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Characterization of the Binding of [^{3}H]-(±)-L-364,718: A New Potent, Nonpeptide Cholecystokinin Antagonist Radioligand Selective for Peripheral Receptors

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SUMMARY

[^{3}H]-(±)-L-364,718 a new, potent and selective nonpeptide peripheral cholecystokinin (CCK) antagonist bound saturably and reversibly to rat pancreatic membranes. The radioligand recognized a single class of binding sites with a high affinity (K_d = 0.23 nM). The binding of [^{3}H]-(±)-L-364,718 was stereospecific in that the more biologically active (−)-enantiomer demonstrated greater potency than the (+)-enantiomer. The rank order of potency of various CCK agonists and antagonists in displacing [^{3}H]-(±)-L-364,718 correlated with their ability to displace [^{125}I]CCK-8 and their known pharmacological activities in peripheral tissues. However, the absolute potencies of agonists were greater in displacing [^{125}I]CCK-8 than [^{3}H]-(±)-L-364,718. As described for other physiologically relevant receptor systems, the potency for displacement of [^{3}H]-(±)-L-364,718 binding by CCK agonists, but not antagonists, was reduced by guanosine 5′-(β,γ-imido)triphosphate and NaCl and enhanced by $MgCl_2$. [^{3}H]-(±)-L-364,718 also demonstrated specific binding to bovine gall bladder tissue but not guinea pig brain or gastric glands, consistent with its selectivity as a peripheral CCK antagonist. [^{3}H]-(±)-L-364,718 binding to pancreatic membranes was not affected by various pharmacological agents known to interact with other common peptide and nonpeptide receptor systems. These data indicate that [^{3}H]-(±)-L-364,718 represents a new potent nonpeptide antagonist radioligand for the study of peripheral CCK receptors which may allow differentiation of agonist and antagonist interactions.

CCK is a recognized peptide hormone and proposed neurotransmitter which is found in the gut and the central nervous system (1). Distinct CCK receptors in peripheral and brain tissues have been characterized using radiolabeled CCK analogues (2–6). However, neither nonpeptide radioligands, antagonist radioligands, nor peripherally selective radioligands are available for the study of CCK receptor interactions. Recently, L-364,718, an extremely potent, nonpeptide competitive antagonist of CCK receptors with high selectivity for peripheral tissues, was developed in our laboratories (7, 8). In the present study, the binding of [^{3}H]-(±)-L-364,718 (Fig. 1) to rat pancreas, a major peripheral target tissue for CCK-8 (1), is characterized. The specific binding of [^{3}H]-(±)-L-364,718 to another peripheral target tissue for CCK, the bovine gall bladder, and its inability to bind to guinea pig brain or gastric fundic mucosal glands are also reported.

Materials and Methods

Radioligands. [^{3}H]-(±)-L-364,718 was prepared from its N-demethylated precursor, using tritium-labeled methyliodide, by Dr. Avery Rosegay of Merck Sharp & Dohme Research Laboratories. The specific activity of [^{3}H]-(±)-L-364,718 was determined to be 67.4 Ci/mmol. [^{125}I]CCK-8 labeled with Bolton Hunter reagent (2200 Ci/mmol) was purchased from New England Nuclear (Boston, MA). The difference in specific activities of the two radioligands had no qualitative or quantitative effect upon the results obtained as demonstrated below.

Radioligand binding assays. Male Sprague-Dawley rats weighing 250–350 g were sacrificed by decapitation and the pancreas was removed. The pancreas was dissected free from adipose and connective tissue and homogenized for 20 sec in 50 volumes of Tris-HCl buffer (50 mM, pH 7.7 at 25°) using a Polytron homogenizer (Brinkmann PT-10, setting 6). The homogenate was centrifuged at 50,000 × *g* for 10 min, and the supernatant was discarded. The pellet was washed by resuspension in the original volume of fresh Tris-HCl buffer and again centrifuged as above. The final pellet was resuspended in the desired volume (routinely, 1 g of original tissue in 4,000 ml) of binding assay buffer containing 50 mM Tris-HCl (pH 7.4 at 37°), 5 mM $MgCl_2$, 5 mM dithiothreitol, 2 mg/ml of bovine serum albumin, and 0.14 mg/ml of bacitracin. To measure [^{3}H]-(±)-L-364,718 binding, 980 μl of tissue membranes were added to triplicate tubes containing 10 μl of either buffer (for total binding), unlabeled (±)-L-364,718 (0.3 μM final concentration, for nonspecific binding), or displacers (at the desired final concentrations) and 10 μl of [^{3}H]-(±)-L-364,718 (0.2 nM final concentration unless indicated otherwise). After incubation at 37° for 30 min

ABBREVIATIONS: CCK, cholecystokinin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*^{1},*N*^{1}-tetraacetic acid; MES, 4-morpholineethanesulfonic acid; TLC, thin layer chromatography; GppNHp, guanosine 5′-(β-γ-imido)triphosphate; CBZ-CCK(26-32)-amide, *N*-carboxybenzoxy-cholecystokinin(26–32)amide.

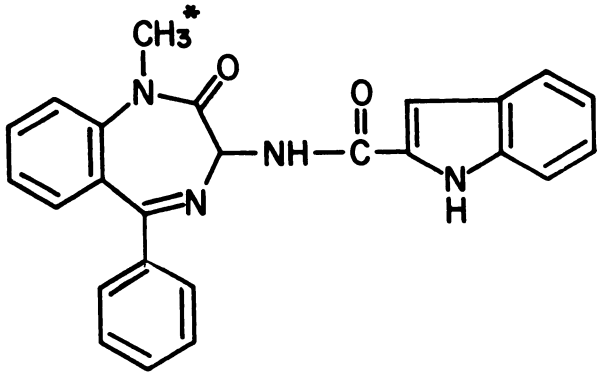


Fig. 1. Structure of (±)-L-364,718. *, position of the tritium label.

(various time intervals were used in association rate studies), the incubation was terminated by filtration under reduced pressure through glass fiber GF/B filters and washed immediately four times with 4 ml of ice-cold Tris buffer (50 mM, pH 7.4). The radioactivity trapped on the filters was counted in 10 ml of Universol Cocktail (ICN, Irving, CA). Specific binding was defined as the radioactivity bound after subtracting nonspecific binding determined in the presence of 0.3 μM unlabeled (±)-L-364,718. [^{125}I]CCK-8 binding to rat pancreatic membranes was performed as described for [^{3}H]-(±)-L-364,718 binding with the exception that [^{125}I]CCK-8 (15 pM) was used instead of [^{3}H]-(±)-L-364,718 and 1 μM unlabeled CCK-8 was used to define nonspecific binding.

To prepare bovine gall bladder membranes, freshly obtained tissues were cut into small pieces with scissors and homogenized with 50 volumes of Tris-HCl (50 mM, pH 7.4) using a Polytron homogenizer (Brinkmann PT 10, maximal setting for 30 sec). The homogenates were centrifuged at 50,000 × *g* for 10 min, and the pellets were resuspended in the same buffer and centrifuged as above. The resulting pellets were resuspended in 25 volumes of the binding assay buffer as described for [^{3}H]-(±)-L-364,718 binding in rat pancreatic membranes. The [^{125}I]CCK-8 binding assay buffer contained 10 mM HEPES (pH 6.5), 1 mM EGTA, 5 mM $MgCl_2$, 130 mM NaCl, 0.25 mg/ml of bacitracin, and 0.2 mg/ml of soybean trypsin inhibitor. This buffer was the same as that employed by others (9) for [^{125}I]CCK binding in bovine gallbladder membranes with the exception that, later, HEPES was used instead of MES, and KCl and bovine serum albumin were omitted. These changes resulted in an improved ratio of specific to nonspecific binding in our laboratories. The [^{3}H]-(±)-L-364,718 and [^{125}I]CCK-8 binding assays using gall bladder membranes were performed as described for pancreatic membranes with the exception that, for [^{125}I]CCK-8 binding, the incubation was at 25° for 2 hr.

Stability of [^{3}H]-(±)-L-364,718. To determine the stability of [^{3}H]-(±)-L-364,718 under the assay conditions, the ligand was incubated with pancreatic membranes according to the methods used in the binding assays. Free and bound [^{3}H]-(±)-L-364,718 was separated by microcentrifugation (Brinkmann Eppendorf 5431). The pellet containing bound [^{3}H]-(±)-L-364,718 was extracted with 1 ml of methanol, and the extract was concentrated with a stream of nitrogen before applying to TLC plates. The supernatant containing free [^{3}H]-(±)-L-364,718 was treated with ethanol (final concentration 70%) to precipitate proteins and the centrifuged supernatant was used for TLC. TLC was carried out on silica gel G using the following solvent systems (tolune:methanol, 25:1.7, or chloroform:acetone, 19:1). The TLC plates after development were cut into pieces and the radioactivity was measured. The mobilities of both samples were comparable with that of authentic [^{3}H]-(±)-L-364,718 demonstrating the stability of the ligand under these conditions.

Results

Tissue linearity. Specific [^{3}H]-(±)-L-364,718 binding increased linearly with the concentration of pancreatic tissue over the range of 0.06–0.5 mg of wet tissue weight/ml (5–40 μg/ml of protein) (Fig. 2). A tissue concentration of 0.25 mg/ml was subsequently used for routine binding assays.

Saturation analysis of [^{3}H]-(±)-L-364,718 binding. The binding of [^{3}H]-(±)-L-364,718 to pancreatic tissue was saturable (Fig. 3A). The ratio of total [^{3}H]-(±)-L-364,718 binding to nonspecific binding was about 20 at a [^{3}H]-(±)-L-364,718 concentration of 0.2 nM, which was used for routine binding assays. Nonspecific binding increased linearly with the [^{3}H]-(±)-L-364,718 concentration. Specific [^{3}H]-(±)-L-364,718 binding began to plateau at about 0.8 nM and half-maximal binding was apparent at approximately 0.1–0.2 nM [^{3}H]-(±)-L-364,718. Scatchard analysis (10) of these data indicated a single population of binding sites, with a dissociation constant of 0.23 ± 0.048 nM (Fig. 3B). The maximal number of binding sites for specific [^{3}H]-(±)-L-364,718 binding was 270 ± 25 pmol/g of pancreas wet weight (3.1 ± 0.29 pmol/mg of protein) (Fig. 3B). The maximal number of binding sites for [^{3}H]-(±)-L-364,718 was 4.4 ± 0.6 times the maximal number of binding sites determined using [^{125}I]CCK-8 (Fig. 3B). The maximal number of binding sites and the dissociation constant of [^{125}I]CCK was 72 ± 12 pmol/g (0.84 ± 0.14 pmol/mg of protein) and 0.09 ± 0.013 nM, respectively. A Hill plot of the [^{3}H]-(±)-L-364,718 data gave a Hill coefficient of 1.01 ± 0.06, indicating a single class of binding sites and the absence of positive or negative cooperative interaction (Fig. 3C).

Kinetics of [^{3}H]-(±)-L-364,718 binding. The specific binding of [^{3}H]-(±)-L-364,718 to rat pancreatic membranes was time dependent, reaching steady state in 10 min at 37° (Fig. 4). The calculated association rate constant (K_1) was 0.91 min^{-1} nM^{-1}. The rate of dissociation was examined by incubating membranes to equilibrium at 37° and then adding 0.3 μM unlabeled (±)-L364,718 to prevent rebinding of dissociated [^{3}H]-(±)-L-364,718. The remaining bound [^{3}H]-(±)-L-364,718 was measured at different time intervals (Fig. 5A). When plotted on a semilogarithmic scale (Fig. 5B), the dissociation was linear, indicating a first order process. The dissociation rate constant (K_{-1}) was calculated to be 0.095 min^{-1}. The dissociation con-

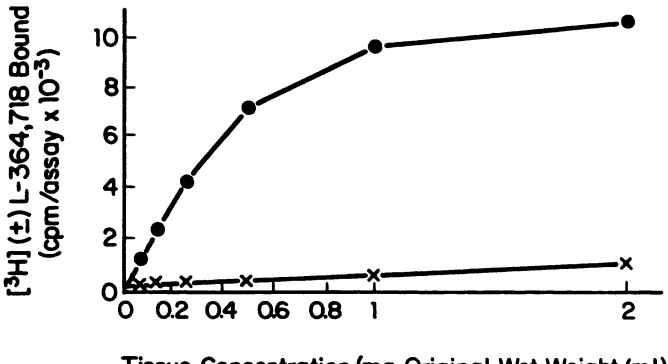
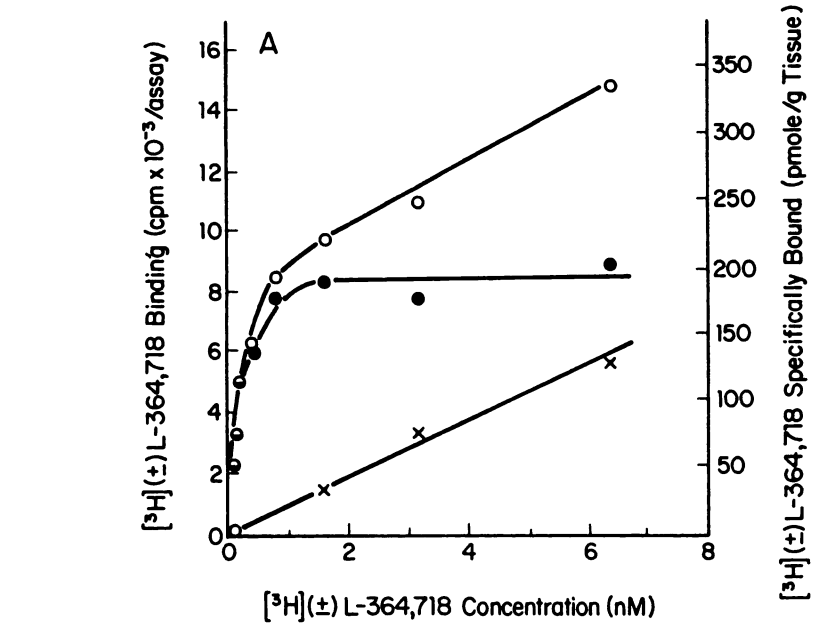


Fig. 2. [^{3}H]-(±)-L-364,718 binding as a function of increasing tissue concentrations. Various concentrations of pancreatic membranes were incubated with 0.2 nM [^{3}H]-(±)-L-364,718 for 30 min at 37° as described in Materials and Methods. The amount of protein from each gram of pancreatic tissue was 86 ± 7 mg. Nonspecific binding (×) was defined in the presence of 0.3 μM unlabeled (±)-L-364,718. Specific binding (●) is the difference between total binding and nonspecific binding. Each *point* represents the mean of triplicate determinations which varied less than 10% in each experiment. This experiment was duplicated and similar results were obtained.



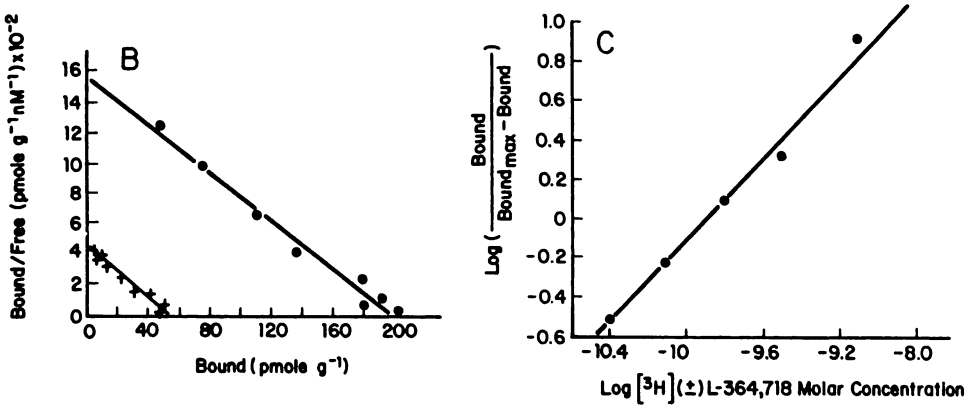


Fig. 3. [^{3}H]-(±)-L-364,718 binding as a function of increasing concentrations of [^{3}H]-(±)-L-364,718. The binding assays were performed as described in Materials and Methods using various concentrations of [^{3}H]-(±)-L-364,718 (0.05–6.4 nM). The *points* shown are means of triplicate determinations which varied less than 10% in each experiment. This experiment was replicated four times with similar results. A. ○, total binding; ×, nonspecific binding, ●, specific binding. Nonspecific binding was defined using 0.3 μM unlabeled (±)-L-364, 718. Specific binding is the difference between total and nonspecific binding. B. Scatchard plot for [^{3}H]-(±)-L-364,718 (●) and [^{125}I]CCK-8 (×). The calculated mean (± SE) K_d value and estimated maximal number of binding sites of the four experiments are given in the text. C. Hill plot for [^{3}H]-(±)-L-364,718 binding. In each of the four experiments, the slope of the Hill plot approximated unity; the mean slope was 1.01 ± 0.06.

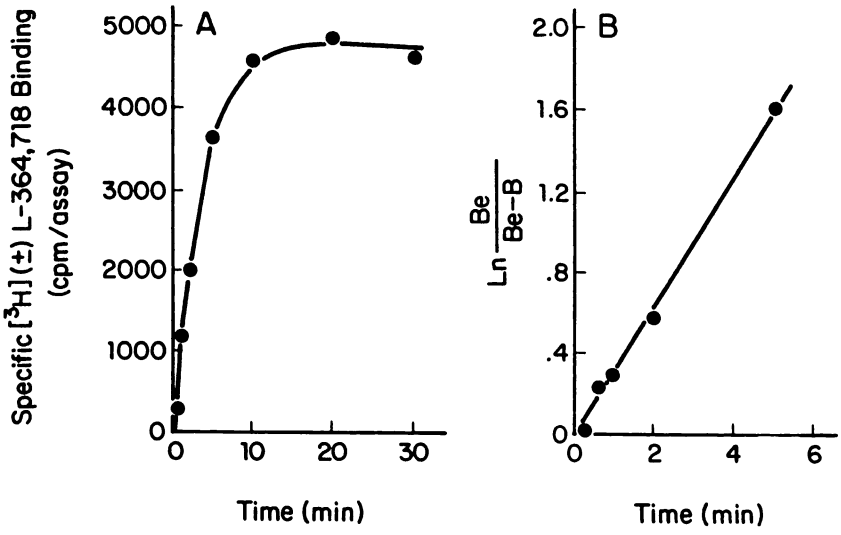


Fig. 4. Time course of association of [^{3}H]-(±)-L-364,718 binding. The association of [^{3}H]-(±)-L-364,718 binding to pancreatic membranes (0.25 mg of original tissue wet weight) was determined at various time intervals as described in Materials and Methods. Specific binding was defined as the difference between binding obtained in the presence and absence of 0.3 μM unlabeled (±)-L-364,718. The *points* shown are those obtained in a single experiment, performed in triplicate. The triplicate values varied less than 10%. The experiments were replicated three times with similar results. A. Specific [^{3}H]-(±)-L-364,718 binding as a function of time. B. Pseudo-first order kinetic plots of initial specific [^{3}H]-(±)-L-364,718 binding. On the *ordinate*, *B* is the amount of specific [^{3}H]-(±)-L-364,718 binding at time *t* and *Be* is the amount of specific [^{3}H]-(±)-L-364,718 binding at equilibrium. The slope of the plot is the observed rate constant (k_{ob}) for the pseudo-first order reaction. The second-order association rate, K_1, calculated from $K_1 = (k_{ob} - k_{-1})/([^3H]\text{-}(\pm)\text{-L-364,718})$, was 0.91 min^{-1} nM^{-1}. K_{-1} is the first order rate constant for dissociation (from Fig. 5) and ([^{3}H]-(±)-L-364,718) is the concentration of radioligand used in the experiment (0.2 nM).

stant calculated from the ratio k_{-1}:k_1 was 0.10 nM, similar to the dissociation constant determined in saturation studies.

Effect of CCK agonists and antagonists on specific [^{3}H]-(±)-L-364,718 binding in pancreas. Specific [^{3}H]-(±)-L-364,718 binding to pancreatic membranes was inhibited by both the (−)- and (+)-enantiomers of L-364,718 (Table 1). The affinity of the more biologically active (−)-enantiomer (7, 8) (K_i = 0.036 nM) was approximately 100 times greater than that of the (+)-enantiomer, thus demonstrating the stereoselectivity of the bound radioligand. Other CCK antagonists,

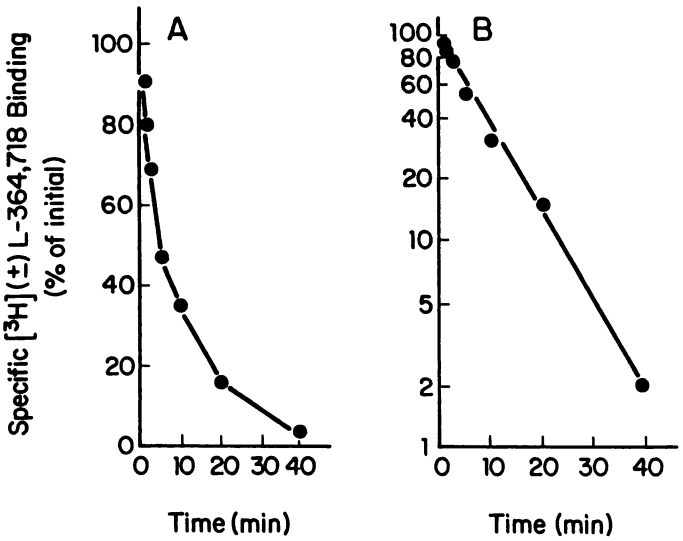


Fig. 5. Dissociation of specific [^{3}H]-(±)-L-364,718 binding to rat pancreatic membranes. Specific [^{3}H]-(±)-L-364,718-binding assays were performed as described in Materials and Methods. The *points* shown are obtained in a single experiment, performed in triplicate. The triplicate values varied less than 10%. The experiments were replicated three times with similar results. For dissociation studies, [^{3}H]-(±)-L-364,718 was first allowed to associate for 30 min at 37°, whereupon 0.3 μM unlabeled (±)-L-364,718 was added to prevent rebinding of dissociated [^{3}H]-(±)-L-364,718. The dissociation reaction was measured at various time intervals after the addition of unlabeled (±)-L-364-718 by rapid filtration as described in Materials and Methods. A linear plot (A) and a semilog plot (B) of B/Be versus t where Be and B are binding at equilibrium and time t, and t is the time after the addition of excess unlabeled (±)-L-364,718. The dissociation rate constant (k_{-1}), calculated according to the formula, $k_{-1} = 2.3 \times$ slope, was 0.095 min^{-1}.

including asperlicin (6), CBZ-CCK(26-32)-amide (11),[1] dibutyryl-cGMP (12), benzotript, and proglumide (13), also displaced [^{3}H]-(±)-L-364,718 binding with relative potencies corresponding to their known pharmacological activities in peripheral tissues (Table 1). The K_i values of all the CCK antagonists for inhibiting specific [^{3}H]-(±)-L-364,718 binding were in good agreement with their K_i values for inhibiting [^{125}I]CCK-8 binding in pancreatic tissues (Table 1). The K_i value of (−)-L364,718 (0.04 nM) was also in reasonable agreement with the K_d values (0.1–0.2 nM) determined above for [^{3}H]-(±)-L-364,718 since this ligand is a 50% mixture of the enantiomers.

The CCK receptor agonists, including CCK-8, caerulein, and CCK-33, also potently inhibited specific [^{3}H]-(±)-L-364,718 binding with K_i values of 2.0, 2.0, and 16 nM, respectively (Table 1). Complete inhibition of specific [^{3}H]-(±)-L-364,718 binding was achieved with the high concentrations of these agonists. The K_i values for CCK-8, caerulein, and CCK-33 for inhibiting specific [^{3}H]-(±)-L-364,718 binding were 13–40-fold higher than their K_i values for [^{125}I]CCK-8 binding (Table 1). Similarly, CCK-8-desulfate, CCK-4, and gastrin were ineffective in displacing [^{3}H]-(±)-L-364,718 at concentrations which exceeded their K_i values for displacing [^{125}I]CCK-8 binding (Table 1). The Hill coefficients (n_H) of most CCK antagonists for displacement of [^{3}H]-(±)-L-364,718 or [^{125}I]CCK-8 binding approximated unity (Table 1). In contrast, the Hill coefficients for most CCK agonists in displacing specific [^{125}I]CCK-8 binding, and particularly, [^{3}H]-(±)-L-364,718 binding appeared to deviate from unity (Table 1).

Effect of various pharmacological agents on [^{3}H]-(±)-L-364,718 binding in rat pancreas. Although the chemical structure of L-364,718 contains a benzodiazepine moiety (8), specific [^{3}H]-(±)-L-364,718 binding in rat pancreas was not affected by the central or peripheral benzodiazepine receptor ligands, diazepam and RO-5-4864, respectively, at concentrations up to 10 μM. Other pharmacological agents, including α- or β-adrenergic, serotonergic, histaminergic, dopaminergic, cholinergic, opiate and GABAergic agonists and/or antagonists or the endogenous peptides, substance P, thyrotropin releasing hormone, Leu-enkephalin, bradykinin, neurotensin, arginine-

[1] J. Gardner and R. Jensen, personal communication.

TABLE 1

Displacement of specific [^{3}H]-(±)-L-364,718 and [^{125}I]CCK-8 pancreatic binding by various CCK agonists and antagonists

Values are means ± standard errors from three to four experiments performed in triplicate. K_i values were calculated according to the formula

$$K_i = \frac{IC_{50}}{1 + \frac{[L]}{K_d}}$$

where $[L]$ is the radioactive ligand concentration and K_d the dissociation constant of radioactive ligand.

Displacers	[^{3}H]-(±)-L-364,718 binding		[^{125}I]CCK-8 binding	
	K_i	n_H[a]	K_i	n_H
	nM		nM	
Antagonists				
(−)-L-364,718	0.036 ± 0.003	0.9 ± 0.02	0.073 ± 0.015	1.0 ± 0.02
(+)-L-364,718	4.5 ± 0.45	0.8 ± 0.01	8.3 ± 0.79	0.9 ± 0.02
Asperlicin	312 ± 100	0.7 ± 0.1	170 ± 58	0.7 ± 0.1
CBZ-CCK (26-32)-amide	7,500 ± 900	0.8 ± 0.1	4,100 ± 320	0.6 ± 0.1
dibutyryl-cGMP	25,000 ± 9,300	1.3 ± 0.1	22,000 ± 2,600	1.2 ± 0.04
Benzotript	41,000 ± 1,000	1.0 ± 0.1	60,000 ± 30,000	1.0 ± 0.03
Proglumide	510,000 ± 120,000	0.8 ± 0.1	240,000 ± 35,000	0.7 ± 0.01
Agonists				
CCK-8	2.0 ± 0.45	0.36 ± 0.02	0.15 ± 0.01	0.8 ± 0.02
Caerulein	2.0 ± 0.28	0.37 ± 0.01	0.05 ± 0.02	0.6 ± 0.1
CCK-33	16 ± 4.5	0.37 ± 0.02	0.56 ± 0.09	0.7 ± 0.1
CCK-8-desulfate	>800		140 ± 72	0.6 ± 0.1
Gastrin	>2,000		1,900 ± 1,200	0.6 ± 0.1
CCK-4	>3,000		2,200 ± 730	0.6 ± 0.1

[a] n_H, Hill coefficient.

vasopressin, bombesin, secretin, and angiotensin II, also had no effect on the specific [^{3}H]-(±)-L-364,718 binding at concentrations generally considered to be pharmacologically effective (1–100 μM).

Differential effect of guanyl nucleotides and ions on the potencies of CCK agonists and antagonists to displace [^{3}H]-(±)-L-364,718 binding. Addition of GppNHP (100 μM) or sodium chloride (100 mM) or the omission of $MgCl_2$ (5 mM) in the binding assay buffer had no effect on specific pancreatic [^{3}H]-(±)-L-364,718 binding (data not shown). However, the potencies of CCK agonists in displacing [^{3}H]-(±)-L-364,718 binding was altered by such treatments (Table 2). GppNHP (100 μM) increased the K_i value of CCK-8 and caerulein by approximately 5-fold. In contrast, the addition of GppNHP or sodium chloride had no effect on the K_i of the CCK antagonists L-364,718 or CBZ-CCK(26-32)-amide (Table 2). Omission of $MgCl_2$ also did not affect the K_i value of (−)-L-364,718 but increased the value of CBZ-CCK(26-32)-amide by approximately 5-fold.

[^{3}H]-(±)-L-364,718 binding to other tissues. Specific [^{3}H]-(±)-L-364,718 binding was also observed in bovine gall bladder, another peripheral target tissue of CCK. Specific [^{3}H]-(±)-L-364,718 binding (70–80% of total binding) in gall bladder membranes was inhibited by (−)-L-364,718 and (+)L-364,718 in a stereospecific manner, with IC_{50} values of 0.11 ± 0.023 and 13 ± 4.6 nM, respectively (Table 3). CCK-8 and the CCK antagonists, proglumide and asperlicin, also inhibited specific [^{3}H]-(±)-L-364,718 binding. The potencies of the antagonists in displacing specific [^{3}H]-(±)-L-364,718 binding were comparable to their potencies as inhibitors of specific [^{125}I]CCK-8 binding in this tissue. In contrast, the IC_{50} value of CCK-8 for inhibition of specific [^{3}H]-(±)-L-364,718 binding was much greater than its IC_{50} for inhibiting [^{125}I]CCK-8 binding (Table 3).

Only negligible amounts of specific [^{3}H]-(±)-L-364,718 binding (<10% of total binding) was detected in guinea pig cortex membranes or gastric glands under conditions in which appreciable specific [^{125}I]CCK-8 and ^{125}I-gastrin binding is readily observed in our laboratories (6, 7).

Discussion

The binding of the CCK antagonist [^{3}H]-(±)-L-364,718 to rat pancreatic membranes is rapid, time and tissue concentration dependent, reversible, and saturable. The Scatchard and Hill plot analysis data indicate that [^{3}H]-(±)-L-364,718 binds with high affinity (K_d = 0.23 nM) and recognizes a single class of binding sites. The stereoselectivity of the [^{3}H]-(±)-L-364,718 binding is demonstrated by the much greater potency of the more biologically active (−)-enantiomer compared to the (+)-enantiomer (7, 8) as a displacing agent. The rank order of potencies of both CCK agonists (caerulein, CCK-8, CCK-33, desulfated CCK, and gastrin) and antagonists [(−)-L-364,718, asperlicin, CBZ-CCK(26-32)-amide, dibutyryl-cGMP, benzotript, and proglumide] in competing for [^{3}H]-(±)-L-364,718 and [^{125}I]CCK-8 binding were similar and corresponded to their reported biological activities in peripheral tissues (4, 7, 14), indicating that both radioligands interact with the same physiologically relevant receptor. Collectively, these data indicate that [^{3}H]-(±)-L-364,718 represents a new nonpeptide antagonist radioligand suitable for studying CCK receptor interactions.

Although the rank order of potentices of CCK agonists was similar when either [^{3}H]-(±)-L-364,718 or [^{125}I]CCK-8 was used as a radioligand, the absolute potencies of the agonists in displacing [^{3}H]-(±)-L-364,718 binding were less than for displacement of [^{125}I]CCK-8. A reduced potency for agonists, but not antagonists, in competing for radiolabeled antagonist ligands compared to agonist ligands has also been reported for the muscarinic cholinergic receptor (15, 16). Our results may suggest that, as proposed for the cholinergic receptor (15, 16) or other receptor systems (17, 18), the CCK receptor may have either two classes of binding sites or two conformational states which have different affinities for agonists but not antagonists. The tendency for GppNHp to shift the Hill coefficients for agonists closer to unity in displacing [^{3}H]-(±)-L-364,718 binding and, thereby, approximating their Hill coefficients for displacing [^{125}I]CCK-8 binding (Tables 1 and 3) may suggest two conformational states. Interestingly, it has recently been reported that digitonin-solubilized CCK receptors exhibit reduced affinities for agonists and not antagonists when compared to membrane-bound CCK receptors (19).

The ability of guanyl nucleotides and ions to selectively affect agonist and not antagonist binding is well documented in several neurotransmitter receptor systems (20–22). Similarly, the addition of GppNHp and NaCl or the omission of $MgCl_2$ reduced the affinity of CCK agonists for displacing [^{3}H]-(±)-L-364,718 binding to pancreatic membranes. In contrast, the potencies of the CCK antagonists, (−)-L-364,718 and CBZ-CCK(26-32)-amide were not affected by GppNHp or NaCl, and only CBZ-CCK(26-32)-amide was affected by omission of $MgCl_2$. The reduced potency of CBZ-CCK(26-32)-amide in the absence of $MgCl_2$ may be related to its reported partial agonist properties (23)[2]. In any event, the differential effects of GppNHp and NaCl on the displacement of [^{3}H]-(±)-L-364,718 binding by CCK agonists but not antagonists may provide a new method for determination of agonist and antagonist interactions with CCK receptors utilizing a binding assay.

The maximal number of specific pancreatic binding sites

[2] J. Gardner and R. Jensen, personal communication.

TABLE 2

Effect of GppNHp, NaCl, or omission of $MgCl_2$ on the potency of CCK agonist and antagonists in inhibiting specific [^{3}H]-(±)-L-364,718 binding in rat pancreatic membranes

	CCK-8		Caerulein		L-364,718		CBZ-CCK (26-32)	
	K_i	n_H	K_i	n_H	K_i	n_H	K_i	n_H
	nM		nM		nM		nM	
Control	1.9 ± 0.23	0.4 ± 0.02	2.0 ± 0.28	0.4 ± 0.01	0.044 ± 0.001	0.9 ± 0.03	7200 ± 1100	0.7 ± 0.07
+GppNHp	63 ± 18[a]	0.6 ± 0.01	39 ± 11[a]	0.7 ± 0.09	0.042 ± 0.001	0.9 ± 0.04	7000 ± 1100	0.8 ± 0.2
+NaCl	10 ± 1.2[a]	0.4 ± 0.03	9.5 ± 3.3[a]	0.4 ± 0.02	0.042 ± 0.001	0.9 ± 0.02	7000 ± 1100	0.7 ± 0.04
−$MgCl_2$	9.2 ± 2.2[a]	0.4 ± 0.04	11 ± 1.9[a]	0.4 ± 0.04	0.050 ± 0.007	1.0 ± 0.07	35000 ± 420	1.0 ± 0.2

[a] $p \leq 0.05$ (Student's t test) compared to control.

TABLE 3

Displacement of specific [^{3}H]-(±)-L-364,718 and [^{125}I]CCK-8 binding in bovine gall bladder by CCK and antagonists

Values are the means ± standard errors from at least three experiments determined in triplicate.

	IC_{50} (nM)	
	[^{3}H]L-364,718	[^{125}I]CCK-8
(−)-L-364,718	0.11 ± 0.023	0.072 ± 0.024
(+)-L-364,718	13 ± 4.6	4.1 ± 4.2
Asperlicin	810 ± 160	220 ± 11
Proglumide	2,400,000 ± 490,000	200,000 ± 42,000
CCK-8	387 ± 54	0.062 ± 0.0052
CCK-8-desulfate	>2000	110

determined using [^{125}I]CCK-8 was considerably less than the number of binding sites estimated using [^{3}H]-(±)-L-364,718. A lower maximum number of binding sites has also been reported when using radioligand agonists compared to radioligand antagonists in binding studies on the muscarinic-cholinergic ([^{3}H]oxotremorine versus [^{3}H]quinuclidinyl benzilate) and α_2-adrenergic ([^{3}H]-*p*-aminoclonidine versus [^{3}H]yohimbine) systems (16, 24). The reason for the currently observed differences in the number of pancreatic CCK-binding sites estimated using [^{125}I]CCK and [^{3}H]-(±)-L-364,718 is as yet unexplained. However, considering the different possible polypeptide chains reported for CCK receptors (25–27), the data may suggest that these two ligands interact with different sites or subunits on the CCK receptor.

(−)-L-364,718 has previously been reported to exhibit a high selectivity for peripheral CCK receptors compared to brain CCK receptors or the related gastrin receptors (7). In addition to rat pancreatic tissue, [^{3}H]-(±)-L-364,718 also bound in a specific manner to another peripheral target tissue for CCK, namely, the bovine gall bladder. In contrast, specific binding of [^{3}H]-(±)-L-364,718 was not observed in guinea pig brain or gastric fundic mucosal tissues. Moreover, specific [^{3}H]-(±)-L-364,718 binding to rat pancreatic tissue was not displaced by several pharmacological agents known to interact with other common peptide and nonpeptide receptor systems. These results are in agreement with the reported high selectivity of (−)-L-364,718 as a peripheral CCK antagonist and further demonstrate the utility of [^{3}H]-(±)-L-364,718 as a selective ligand for peripheral CCK receptors.

In summary, [^{3}H]-(±)-L-364,718 appears to represent a new potent nonpeptide antagonist radioligand for the study of CCK receptors. Its high selectivity for peripheral as opposed to brain CCK receptors should provide a new tool for identification of these proposed subtypes of CCK receptors in various tissues. The differential effects of GppNHp and NaCl on agonist and antagonist displacement of [^{3}H]-(±)-L-364,718 binding may be useful in defining CCK agonist and antagonist receptor interactions utilizing a binding assay.

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