist for the pancreatic acinar cell CCK receptor (31). Interestingly, this physiologic event occurs with a concentration dependence not unlike the phosphorylation of the $M_r = 85,000-95,000$ protein observed in our current work. Here, we see phosphorylation enhanced by high concentrations of hormone. Further characterization of this work is unclear at the present time. In analogous receptor systems, such as the $\beta$-adrenergic receptor, serine phosphorylation has been a mechanism for receptor desensitization (30). Desensitization has also been shown to exist for the pancreatic acinar cell CCK receptor (31). Interestingly, this physiologic event occurs with a concentration dependence not unlike the phosphorylation of the $M_r = 85,000-95,000$ protein observed in our current work. Here, we see phosphorylation enhanced by high concentrations of hormone. Further characterization of this work is unclear at the present time.

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