

The CCK receptor on pancreatic plasma membranes: Binding characteristics and covalent cross-linking

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The cholecystokinin (CCK) receptor in purified plasma membranes prepared from mouse pancreatic acini had a binding affinity of 1.8 nM, an acid pH optimum between 6.0 and 6.5, and an analog specificity of $\text{CCK}_8 > \text{CCK}_{33} > \text{desulphated CCK}_8 > \text{CCK}_4$. Binding of CCK to its receptor was abolished by pretreatment of plasma membranes with trypsin. When [^{125}I]CCK was cross-linked to its receptors with disuccinimidyl suberate, and the preparation solubilized and subjected to gel electrophoresis and autoradiography, the hormone was associated with M_r 80000 protein in both the presence and absence of the reducing agent dithiothreitol.

Cholecystokinin Pancreas Receptor

1. INTRODUCTION

Cholecystokinin (CCK) is the major hormonal regulator of pancreatic acinar cell function [1,2]. Employing the biologically active ligand, [^{125}I]BH-CCK₃₃, we have demonstrated the presence of specific receptors for CCK both in intact pancreatic acini [3] and on particulate fractions prepared from the whole pancreas [4]. However the nature and location of the CCK receptor is unknown. Recent autoradiographic studies suggest the plasma membrane as the major site for the binding of radioiodinated CCK to pancreatic acini [5]. Accordingly, we prepared purified plasma membranes from mouse pancreatic acini and measured the characteristics of CCK binding to this subcellular organelle. Moreover, we utilized the bifunctional cross-linker, disuccinimidyl suberate, to determine the size and subunit structure of the CCK receptor.

2. MATERIALS AND METHODS

2.1. Preparation of pancreatic plasma membranes

Male Swiss Webster mice (25 g) were fasted 18 h before study. Isolated pancreatic acini were prepared according as in [6]. Plasma membranes were then prepared as in [7] and stored at -80°C . The protein concentration of the membrane was determined using a Coomassie Blue (Bio-Rad) protein assay reagent [8].

2.2. Binding of [^{125}I]BH-CCK to pancreatic plasma membrane

Pure natural porcine cholecystokinin (CCK₃₃) was iodinated to specific activities of 200–300 $\mu\text{Ci}/\mu\text{g}$ by conjugation with *N*-succinimidyl-3-(4-hydroxy,5-[^{125}I]-iodophenyl) propionate ([^{125}I]-BH-reagent) as in [9]. Pancreatic plasma membranes, at 15 μg protein/ml, were incubated with 50 pM [^{125}I]BH-CCK in HEPES buffer at pH 7.4 [4] and enriched with 5 mg/ml bovine serum albumin, 1 mg/ml bacitracin and 0.2 mg/ml soybean trypsin inhibitor. To determine non-specific binding, CCK₈ at 100 nM was added. Samples

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were incubated at 24°C and then centrifuged at $10000 \times g$ for 1 min. The binding supernatants were used to determine the degradation of [125 I]BH-CCK with trichloroacetic acid added at a final concentration of 10% [3].

The dissociation of [125 I]BH-CCK from the membrane protein was studied under two conditions:

- (i) by a 100-times dilution of the hormone-receptor complex [10];
- (ii) by dilution to the same extent in a medium containing an excess of unlabeled hormone (100 nM CCK₈).

After incubation at 24°C, the diluted membranes were collected by filtration through cellulose acetate membrane filters (Oxoid, 0.45 μ m pore size).

2.3. Affinity cross-linking of CCK receptors

Affinity cross-linking was performed by incubating membranes (at 0.05 mg protein/ml in HEPES-buffered solution (pH 6.5) with 1 nM [125 I]BH-CCK for 30 min at 24°C. After incubation, membranes were washed, and resuspended in phosphate-buffered saline at pH 7.4 and then incubated with 5 mM disuccinimidyl suberate for 15 min at 4°C. The cross-linking reaction was quenched by addition of excess 10 mM Tris (pH 7.4) with 1 mM EDTA at 4°C. Finally, the cross-linked materials were pelleted at $25000 \times g$ for 20 min, resuspended in 62.5 mM Tris, 2.3% sodium dodecyl sulfate, and 10% glycerol, at pH 6.8. Electrophoresis was carried out using 7.5% acrylamide in the presence or absence of 50 mM dithiothreitol. Dried gels were exposed to Kodak X-omat AR film for 4 weeks.

3. RESULTS

3.1. Characterization of CCK binding to plasma membrane receptors

The binding of 50 pM [125 I]BH-CCK was rapid with binding occurring after 30 min (fig.1). Non-specific binding was negligible. Degradation of CCK was linear throughout the incubation, but was <10% after 60 min. When [125 I]BH-CCK was bound to plasma membranes for 30 min and then the reaction mixture diluted 100-fold, the labeled CCK dissociated with a one-half time of 60 min (fig.1). The addition of excess unlabeled CCK₈ after dilution greatly accelerated the dissociation

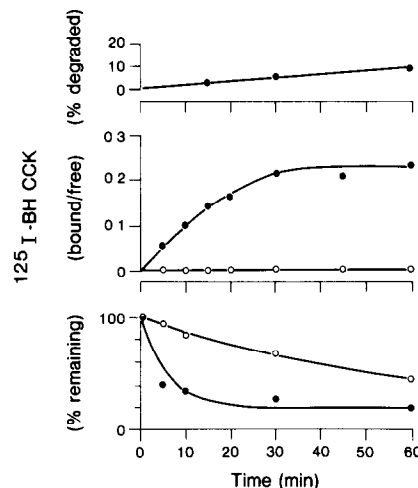


Fig. 1. Time course of [125 I]BH-CCK binding, degradation and dissociation in pancreatic plasma membranes. Top: degradation; Middle: total binding (●), and non-specific binding (○); Bottom: dissociation by dilution only (○), and dissociation by dilution plus unlabeled CCK₈ (●).

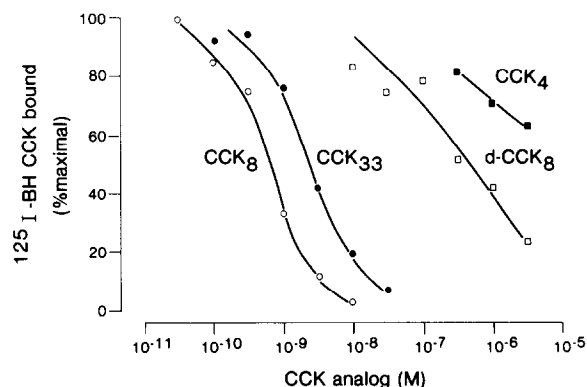


Fig. 2. Analog specificity of [125 I]BH-CCK-binding to pancreatic plasma membranes.

of the radiolabeled CCK, suggesting the presence of negative cooperative interactions.

Scatchard analysis of binding of [125 I]BH-CCK at steady state revealed a single order of binding sites with K_d of 1.83 ± 0.29 nM (mean $\pm$ SE, $n = 4$). Total binding capacity was 22.9 ± 4.5 pmol/mg protein. CCK analog studies revealed a potency of CCK₈ > CCK₃₃ > desulfated CCK₈ > CCK₄ as defined by their ability to compete with [125 I]BH-CCK for the pancreatic CCK receptor (fig.2).

To study whether the plasma membrane CCK receptor was a protein, we incubated the plasma membranes with 0.1 mg trypsin/ml followed by an excess of trypsin inhibitor. This treatment reduced CCK binding by >95%.

3.2. Covalent cross-linking of ^{125}I -BH-CCK to its receptors

To cross-link CCK to its receptors, plasma membranes were first incubated with 1 nM ^{125}I -BH-CCK for 30 min, washed, and then treated with

5 mM disuccinimidyl suberate; this concentration of cross-linker was found to give optimal results. Autoradiographs of SDS gels revealed that a major band of radioactivity was present at M_r of 80000. Several minor bands were present which may have represented partially degraded receptor. The appearance of both major and minor bands were totally eliminated by the presence of 100 nM CCK₈ during the ^{125}I -BH-CCK binding period. Virtually the same patterns were observed when 50 mM dithiothreitol was added to the solubilization solution.

4. DISCUSSION

These studies demonstrate the existence of specific binding sites for ^{125}I -BH-CCK on purified mouse pancreatic acinar plasma membranes. This binding was time-dependent, reversible, inhibited by CCK analogs in proportion to their known biological activities, and it showed evidence for negative cooperative effects [10,11]. These binding characteristics are identical to those observed with both isolated pancreatic acini and particulate fractions prepared from whole pancreas.

Scatchard analysis showed a single order of binding sites with an affinity constant similar to that seen for pancreatic particles [4]. In contrast, the number of binding sites/unit protein was 200-times greater in plasma membranes, a finding that is consistent with the concept that the major site of binding of CCK in the pancreas is to the plasma membrane. Furthermore, studies with trypsin indicated that the CCK receptor is a protein.

To study the nature of this protein ^{125}I -BH-CCK was cross-linked to its receptor with disuccinimidyl suberate, a procedure previously used to study the nature of the receptor for insulin and other peptide hormones [12]. These data suggested that the receptor was a single protein with a M_r of 80000. Studies with the reducing agent dithiothreitol also suggested that the CCK receptor was not a polymer connected by sulphhydryl bonds. The size of this receptor is thus similar to the receptors for glucagon [13], prolactin [14], and gonadotropin [15], but smaller than those for insulin, EGF and IGF [12,16,17]. In [18] it was reported that cross-linking of ^{125}I -BH-CCK to rat pancreatic plasma membranes labels an M_r 85000 protein.

We find that purified pancreatic acinar plasma

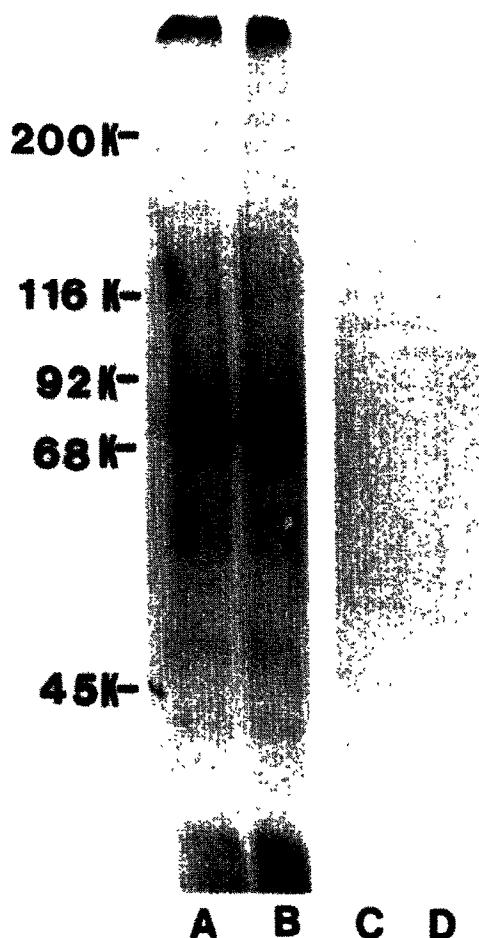


Fig. 3. Autoradiogram of a SDS polyacrylamide gel of pancreatic plasma membranes cross-linked to ^{125}I -BH-CCK with disuccinimidyl suberate. (A) ^{125}I -BH-CCK; (B) ^{125}I -BH-CCK + dithiothreitol; (C) ^{125}I -BH-CCK + unlabeled CCK₈; (D) ^{125}I -BH-CCK + unlabeled CCK₈ + dithiothreitol.

membranes have a large number of receptor sites for CCK. The receptor is protein in nature and has an app. M_r of 80000. Therefore, these data agree with observations suggesting that the initial step in the action of CCK is binding to a specific receptor localized on the plasma membrane of pancreatic acinar cells [2].

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