Effects of Cholecystokinin (CCK)-JMV-180 on the CCK Receptors of Rabbit Pancreatic Acini and Gallbladder Smooth Muscle

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ABSTRACT—Effects of cholecystokinin (CCK)-JMV-180, a CCK analog, on the CCK receptor functions of isolated rabbit pancreatic acini and gallbladder smooth muscle were studied. When the pancreatic acini were incubated with increasing concentrations of CCK-8, stimulation of amylase release reached a maximum at 3 nM and then declined with the increasing concentration of CCK-8. CCK-JMV-180 also caused a dose-dependent amylase release stimulation, which plateaued and remained unchanged above 300 nM at about 50% of the maximal stimulation by CCK-8. CCK-JMV-180 above 100 nM caused a rightward shift of the downstroke of the dose-response curve for CCK-8 (pA2 = 7.5). In the gallbladder smooth muscle, CCK-8 caused a dose-dependent contraction, but CCK-JMV-180 totally lacked this property. Instead, CCK-JMV-180 caused a rightward shift of the dose-response curve for CCK-8 (pA2 = 7.9). These results suggest that CCK-JMV-180 distinguishes between the CCKA receptors associated with pancreatic exocrine secretion in the acini and those involved in contraction of the isolated gallbladder smooth muscle in rabbits.

Keywords: Cholecystokinin (CCK), Cholecystokinin (CCK)-JMV-180, Pancreatic acini, Gallbladder smooth muscle

In isolated pancreatic acini, cholecystokinin (CCK) has been shown to stimulate amylase release in a biphasic manner (1). It has been proposed that the dose-dependent stimulation of amylase release by low concentrations of CCK is attributed to occupancy of the high affinity CCKA receptor site and the inhibition of amylase release by high concentrations of CCK is due to occupancy of the low affinity CCKA receptor site (2, 3). CCK-JMV-180, a new heptapeptide analog of CCK (4), has been shown to stimulate amylase release from rat pancreatic acini as an agonist at the high affinity CCKA receptor site and block the CCK-induced supramaximal inhibition of amylase release as an antagonist at the low affinity site (5, 6).

In gallbladder smooth muscle, CCK octapeptide (CCK-8)-induced contraction is thought to be mediated by the CCKA receptor (7), but the affinity subtype of the CCKA receptor has not been clearly defined. It has been reported that CCK-JMV-180 exhibited antagonistic activity against CCK-8-induced contraction of the isolated guinea pig gallbladder smooth muscle (8). However, there has so far been no report dealing with the interaction of CCK-JMV-180 with the CCK receptors of pancreatic acini and gallbladder smooth muscle in one animal species.

In the present study, we examined the effects of CCK-JMV-180 on the functions of isolated rabbit pancreatic acini and gallbladder smooth muscle in order to characterize the subtypes of the CCK receptor mediating the CCK action to stimulate exocrine secretion from the pancreatic acini and contract the gallbladder smooth muscle.

MATERIALS AND METHODS

Materials

125I-Bolton-Hunter-labeled CCK-8 (125I-BH-CCK-8, 2,200 Ci/mmol) was purchased from DuPont-New England Nuclear (Boston, MA, USA). L-364,718 [3S(-)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepine-3-yl)-1H-indole-2-carboxamide] and L-365,260 [(3R(+)-2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-N-(3-methylphenyl)-urea] were synthesized at the Organic Chemistry Research Laboratory of Tanabe Seiyaku Co., Ltd. (Toda). CCK-8 and CCK-4 were purchased from the Peptide Institute (Osaka). CCK-JMV-180 [Butyloxycarbonyl-Try(SO3H)-Nle-Gly-Trp-Nle-Asp-2-phenylethylester] was purchased from Research Plus (Bayonne, NJ, USA). All other chemicals used were of reagent grade.
**Animals**

Male Japanese white rabbits weighing 1.5 - 2.5 kg were killed by injecting an overdose of sodium pentobarbital into the ear vein. The pancreas and gallbladder were immediately removed and used for the experiments.

**Receptor binding assay**

Preparation of the pancreatic membranes and a receptor binding study were performed according to the methods of Chang and Lotti (9).

The pancreatic tissue was homogenized in 20 vol. of ice-cold 50 mM Tris-HCl buffer (pH 7.4 at 25°C) containing 0.01% soybean trypsin inhibitor (Sigma, St. Louis, MO, USA; type II-S) and centrifuged at 50,000 x g for 10 min. The pellet was resuspended in the same volume of the buffer and then recentrifuged at 50,000 x g for 10 min. The resulting pellet was resuspended in incubation medium (50 mM Tris, 5 mM MgCl₂ and 5 mM dithiothreitol) containing 0.14 mg/ml bacitracin, 0.1% bovine serum albumin, 0.01% soybean trypsin inhibitor (Sigma, type II-S), and pH was adjusted to 7.4 at 25°C. All the procedures were conducted at 4°C.

The competitive binding study was performed by incubating the membrane preparations (3 mg wet tissue/ml) in the incubation medium (300 μl) containing 125I-BH-CCK-8 (50 pM) and various concentrations of drugs. Each determination was carried out in duplicate. Incubation was continued for 90 min at 25°C and terminated by adding ice-cold Tris-HCl buffer. Free and bound 125I-BH-CCK-8 were separated by filtration through a Whatman GF/B glass fiber filter. Nonspecific binding was estimated from a parallel assay in the presence of a large excess (1 μM) of CCK-8. Specific 125I-BH-CCK-8 binding, defined as the difference between the total and nonspecific binding, was about 85–95% of the total binding.

**Preparation of pancreatic acini and amylase release assay**

Preparation of the pancreatic acini and amylase release experiments were performed according to the procedures described previously (10, 11) with minor modifications.

The medium used for the preparation of pancreatic acini was a modified Krebs-Henseleit bicarbonate (KHB) buffer (110 mM NaCl, 32.5 mM NaHCO₃, 4.7 mM KCl, 1.13 mM MgCl₂, 1 mM Na₂HPO₄, 2 mM glutamine and 11.1 mM glucose) containing 0.01% soybean trypsin inhibitor (Sigma, type I-S) and minimal Eagle’s medium amino acid supplement. The medium used for the assay of amylase release was a HEPES-Ringer (HR) buffer (127 mM NaCl, 4.7 mM KCl, 1.2 mM CaCl₂, 0.56 mM MgCl₂, 1 mM Na₂HPO₄, 10 mM HEPES, 2 mM glutamine and 11.1 mM glucose) containing 0.01% soybean trypsin inhibitor (Sigma, type I-S), minimal Eagle’s medium amino acid supplement and 0.5% bovine serum albumin. All media were equilibrated with 95% O₂ : 5% CO₂ and the pH was adjusted to 7.4.

The KHB buffer containing 200 U/ml purified collagenase, 0.03 mg/ml chymotrypsin (both from Worthington Biochemicals, Freehold, NJ, USA) and 1.8 mg/ml hyaluronidase (Sigma, type I-S) was injected into the pancreas to make it swell, and then it incubated at 37°C for 10 min. The swollen pancreas was minced and reincubated with fresh medium under the same conditions for 30 min. The digested pancreas was then dissociated by aspirating up and down through pipettes with decreasing orifice size (first one with a 2-mm diameter and then one with a 1-mm diameter) and filtered through a nylon sieve (mesh size, 150 μm). The acini were purified by centrifugation (4 min at 50 x g) in KHB buffer containing 4% bovine serum albumin and 0.5 mM CaCl₂. Finally, the acini were resuspended in HR buffer (0.3–0.5 mg protein/ml) and preincubated for 30 min at 37°C before each experiment.

The biological activities of CCK-8 and CCK-JMV-180 were determined by measuring amylase release from the acini over a 30-min period at 37°C using blue starch as a substrate (12). Amylase release was expressed as percent of the amylase release from the acini incubated with 3 nM CCK-8. Protein concentration was determined by the method of Bradford (13) using bovine serum albumin as the standard.

**Contraction of gallbladder smooth muscle**

Circular ring muscle strips (2 - 3 mm in width and 3 - 5 mm in diameter) were prepared from the gallbladder. The strip was suspended in a 15-ml organ bath and connected via surgical silk to a force-displacement transducer for monitoring changes in isometric tension. The organ bath was filled with Tyrode solution (137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 1.0 mM MgCl₂, 0.4 mM NaH₂PO₄, 11.9 mM NaHCO₃ and 5.5 mM glucose) kept at 29°C and gassed with 95% O₂ : 5% CO₂. The muscle strip was equilibrated for 60–90 min under an initial tension of 1.5 g.

Agonistic effects of CCK-8, CCK-4 and CCK-JMV-180 were examined by cumulative application of each peptide. Antagonistic effects were evaluated by obtaining the cumulative dose-response curves for CCK-8 before and after a 60-min treatment with the drugs.

**RESULTS**

**Effects of CCK-8 and CCK-JMV-180 on the specific 125I-BH-CCK-8 binding to pancreatic membranes**

The competitive binding study showed that both CCK-8 and CCK-JMV-180 dose-dependently inhibited specific 125I-BH-CCK-8 (50 pM) binding to the pancreatic mem-
branes. Unlabeled CCK-8 caused a detectable inhibition of the binding of \(^{125}\text{I}-\text{BH-CCK-8}\) at 10 pM and complete inhibition at 10 nM (Fig. 1). CCK-JMV-180 caused a detectable inhibition at 300 pM and complete inhibition at 300 nM (Fig. 1). The IC\(_{50}\) values for CCK-8 and CCK-JMV-180 were 180±30 pM (n=4) and 6.1±1.0 nM (n=4), respectively.

**Effects of CCK-8 and CCK-JMV-180 on amylase release from pancreatic acini**

When the pancreatic acini were incubated with increasing concentrations of CCK-8 (10 pM–1 \(\mu\)M), stimulation of amylase release reached a maximum at 3 nM and then declined as the concentration of CCK-8 was further increased (Fig. 2). CCK-JMV-180 (1 nM–10 \(\mu\)M) also caused a dose-dependent amylase release stimulation, which plateaued and remained unchanged above 300 nM at about 50% of the maximal stimulation by CCK-8 (Fig. 2).

CCK-JMV-180 (100 nM–1 \(\mu\)M) dose-dependently caused rightward shifts of the downstroke of the dose-response curve for CCK-8 and depressed the maximal response by CCK-8 (Fig. 3A). The pA\(_{2}\) value obtained by Schild plot analysis was 7.5 (Fig. 3B).

**Effects of CCK-8 and CCK-JMV-180 on the contractile response of isolated gallbladder smooth muscle**

CCK-8 (100 pM–1 \(\mu\)M) caused a dose-dependent contraction of the isolated gallbladder smooth muscle, but CCK-JMV-180 (1 nM–10 \(\mu\)M) was devoid of this effect (Fig. 4). CCK-4 (1 nM–1 \(\mu\)M) also did not cause a contraction (data not shown). The dose-response curve for CCK-8 was shifted to the right 5- and 49-fold in the presence of 3 and 10 nM L-364,718, a CCK\(_{A}\)-receptor antagonist, respectively (Fig. 5). A high concentration of L-365,260 (100 nM), a CCK\(_{B}\) receptor antagonist, caused only a twofold rightward shift of the dose-response curve for CCK-8 (Fig. 5).

The dose-response curve for CCK-8 was progressively shifted to the right in the presence of increasing concentrations of CCK-JMV-180 (30–300 nM) without depression of the maximum response (Fig. 6A). The pA\(_{2}\) value obtained by Schild plot analysis was 7.9 (Fig. 6B).

**DISCUSSION**

In recent years, identification of the high and low affinity CCK\(_{A}\) receptor subtypes has become possible with the use of CCK-JMV-180, which can discriminate between those receptor subtypes in rat pancreas. In the present study, we examined the effects of CCK-JMV-180 on the CCK receptor functions of isolated rabbit pancreatic acini and gallbladder smooth muscle.

CCK-JMV-180 dose-dependently inhibited the specific \(^{125}\text{I}-\text{BH-CCK-8}\) binding to the pancreatic membranes and caused a complete inhibition at 300 nM. In isolated pancreatic acini, CCK-JMV-180 (1 nM–300 nM) caused...
a dose-dependent amylase release stimulation, which plateaued and remained unchanged above 300 nM at 50% of the maximal stimulation by CCK-8. The data from the competitive binding and amylase release studies suggest that CCK-JMV-180 acts as a partial agonist at the high affinity CCK_A receptor site of the pancreatic acini.

CCK-JMV-180 did not decrease the amylase release at the supramaximal concentrations (above 300 nM), and CCK-JMV-180 (100 nM - 1 μM) caused a rightward shift of the downstroke of the dose-response curve for CCK-8. These results indicate that CCK-JMV-180 acts as a competitive antagonist at the low affinity CCK receptor site of the pancreatic acini. The maximum response of amylase
release stimulated by CCK-8 was dose-dependently depressed by CCK-JMV-180. The possibility that CCK-JMV-180 binds irreversibly to the high affinity site or the dissociation of the bound CCK-JMV-180 is much slower than CCK-8 may explain this result.

In the isolated gallbladder smooth muscle, CCK-8 caused a dose-dependent contraction, but CCK-JMV-180 did not show this effect. It has been reported that CCK-8 acts as an agonist and CCK-JMV-180 as an antagonist at the CCKB receptor on small cell lung cancer (H345) cells (14). If so, contraction induced by CCK-8 could be mediated by CCKB receptors. The CCKA and CCKB receptors are characterized by the relative affinities of CCK-8 and CCK-4: the former shows equal affinities to the CCKA and CCKB receptors, while the latter shows an about 2000-fold higher affinity to the CCKB than to the CCKA receptors (15). CCK-8 (100 nM-1 μM) caused a dose-dependent contraction of the isolated gallbladder smooth muscle, but CCK-4 (1 nM-1 μM) did not show this effect. Furthermore, the contraction caused by CCK-8 was much more potently inhibited by the CCKA-receptor antagonist L-364,718 (9) than by the CCKB-receptor antagonist L-365,260 (16). CCK-JMV-180 caused a rightward shift of the dose-response curve for CCK-8. These results altogether suggest that the CCK-8-induced contraction of the rabbit gallbladder smooth muscle is mediated by the CCKA receptor as described in the guinea pig gallbladder (7) and that CCK-JMV-180 acts as a competitive antagonist at the CCKA receptor.

In comparison with exocrine secretion from the pancreatic acini, contraction of the gallbladder smooth muscle was observed at higher concentrations of CCK-8. CCK-JMV-180 acted as a competitive antagonist at the CCKA receptor in the gallbladder as it did at the low affinity CCKA receptor in the pancreas with approximately equal pA2 values. These findings suggest that the CCKA receptor associated with contraction of the gallbladder smooth muscle might be of the low affinity subtype.

Recently, Weerth et al. (17) have reported that guinea pig gallbladder and pancreas possess an identical CCKA-receptor subtype because of an identical nucleotide sequence of the cloned cDNA. Their study was not in accord with the previous proposals by Yanaihara et al. (18) and Jensen et al. (19) that the pancreas and gallbladder might possess different subtypes of the CCKA receptor. However, a study on Chinese hamster ovary cells transfected with the cloned rat CCKA receptor performed by Yule et al. (20) suggested that a single CCKA receptor protein may respond differently to different ligands such as CCK-8 and CCK-JMV-180 and then activate discrete transduction pathways. This possibility could explain our findings that CCK-8 and CCK-JMV-180 exert different pharmacological effects through the CCKA receptor in different tissues.
sparing receptors.

In summary, CCK-JMV-180 acts as a partial agonist at the high affinity CCKA receptor of pancreatic acini and acts as an antagonist at the low affinity CCKA receptor of the pancreatic acini and the gallbladder smooth muscle in rabbits. CCK-JMV-180 distinguishes between the CCKA receptors associated with pancreatic exocrine secretion in the acini and those involved in contraction of the isolated gallbladder smooth muscle.

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