ELSEVIER

European Journal of Pharmacology 304 (1996) 147–154

ejp

Pharmacological profile of T-0632, a novel potent and selective CCK_A receptor antagonist, in vitro

Hiroyuki Taniguchi *, Naoko Yazaki, Toshio Endo, Masaaki Nagasaki

Lead Optimization Research Laboratory, Tanabe Seiyaku Co., Ltd., Toda, Saitama 335, Japan

Received 26 October 1995; revised 22 January 1996; accepted 29 January 1996

Abstract

The pharmacological profile of a new CCK_A receptor antagonist, T-0632 [sodium (*S*)-3-[1-(2-fluorophenyl)-2,3-dihydro-3-[(3-isoquinolinyl)-carbonyl]amino-6-methoxy-2-oxo-1-H-indole]propanoate], was examined in in vitro studies and compared with those of L-364,718 [3*S*(−)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepine-3-yl)-1H-indole-2-carboxamide] and loxiglumide [D,L-4-(3,4-dichlorobenzoylamino)-5-(*N*-3-methoxypropyl-pentylamino)-5-oxopentanoic acid]. T-0632 inhibited the specific binding of [^{125}I]CCK-8 to rat pancreatic CCK_A receptor in a concentration-dependent and competitive manner. The K_i value of T-0632 for the CCK_A receptor was estimated to be 0.24 nM, which was 23 000-fold less than the K_i value (5600 nM) for guinea pig CCK_B receptor. L-364,718 and loxiglumide were 1500- and 64-fold selective for CCK_A over CCK_B receptor, respectively. T-0632, L-364,718 and loxiglumide inhibited CCK-8 (100 pM)-stimulated amylase release from rat pancreatic acini in a concentration-dependent manner with IC_{50} values of 5.0 nM, 5.0 nM and 3.0 μM, respectively. In the isolated rabbit gallbladder smooth muscle, T-0632 and loxiglumide competitively inhibited CCK-8-induced contraction with pA_2 values of 8.5 and 7.0, respectively. However, L-364,718 showed an apparent non-competitive antagonism. The IC_{50} values of T-0632, L-364,718 and loxiglumide for CCK-8 (30 nM)-induced contraction were 31 nM, 4.9 nM and 1300 nM, respectively. The inhibitory effects of T-0632 and loxiglumide in gallbladder smooth muscle were readily reversible, but L-364,718 showed a long-lasting inhibition. These results suggest that T-0632 is a potent, reversible and more selective CCK_A receptor antagonist compared with L-364,718 and loxiglumide.

Keywords: CCK_A receptor; Pancreas; Gallbladder; Amylase release; Gallbladder contraction; T-0632

1. Introduction

Cholecystokinin (CCK) is a gastrointestinal hormone and neurotransmitter that is found in the digestive tract and the central nervous system (review in Liddle, 1994). Receptors for CCK are classified into two types, CCK_A and CCK_B receptors (Innis and Snyder, 1980). CCK_A receptor is found mainly in the peripheral organs and also found in the restricted region of the brain, while CCK_B receptor is found mainly in the brain, but in periphery, gastrin receptor has been reported to be identical with CCK_B receptor in molecular structure (Pisegna et al., 1992; Lee et al., 1993).

In the gastrointestinal tract, CCK plays an important role in the control of gallbladder contraction, pancreatic secretion and gut motility via the CCK_A receptor (review in Crawley and Corwin, 1994). Recent studies have also demonstrated the possible importance of CCK in a wide variety of gastrointestinal disorders such as gastroesophageal reflux disease (Perdikis et al., 1994), non-ulcer dyspepsia (Chua et al., 1993), pancreatitis (Modlin et al., 1989; Cerezo et al., 1991), biliary colic (Beglinger et al., 1989) and irritable bowel syndrome (Cann et al., 1993).

Recently, a number of selective antagonists for CCK_A receptors have been reported including L-364,718 (Chang and Lotti, 1986) and loxiglumide (Setnikar et al., 1987). Although these compounds have provided valuable informations on the role of CCK_A receptors, they have some limitations in their use as pharmacological tools and therapeutic agents. For example, L-364,718 shows poor aqueous solubility and loxiglumide possesses relatively weak affinity and low selectivity for CCK_A receptor.

T-0632 (Fig. 1) is a novel non-peptide and water-soluble CCK_A receptor antagonist synthesized in our laboratory. In the present report, the pharmacological properties of T-0632 were examined in in vitro studies and compared

* Corresponding author. Lead Optimization Research Laboratory, Tanabe Seiyaku Co., Ltd., 2-2-50 Kawagishi, Toda, Saitama 335, Japan. Tel.: (81) (48) 433-8046; fax: (81) (48) 433-8157.

PII S0014-2999(96)00091-X

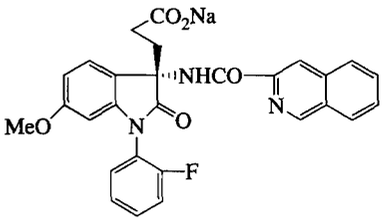

Fig. 1. Chemical structure of T-0632.

with those of representative CCK_A receptor antagonists, L-364,718 and loxiglumide. Our results demonstrate that T-0632 is a potent, reversible and more selective CCK_A receptor antagonist than L-364,718 and loxiglumide.

2. Materials and methods

2.1. Receptor binding studies for CCK_A and CCK_B receptors

Receptor binding studies were performed according to those discribed by others (Sankaran et al., 1982; Dijk et al., 1984; Chang and Lotti, 1986) for CCK_A receptor using rat pancreas and CCK_B receptor using guinea pig cerebral cortex with slight modifications.

The pancreatic tissue from Sprague-Dawley rat was homogenized in 20 vols. of an ice-cold 50 mM Tris-HCl buffer (pH 7.4 at 25°C) containing 0.01% soybean trypsin inhibitor (Sigma, type II-S) and centrifuged at $50\,000 \times g$ for 10 min. The pellet was resuspended in the same volume of the buffer and then recentrifuged at $50\,000 \times g$ for 10 min. The resulting pellet was resuspended in an incubation medium (mM: Tris 50, $MgCl_2$ 5, dithiothreitol 5) containing 0.14 mg/ml bacitracin, 0.1% bovine serum albumin, 0.01% soybean trypsin inhibitor and pH was adjusted to 7.4 at 25°C. The cerebral cortex from Hartley guinea pig was homogenized in 20 vols. of an ice-cold 320 mM sucrose, centrifuged at $1000 \times g$ for 10 min, followed by recentrifugation of the supernatant at $140\,000 \times g$ for 45 min. The pellet was resuspended in the same volume of HEPES buffer (mM: HEPES 10, NaCl 130, $MgCl_2$ 5; pH 7.4 at 25°C), centrifuged at $48\,000 \times g$ for 10 min. The resulting pellet was resuspended in the incubation medium (HEPES buffer containing 0.2 mg/ml bacitracin, 1 μg/ml phenylmethylsulfonyl fluoride). All the procedures were conducted at 4°C. Protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as the standard.

The competitive binding study was performed by incubating the membrane preparations (1 mg wet tissue/ml for pancreas and 10 mg wet tissue/ml for brain) in each incubation medium (300 μl) containing [^{125}I]CCK-8 (50 pM) and various concentrations of the drugs. For the saturation binding study, the membrane preparations were incubated with various concentrations of [^{125}I]CCK-8 (0.02–1 nM). Incubation was continued for 90 min at 25°C and terminated by adding ice-cold Tris-HCl buffer. Free and bound [^{125}I]CCK-8 were separated by filtration through the Whatman GF/B glass fiber filter. Non-specific binding was estimated from a parallel assay in the presence of a large excess (1 μM) of CCK-8. Each determination was carried out in duplicate. Specific [^{125}I]CCK-8 binding in the competition binding study, defined as the difference between the total and non-specific binding, was about 85–95% and 80–90% of the total binding in pancreas and brain, respectively.

2.2. Receptor binding studies for other receptors

Receptor binding studies for α_1-, α_2- and β-adrenoceptor; muscarinic M_2 and M_3; dopamine D_1 and D_2; histamine H_1; 5-HT_1 and 5-HT_2; μ-, δ- and κ-opioid receptors were performed as described elsewhere using [^{3}H]prazosin (rat brain); [^{3}H]rauwolscine (rat brain); [^{3}H]CGP-12177 (rat brain); [^{3}H]*N*-methyl scopolamine (rat heart); [^{3}H]*N*-methyl scopolamine (rat submaxillary salivary glands); [^{3}H]SCH-23390 (human recombinant); [^{3}H]spiperone (human recombinant); [^{3}H]pyrilamine (guinea pig brain); [^{3}H]5-HT (rat brain); [^{3}H]ketanserin (rat brain); [^{3}H][D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]enkephalin (DAMGO; guinea pig brain); [^{3}H][D-Pen^{2,5}]enkephalin (DPDPE; guinea pig brain); [^{3}H]U-69593 (guinea pig brain), respectively.

2.3. Preparation of pancreatic acini and amylase release assay

Preparation of the rat (Sprague-Dawley) pancreatic acini and amylase release experiments were performed according to the procedures described previously (Williams et al., 1978) with minor modifications (Taniguchi et al., 1995).

The medium used for the preparation of pancreatic acini was a modified Krebs-Henseleit bicarbonate (KHB) buffer (mM: NaCl 110, $NaHCO_3$ 32.5, KCl 4.7, $MgCl_2$ 1.13, Na_2HPO_4 1, glutamine 2, glucose 11.1) containing 0.01% soybean trypsin inhibitor (Sigma, type II-S) and minimal Eagle's medium amino acid supplement. The medium used for the assay of amylase release was a HEPES-Ringer (HR) buffer (mM: NaCl 127, KCl 4.7, $CaCl_2$ 1.2, $MgCl_2$ 0.56, Na_2HPO_4 1, HEPES 10, glutamine 2, glucose 11.1) 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% O_2 : 5% CO_2 and pH was adjusted to 7.4.

The KHB buffer containing 200 U/ml purified collagenase, 0.03 mg/ml chymotrypsin (both from Worthington Biochemicals) and 1.8 mg/ml hyaluronidase (Sigma, type I-S) was injected into the isolated pancreas to make it swell and incubated at 37°C for 10 min. The swollen pancreas was minced and reincubated with fresh medium

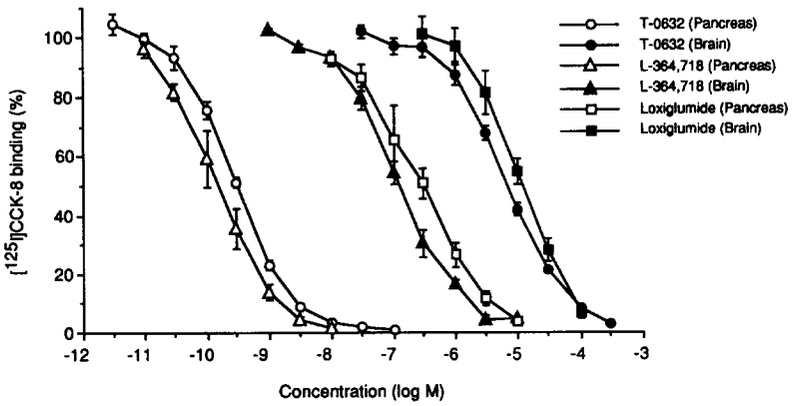

Fig. 2. Inhibition of the specific [^{125}I]CCK-8 binding to rat pancreatic (○, △, □) and guinea pig cerebral cortex (●, ▲, ■) membranes by T-0632, L-364,718 and loxiglumide, respectively. Membranes were incubated with 50 pM [^{125}I]CCK-8 in the presence of increasing concentrations of T-0632 (○, ●), L-364,718 (△, ▲) and loxiglumide (□, ■) for 90 min at 25°C. Each point indicates the mean ± S.E.M. of three to four experiments, each performed in duplicate.

under the same conditions for 30 min. The digested pancreas was then dissociated by sucking up and down through pipettes with decreasing orifice size (first with a 2-mm and then a 1-mm in diameter) and filtered through a nylon sieve (mesh size 150 μm). The acini were purified by centrifugation (4 min at 50 × *g*) in KHB buffer containing 4% bovine serum albumin and 0.5 mM $CaCl_2$. Finally, the acini were resuspended in HR buffer (0.3–0.6 mg protein/ml) and preincubated for 30 min at 37°C before each experiment.

Amylase release was expressed as percent of the amylase release from the acini incubated with 100 pM CCK-8 over a 30-min period at 37°C measured by using blue starch as a substrate (Ceska et al., 1969). The drugs were added 10 min prior to the incubation with CCK-8. Protein concentration was determined by the method of Bradford (1976) as described above.

2.4. Contraction of gallbladder smooth muscle

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

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 (mM: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.4, $NaHCO_3$ 11.9, glucose 5.5) kept at 29°C and gassed with 95% O_2 : 5% CO_2. The muscle strip was equilibrated for 60–90 min under an initial tension of 1.5 g.

Antagonistic effects were evaluated by obtaining the cumulative concentration-response curves for CCK-8 before and after a 60 min treatment with the drugs. The IC_{50} values were estimated from the inhibitory effects on CCK-8 (30 nM)-induced contraction.

To examine the reversibility of inhibitory effects of the drugs, CCK-8 (30 nM)-induced contraction was repeatedly evoked every 55 min before, during and after the treatment of drugs for 10 or 40 min.

2.5. Drugs

[^{125}I]CCK-8 (2200 Ci/mmol) was purchased from Du Pont-New England Nuclear. T-0632 [sodium (*S*)-3-[1-(2-fluorophenyl)-2,3-dihydro-3-[(3-isoquinolinyl)-carbonyl]-amino-6-methoxy-2-oxo-1-H-indole]propanoate], L-364,718 [3*S*(−)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepine-3-yl)-1H-indole-2-carboxamide]

Table 1
K_i values of T-0632, L-364,718 and loxiglumide for the specific binding of [^{125}I]CCK-8 to rat pancreas and guinea pig brain membranes

Compound	Pancreas		Brain		Selectivity
	K_i nM	n_H [a]	K_i nM	n_H	Brain/pancreas
T-0632	0.24 (0.10–0.57)	0.99 (0.85–1.1)	5600 (2100–16000)	0.93 (0.65–1.2)	23000
L-364,718	0.087 (0.036–0.21)	1.1 (0.84–1.3)	130 (71–250)	0.87 (0.78–0.95)	1500
Loxiglumide	150 (43–500)	0.85 (0.71–0.99)	9600 (3900–24000)	1.1 (0.64–1.5)	64

Values are given as mean and 95% confidence limits in parentheses from three to four experiments. K_i values were calculated according to the formula $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the radioligand concentration, and K_d is the dissociation constants of radioligand. [a] n_H, pseudo-Hill coefficient.

and loxiglumide [D,L-4-(3,4-dichlorobenzoylamino)-5-(*N*-3-methoxypropyl-pentylamino)-5-oxopentanoic acid] were synthesized at the Lead Optimization Research Laboratory of Tanabe Seiyaku. CCK-8 was purchased from the Peptide Institute (Osaka, Japan). All other chemicals used were of reagent grade.

2.6. Statistics

Regression analysis was used for estimation of IC_{50} values and 95% CL. Means were compared using Student's *t*-test. Probabilities of less than 5% ($P < 0.05$) were considered significant.

3. Results

3.1. Receptor binding studies for CCK_A and CCK_B receptor

T-0632, L-364,718 and loxiglumide inhibited [^{125}I] CCK-8 binding to both rat pancreatic and guinea pig cerebral cortex membranes in a concentration-dependent manner (Fig. 2). The inhibition constants (K_i) of T-0632 and L-364,718 for the rat pancreatic CCK_A receptor were 0.24 nM and 0.087 nM, respectively (Table 1). The affinity of T-0632 for the CCK_A receptor was about 600-fold higher than that of loxiglumide (K_i = 150 nM; Table 1). In the guinea pig cerebral cortex CCK_B receptor, however, T-0632 showed very low affinity (K_i = 5600 nM) as compared with L-364,718 (K_i = 130 nM) (Fig. 2, Table 1). T-0632 possessed 23000-fold higher selectivity for the CCK_A receptor than for the CCK_B receptor (Table 1).

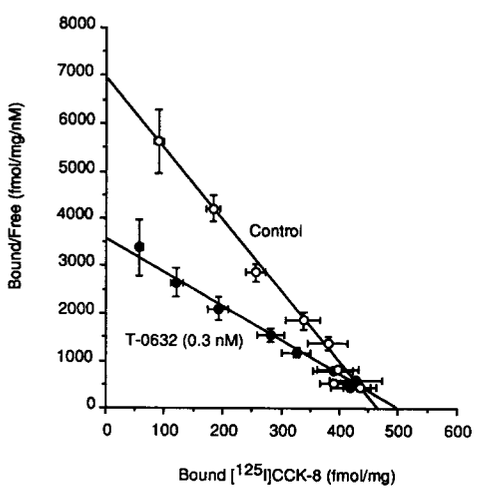

Fig. 3. Scatchard plots for the specific [^{125}I]CCK-8 binding to rat pancreatic membranes. Membranes were incubated with various concentrations of [^{125}I]CCK-8 (0.02–1 nM) for 90 min at 25°C in the absense (○) and presence (●) of 0.3 nM T-0632. Each value is the mean ± S.E.M. of five experiments, each performed in duplicate.

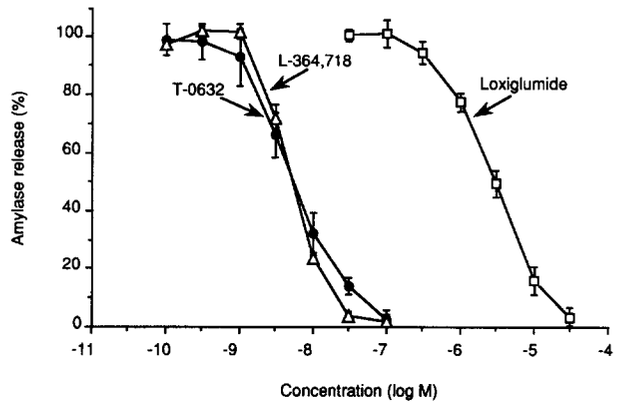

Fig. 4. Effects of T-0632, L-364,718 and loxiglumide on CCK-8 (100 pM)-stimulated amylase release from rat pancreatic acini. In each experiment, basal release was subtracted and stimulated release in the presence of T-0632 (●), L-364,718 (△) and loxiglumide (□) was calculated as percentage of the release in the absence of antagonists. Each point indicates the mean ± S.E.M. of four to five experiments, each performed in duplicate.

T-0632 also exhibited very low affinity for CCK_B receptor in the rat cerebral cortex (IC_{50} = 15000 (95% CL; 11000–20000) nM, n = 3) and had no affinity (IC_{50} > 10 μM) for α_1-, α_2- and β-adrenoceptor; muscarinic M_2 and M_3; dopamine D_1 and D_2; histamine H_1; 5-HT_1 and 5-HT_2; μ-, δ- and κ-opioid receptors (data not shown).

Fig. 3 shows Scatchard analysis of the specific [^{125}I]CCK-8 binding to the rat pancreatic membranes in the absence (control) and presence of T-0632 (0.3 nM). The equilibrium dissociation constant (K_d) in the control and T-0632-treated membranes were 0.067 (95% CL; 0.047–0.095) nM and 0.14 (95% CL; 0.068–0.29) nM, respectively. The maximum number of binding sites (B_{max}) in the T-0632-treated membranes was 500 (95% CL; 310–700) fmol/mg protein, which was not significantly different from the value in the control (460 (95% CL; 310–600) fmol/mg protein). The Hill coefficients in the control and T-0632-treated membranes were 0.97 (95% CL; 0.88–1.1) and 1.05 (95% CL; 0.87–1.2), respectively.

3.2. Effects of T-0632, L-364,718 and loxiglumide on CCK-8-stimulated amylase release from pancreatic acini

When the dispersed acini from rat pancreas were incubated with increasing concentration of CCK-8 (1 pM–10 nM), stimulation of amylase release reached a maximum at 100 pM and then declined as the concentration of CCK-8 was further increased (data not shown). Stimulation with 100 pM CCK-8 caused 5.2 ± 0.7-fold increase in amylase release compared with basal release. T-0632, L-364,718 and loxiglumide caused a concentration-dependent inhibition of the amylase release stimulated by CCK-8 (100 pM), and the IC_{50} values were 5.0 nM, 5.0 nM and 3000 nM, respectively (Fig. 4, Table 2).

3.3. Effects of T-0632, L-364,718 and loxiglumide on CCK-8-induced contractile response of isolated gallbladder smooth muscle

CCK-8 (100 pM–1 μM) caused a concentration-dependent contraction of the rabbit isolated gallbladder smooth muscle. The concentration-response curve for CCK-8 was shifted to the right 5-, 10- and 49-fold in the presence of 10, 30 and 100 nM T-0632, respectively (Fig. 5A). L-364,718 (3–30 nM) and loxiglumide (0.3–3 μM) also caused rightward shift of the concentration-response curve for CCK-8 (Fig. 5B,C).

The slope of Schild plots for T-0632 and loxiglumide were not significantly different from unity (0.87 ± 0.08 and 0.81 ± 0.17, respectively), but that for L-364,718 was greater than unity (1.4 ± 0.2). The pA_2 values for T-0632 and loxiglumide were 8.5 and 7.0, respectively. The IC_{50} values of T-0632, L-364,718 and loxiglumide for CCK-8 (30 nM)-induced contraction of the smooth muscle were 31 nM, 4.9 nM and 1300 nM, respectively (Table 2).

Table 2
Inhibition of CCK-8-stimulated amylase release from pancreatic acini and CCK-8-induced contraction of gallbladder smooth muscle

Compound	Pancreas IC_{50} nM	Gallbladder IC_{50} nM	Selectivity gallbladder/pancreas
T-0632	5.0 (1.5–16.5)	31 (24–40)	6.2
L-364,718	5.0 (3.7–6.8)	4.9 (4.4–5.4)	1.0
Loxiglumide	3000 (1700–5400)	1300 (1100–1600)	0.43

Values are given as mean and 95% confidence limits in parentheses from three to five experiments.

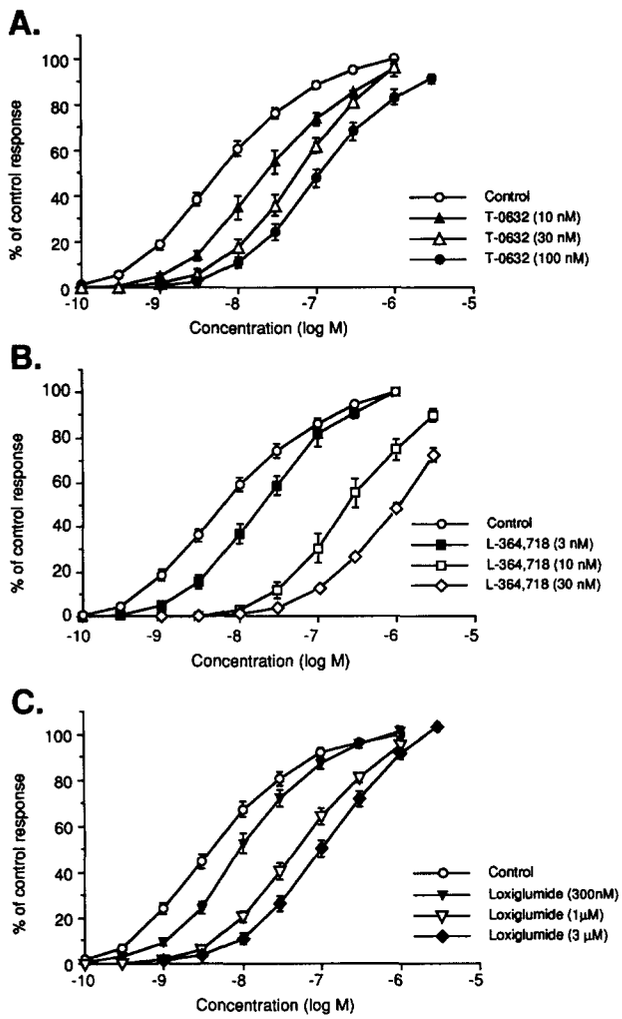

Fig. 5. Antagonistic effects of T-0632, L-364,718 and loxiglumide on the contractile response of the isolated gallbladder smooth muscle to CCK-8. CCK-8 (0.1–1000 nM) was cumulatively applied to obtain the concentration–response curve (control; ○). (A) Effect of T-0632 at 10 nM (▲), 30 nM (△) and 100 nM (●); (B) effect of L-364,718 at 3 nM (■), 10 nM (□) and 30 nM (◇); (C) effect of loxiglumide at 300 nM (▼), 1 μM (▽) and 3 μM (◆). Relative amplitude of contraction was expressed as percentage of the response to 1 μM of CCK-8 in the absence of antagonist. Each value is the mean ± S.E.M. of three to five experiments.

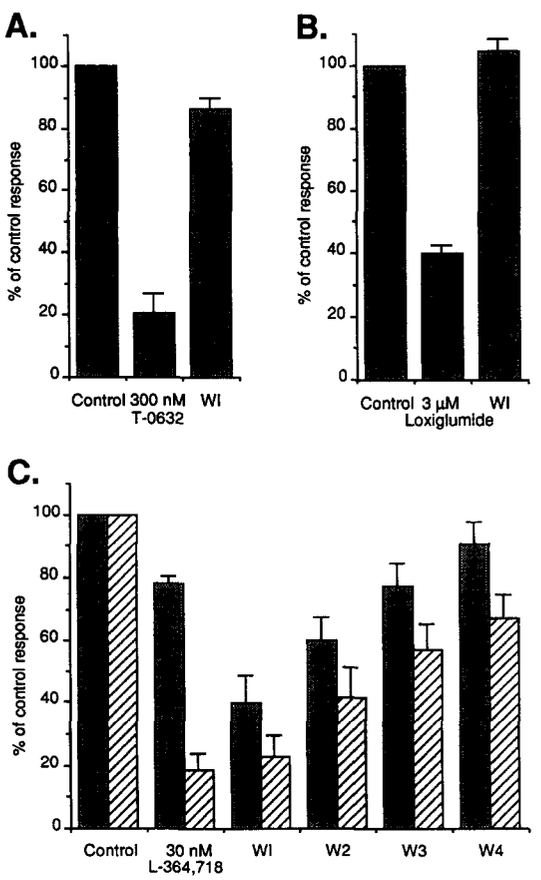

Fig. 6. Reversibility of the inhibitory effects of T-0632, L-364,718 and loxiglumide on CCK-8-induced contractile response of the isolated gallbladder smooth muscle. CCK-8 (30 nM)-induced contraction was repeatedly evoked every 55 min before, during and after the treatment of T-0632 (300 nM; A) and loxiglumide (3 μM; B) for 10 min. (C) The contractile response was evoked before and during the treatment of L-364,718 (30 nM) for 10 min (stippled columns) or 40 min (hatched columns) and after the washout of the drug for 4 times (W1–W4). Each value is the mean ± S.E.M. of four experiments.

3.4. Reversibility of the inhibitory effects of T-0632, L-364,718 and loxiglumide on CCK-8-induced contractile response of isolated gallbladder smooth muscle

The amplitudes of CCK-8 (30 nM)-induced contraction of the gallbladder smooth muscle in the presence of T-0632 (300 nM) or loxiglumide (3 μM) for 10 min were about 20 or 40% of the respective control responses. Both of these effects were readily disappeared by washout of the drugs (Fig. 6A,B). Inhibitory effect of L-364,718 (30 nM) after the first washout of the drug (W1) was greater than that in the presence of L-364,718, and then gradually decreased by the repeated washout. The inhibitory effect of L-364,718 was enhanced by prolonging the incubation time with the drug to 40 min (Fig. 6C).

4. Discussion

In the present study, we evaluated the pharmacological properties of T-0632 in in vitro studies and compared with those of representative CCK_A receptor antagonists, L-364,718 and loxiglumide.

T-0632 inhibited the specific binding of [^{125}I]CCK-8 to CCK_A receptor in rat pancreatic membranes in a concentration-dependent manner with the slope factor of the pseudo-Hill plot being almost unity, indicating that T-0632 have a single class of binding sites and no cooperative interaction. Scatchard analysis showed that the K_d value of CCK-8 for the rat pancreatic CCK_A receptor was 0.067 nM, that was similar to those previously reported (Dijk et al., 1984; Chang and Lotti, 1986). T-0632 (0.3 nM) increased the K_d value of CCK-8 (K_d = 0.067 to 0.15 nM) without modifying the B_{max}. These results indicate that T-0632 competitively inhibits [^{125}I]CCK-8 binding to the CCK_A receptor. The affinity of T-0632 for the rat CCK_A receptor was 23 000 times higher than that for the guinea pig CCK_B receptor. The selectivity of T-0632 for the CCK_A receptor was higher than that of L-364,718 (Chang and Lotti, 1986), loxiglumide (Setnikar et al., 1987), SR 27897 (Gully et al., 1993), 2-NAP (McDonald et al., 1993), KSG-504 (Yamazaki et al., 1993) and FK480 (Ito et al., 1994). To exclude the possibility that the high selectivity for the CCK_A receptor is due to species difference, we examined the affinity of T-0632 for the rat cerebral cortex CCK receptor and confirmed that T-0632 possessed also very low affinity for the rat CCK_B receptor. Furtheremore, the selectivity of T-0632 for the CCK_A receptor was shown by its lack of the affinity for the other receptors in various radioligand binding assays (IC_{50} > 10 μM). These results suggest that T-0632 is a potent and highly selective CCK_A receptor ligand.

In the isolated gallbladder smooth muscle, T-0632 and loxiglumide caused a rightward shift of the concentration-response curves for CCK-8-induced contraction without reducing the maximal responses. The slopes of Schild plots were not significantly different from unity in the both cases, suggesting the competitive antagonism. However, the slope for L-364,718 was 1.4, indicating that L-364,718 non-competitively inhibited the CCK-8-induced contraction. In receptor binding study, however, L-364,718 have been reported to interact competitively with CCK_A receptor (Chang and Lotti, 1986). This discrepancy may be explained by supposing the dissociation rate of L-364,718 from CCK_A receptor is much slower than that of CCK-8. Similar observations and explanations have been reported on other receptors such as 5-HT (Bond et al., 1989), histamine H_2 (Pendleton et al., 1983; Muramatsu et al., 1991) and angiotensin II (Wienen et al., 1992; Chang et al., 1992; Noda et al., 1993).

To confirm the above-mentioned hypothesis, reversibility of the inhibitory effects of the antagonists on CCK-8-induced contractile response of the isolated gallbladder smooth muscle were examined. The inhibitory effect of T-0632 was fully reversible. Loxiglumide also showed good reversibility as in the case that reported in isolated rat pancreatic acini (Otsuki et al., 1989). In contrast to T-0632 and loxiglumide, after the first washout of L-364,718, the residual inhibitory effect was greater than that in the presence of the drug. After that, the inhibitory effect was decreased gradually by repeating washout. Moreover, the inhibitory effect of L-364,718 was enhanced by prolonging the incubation time with the drug. Similar observations have been reported on the tachykinin NK_2 receptor antagonist, SR 48968 (Patacchini et al., 1994). Although this interesting phenomenon can not be explained cleary at present, these results may suggest that association and dissociation rates of L-364,718 for CCK_A receptor are slower than those of T-0632 and loxiglumide. The long-lasting inhibitory effects of the benzodiazepine CCK_A receptor antagonists were reported by others in isolated rat pancreatic acini (Akiyama and Otsuki, 1994; Bhat et al., 1994) and in oocyte in which rat pancreatic CCK_A receptor had been expressed (Wank et al., 1992).

The inhibitory effects of L-364,718 and loxiglumide on the gallbladder contraction were almost equivalent to the respective effects on pancreatic amylase release (Table 2). In contrast, the inhibitory effect of T-0632 on gallbladder contraction was about 6-fold less potent than that on pancreatic amylase release. We have recently reported the possibility that the CCK-stimulated pancreatic exocrine secretion and gallbladder contraction may be mediated through the high- and low-affinity CCK_A receptor site, respectively (Taniguchi et al., 1995). These finding may suggest that T-0632 prefer to interact with the high-affinity CCK_A receptor site rather than the low-affinity site. Further work needs to be done to evaluate this speculation, because the selectivity of T-0632 for the pancreatic acini could be due to species difference in the present preparations.

The IC_{50} values for CCK-8-induced amylase release

were higher than those for [^{125}I]CCK-8 displacement from the receptor of pancreatic membranes. For receptor binding study, we used the pancreatic membranes that did not discriminate between acini and islets of Langerhans which also possess binding sites for CCK (Verspohl et al., 1986). The possibility that CCK receptors on pancreatic B cells may be different from those on pancreatic acinar cells in terms of their relative affinities for various antagonists have been reported by others (Verspohl et al., 1986; Okabayashi et al., 1990). The above-mentioned discrepancy between both of the IC_{50} values could be partly explained by the contamination of CCK receptor of pancreatic B cells in the pancreatic membranes preparation.

The present studies demonstrate that T-0632 is an extremely potent, competitive and highly selective antagonist of CCK_A receptors in in vitro radioligand receptor binding and functional isolated tissue assays. Furthermore, T-0632 shows an excellent reversibility of the antagonistic action on CCK_A receptor. Therefore, T-0632 may be useful as a pharmacological tool to investigate the function of CCK_A receptors and also have important clinical utility for gastrointestinal disorders in which CCK is possibly involved.

References

Akiyama, T. and M. Otsuki, 1994, Characterization of a new cholecystokinin receptor antagonist FK480 in in vitro isolated rat pancreatic acini, Pancreas 9, 324.

Beglinger, C., S. Dill, B. Meyer, B. Werth and G. Adler, 1989, Treatment of biliary colic with loxiglumide, Lancet 8655, 167.

Bhat, S.T., V.D. Talkad, D.A. Pollo and J.D. Gardner, 1994, Characterization of a persistent inhibitory action of L-364,718 on cholecystokinin-stimulated enzyme secretion in pancreatic acini, Pancreas 9, 101.

Bond, R.A., A.G. Ornstein and D.E. Clarke, 1989, Unsurmountable antagonism to 5-hydroxytryptamine in rat kidney results from pseudoirreversible inhibition rather than multiple receptors or allosteric receptor modulation, J. Pharmacol. Exp. Ther. 249, 401.

Bradford, M.M., 1976, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72, 248.

Cann, P.A., L.C. Rovati, H. Smart, R.C. Spiller and P.J. Whorwell, 1993, Loxiglumide, a CCK-A antagonist, in irritable bowel syndrome: a pilot multicenter clinical study, Gastroenterology 104, A486.

Cerezo, J.G., R. Codoceo, P.F. Calle, F. Molina, J.M. Tenias and J.J. Vazquez, 1991, Basal and postprandial cholecystokinin values in chronic pancreatitis with and without abdominal pain, Digestion 48, 134.

Ceska, M., K. Birath and B. Brown, 1969, A new and rapid method for the clinical determination of α-amylase activities in human serum and urine. Optimal conditions, Clin. Chim. Acta 26, 437.

Chang, R.S.L. and V.J. Lotti, 1986, Biochemical and pharmacological characterization of an extremely potent and selective nonpeptide cholecystokinin antagonist, Proc. Natl. Acad. Sci. USA 83, 4923.

Chang, R.S.L., P.K.S. Siegl, B.V. Clineschmidt, N.B. Mantlo, P.K. Chakravarty, W.J. Greenlee, A.A. Patchett and V.J. Lotti, 1992, In vitro pharmacology of L-158,809, a new highly potent and selective angiotensin II receptor antagonist, J. Pharmacol. Exp. Ther. 262, 133.

Chua, A., M. Bekkering, L.C. Rovati and P.W.N. Keeling, 1993, Clinical efficacy and prokinetic effect of the CCK-A antagonist loxiglumide in non-ulcer dyspepsia, Gastroenterology 104, A491.

Crawley, J.N. and R.L. Corwin, 1994, Biological actions of cholecystokinin, Peptides 15, 731.

Dijk, A.V., J.G. Richards, A. Trzeciak, D. Gillessen and H. Möhler, 1984, Cholecystokinin receptors: biochemical demonstration and autoradiographical localization in rat brain and pancreas using [^{3}H]cholecystokinin$_8$ as radioligand, J. Neurosci. 4, 1021.

Gully, D., D. Fréhel, C. Marcy, A. Spinazzé, L. Lespy, G. Neliat, J.P. Maffrand and G.L. Fur, 1993, Peripheral biological activity of SR 27897: a new potent non-peptide antagonist of CCK_A receptors, Eur. J. Pharmacol. 232, 13.

Innis, R.B. and S.H. Snyder, 1980, Distinct cholecystokinin receptors in brain and pancreas, Proc. Natl. Acad. Sci. USA 77, 6917.

Ito, H., H. Sogabe, T. Nakarai, Y. Sato, M. Tomoi, M. Kadowaki, M. Matsuo, K. Tokoro and K. Yoshida, 1994, Pharmacological profile of FK480, a novel cholecystokinin type-A receptor antagonist: comparison to loxiglumide, J. Pharmacol. Exp. Ther. 268, 571.

Lee, Y.M., M. Beinborn, E.W. McBride, M. Lu, L.F. Kolakowski, Jr. and A.S. Kopin, 1993, The human brain cholecystokinin-B/gastrin receptor, J. Biol. Chem. 268, 8164.

Liddle, R.A., 1994, Cholecystokinin, in: Gut Peptide, eds. J.H. Walsh and G.J. Dockray (Raven Press, New York) p. 175.

McDonald, I.M., M.J. Bodkin, H.B. Broughton, D.J. Dunstone, S.B. Kalindjian and C.M.R. Low, 1993, 2-NAP: a selective, hydrophilic, non-peptide CCK_A-receptor antagonist derived from the cholecystokinin C-terminal dipeptide, Biomed. Chem. Lett. 3, 1511.

Modlin, I.M., A.J. Bilchik and K.A. Zucker, 1989, Cholecystokinin augmentation of 'surgical' pancreatitis: benefits of receptor blockade, Arch. Surg. 124, 574.

Muramatsu, M., H. Hirose-Kijima, H. Aihara and S. Otomo, 1991, Time-dependent interaction of a new H_2-receptor antagonist, IT-066, with the receptor in the atria of guinea pig, Jpn. J. Pharmacol. 57, 13.

Noda, M., Y. Shibouta, Y. Inada, M. Ojima, T. Wada, T. Sanada, K. Kubo, Y. Kohara, T. Naka and K. Nishikawa, 1993, Inhibition of rabbit aortic angiotensin II (AII) receptor by CV-11974, a new nonpeptide AII antagonist, Biochem. Pharmacol. 46, 311.

Okabayashi, Y., M. Otsuki, T. Nakamura, M. Fujii, S. Tani, T. Fujisawa, M. Koide, H. Hasegawa and S. Baba, 1990, Proglumide analogues CR 1409 and CR 1392 inhibit cholecystokinin-stimulated insulin release more potently than exocrine secretion from the isolated perfused rat pancreas, Pancreas 5, 291.

Otsuki, M., M. Fujii, T. Nakamura, Y. Okabayashi, S. Tani, T. Fujisawa, M. Koide and S. Baba, 1989, Loxiglumide: a new proglumide analog with potent cholecystokinin antagonistic activity in the rat pancreas, Dig. Dis. Sci. 34, 857.

Patacchini, R., R.D. Giorgio, A. Giachetti and C.A. Maggi, 1994, Different mechanism of tachykinin NK_2 receptor blockade by SR 48968 and MEN 10,627 in the guinea-pig isolated gallbladder and colon, Eur. J. Pharmacol. 271, 111.

Pendleton, R.G., M.L. Torchiana, C. Chung, P. Cook, S. Wiese and B.V. Clineschmidt, 1983, Studies on MK-208 (YM-11170) a new, slowly dissociable H_2-receptor antagonist, Arch. Int. Pharmanodyn. 266, 4.

Perdikis, G., P. Wilson, R.A. Hinder, E.J. Redmond, G.J. Wetscher, S. Saeki and T.E. Adrian, 1994, Gastroesophageal reflux disease is associated with enteric hormone abnormalities, Am. J. Surg. 167, 186.

Pisegna, J.R., A.D. Weerth, K. Huppi and S.A. Wank, 1992, Molecular cloning of the human brain and gastric cholecystokinin receptor: structure, functional expression and chromosomal localization, Biochem. Biophys. Res. Commun. 189, 296.

Sankaran, H., I.D. Goldfine, A. Bailey, V. Licko and J.A. Williams, 1982, Relationship of cholecystokinin receptor binding to regulation of biological functions in pancreatic acini, Am. J. Physiol. 242, G250.

Setnikar, I., M. Bani, R. Cereda, R. Chisté, F. Makovec, M.A. Pacini, L. Revel, L.C. Rovati and L.A. Rovati, 1987, Pharmacological characterisation of a new potent and specific nonpolypeptidic cholecystokinin antagonist, Arzneim. Forsch./Drug Res. 37, 703.

Taniguchi, H., M. Nagasaki and H. Tamaki, 1995, Effects of cholecystokinin (CCK)-JMV-180 on the CCK receptors of rabbit pancreatic acini and gallbladder smooth muscle, Jpn. J. Pharmacol. 67, 219.

Verspohl, E.J., H.P.T. Ammon, J.A. Williams and I.D. Goldfine, 1986, Evidence that cholecystokinin interacts with specific receptors and regulates insulin release in isolated rat islets of Langerhans, Diabetes 35, 38.

Wank, S.A., R. Harkins, R.T. Jensen, H. Shapira, A.D. Weerth and T. Slattery, 1992, Purification, molecular cloning, and functional expression of the cholecystokinin receptor from rat pancreas, Proc. Natl. Acad. Sci. USA 89, 3125.

Wienen, W., A.B.M. Mauz, J.C.A. Meel and M. Entzeroth, 1992, Different types of receptor interaction of peptide and nonpeptide angiotensin II antagonists revealed by receptor binding and functional studies, Mol. Pharmacol. 41, 1081.

Williams, J.A., M. Korc and R.L. Dormer, 1978, Action of secretagogues on a new preparation of functionally intact, isolated pancreatic acini, Am. J. Physiol. 235, E517.

Yamazaki, Y., M. Akahane, M. Kobayashi, M. Kitazawa, Y. Kurashina and K. Iizuka, 1993, Pharmacological profile of KSG-504, a new cholecystokinin-A-receptor antagonist, Jpn. J. Pharmacol. 63, 219.