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# Biological Properties of the Benzodiazepine Amidine Derivative L-740,093, a Cholecystokinin-B/Gastrin Receptor Antagonist with High Affinity *In Vitro* and High Potency *In Vivo*

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## SUMMARY

A novel series of 5-amino-1,4-benzodiazepin-2-one derivatives (amidines), which contain a cationic solubilizing group and which are antagonists for the cholecystokinin (CCK)-B receptor, have been identified. Optimization of this series led to the identification of an azabicyclononane amidine, L-740,093 [*N*-[(3*R*)-5-(3-azabicyclo[3.2.2]nonan-3-yl)-2,3-dihydro-1-methyl-2-oxo-1*H*-1,4-benzodiazepin-3-yl]-*N'*-(3-methylphenyl)urea], that bound with high affinity to CCK-B receptors from guinea pig cerebral cortex ($IC_{50}$ of 0.1 nM) and had a CCK-B/CCK-A receptor selectivity of 16,000. In comparison, L-365,260 had 85-fold lower affinity (8.5 nM) and was only 87-fold selective for CCK-B over CCK-A receptors. L-740,093 bound with high affinity to guinea pig gastrin receptors *in vitro* ($IC_{50}$ of 0.04 nM). Electrophysiological studies on slices of rat ventromedial hypothalamic nucleus showed that L-740,093 produced rightward shifts of the concentration-response curve for the CCK-B receptor agonist pentagastrin ($K_b$ of 0.06 nM). L-740,093 blocked pentagastrin-induced gastric acid secretion in anesthetized rats with a 50% inhibitory dose of 0.01 mg/kg, intraperitoneally, showing 100-fold greater activity, compared with L-365,260 (50% inhibitory dose of 1 mg/kg, intraperitoneally). An *ex vivo* binding assay in mice was used to investigate the interaction of L-740,093 with central CCK binding sites. After intravenous administration, L-740,093 inhibited *ex vivo* binding dose dependently, with a 50% effective dose of 0.2 mg/kg. These studies demonstrate that L-740,093 is the most potent and selective CCK-B antagonist yet described and that it has excellent central nervous system penetration.

The peptide CCK is an important hormone and transmitter within both the periphery and the CNS. A number of physiological processes have been suggested to be under the control of this peptide, including neural pathways mediating secretion, motility, analgesia, and satiety (1–3). The identification of at least two subtypes of CCK receptors (CCK-A and CCK-B/gastrin), which have distinct regional distributions and pharmacological specificities (4), has led to interest in the development of nonpeptide antagonists selective for either receptor subtype. An important initial advance was the isolation and identification of asperlicin as a selective CCK antagonist (5). This work led to the subsequent development of the selective benzodiazepine CCK-A antagonist devazepide (6, 7) and the selective CCK-B antagonist L-365,260 (8, 9), which were the first compounds available with high affinity and selectivity for these receptor subtypes. These compounds have been used as important pharmacological tools in many studies and facilitated the first investigations into the physiological roles of CCK-A and CCK-B receptors and the potential therapeutic effects of CCK antagonists.

In the past 2 years a number of other nonpeptide receptor antagonists, including the dipeptoid series based on the parent peptide, e.g., CI-988 (10), and the pyrazolidinone series, e.g., LY 262,691 (11), have also been described. Although these compounds have offered some structural diversity and provided valuable information, they still represent first-generation compounds that have some limitations for their use as potential therapeutic agents. For example L-365,260 and LY 262,691 have limited aqueous solubility, LY 262,691 has relatively weak affinity for CCK-B receptors, and CI-988 has relatively poor oral bioavailability and CNS penetration (12).

We have recently described a series of 1,4-benzodiazepine derivatives containing an acidic functionality on the phenyl urea portion of L-365,260, including the tetrazole derivative L-368,935 (13, 14). The properties of this compound and related compounds from this series included high affinity and selectiv-

**ABBREVIATIONS:** CCK, cholecystokinin; VMH, ventromedial hypothalamus; CNS, central nervous system; BH, Bolton-Hunter; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid; CCK-8S, cholecystokinin octasulfate; $ID_{50}$, 50% inhibitory dose; $ED_{50}$, 50% effective dose.



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ity for CCK-B receptors and increased aqueous solubility. The brain penetration of these compounds was, however, relatively poor, compared with that of L-365,260. To improve this deficiency, we have endeavored to design benzodiazepine-based CCK-B antagonists that would retain the excellent binding and solubility characteristics of this series containing an acidic functionality but that would embody an amine-based cationic functional group within the benzodiazepine framework. Ideally, the goal was to discover compounds in which the amine would possess a p$K_a$ that would be sufficiently high to support salt formation but sufficiently low to ensure a ready pool of neutral (and presumably, therefore, brain-penetrating) molecules at physiological pH (15). A series of amidine benzodiazepine derivatives have resulted from this work, and the properties of key members of this series are described below. The detailed development of the medicinal chemistry of this series of amidines will be described elsewhere (16). In the present communication we describe the biological profile of the homopiperidine L-737,415 and a detailed profile of the optimized amidine, the azabicyclononane derivative L-740,093 [*N*-[(3*R*)-5-(3-azabicyclo[3.2.2]nonan-3-yl)-2,3-dihydro-1-methyl-2-oxo-1*H*-1,4-benzodiazepin-3-yl]-*N'*-(3-methylphenyl)urea]. This latter compound is the most potent and selective CCK-B antagonist yet known.

## Materials and Methods

### Binding Studies

**$^{125}$I-CCK.** Radioligand binding to guinea pig cortical membranes was performed using 50 pM $^{125}$I-BH-CCK-8S in 20 mM HEPES buffer, pH 6.5, containing 150 mM NaCl, 5 mM $MgCl_2$, 1 mM EGTA, and 0.025% bacitracin (7). For pancreatic membranes, assay buffer was supplemented with 0.01% trypsin inhibitor and 0.2% bovine serum albumin. Guinea pig cortical membranes were prepared by homogenization in 0.32 M sucrose with 15 strokes of a glass/Teflon homogenizer, centrifugation (1000 × *g* for 10 min) and recentrifugation of the supernatant (20,000 × *g* for 20 min), and resuspension of the $P_2$ pellet in assay buffer (1 g of wet weight/120 ml). Rat pancreatic membranes were prepared in 10 mM HEPES/0.01% trypsin inhibitor, pH 7.4, by homogenization using a Polytron homogenizer (setting 6, for 30 sec), centrifugation (50,000 × *g* for 10 min), and resuspension of the pellet in assay buffer at a 1/2000 dilution. Specific binding in all cases was defined using 1 μM CCK-8S, and the reaction was terminated by filtration through Whatman GF/C filters, using a Brandel cell harvester, with three 3-ml washes with ice-cold 100 mM saline wash buffer. Filters were counted with an LKB γ counter.

**$^{125}$I-Gastrin.** $^{125}$I-Gastrin binding to guinea pig gastric glands was determined using a modification of the method described by Chang and Lotti (7).

**[$^3$H]Ro 15–1788.** Binding activity for the benzodiazepine site on the γ-aminobutyric acid type A receptor was determined using the method previously described by Tricklebank *et al.* (17).

### *In Vitro* Antagonism of Pentagastrin-Induced Excitation of VMH Neurons

Male Sprague-Dawley rats (60–120 g) were killed by decapitation and their brains were rapidly removed. Coronal sections (350-μm thick) containing the VMH were cut using an Oxford vibratome and placed in a small chamber, where they were perfused continually with oxygenated warmed artificial cerebrospinal fluid. Extracellular recordings of action potential firing rate were made from single VMH neurons using glass micropipettes filled with 3 M NaCl and having resistances of 10–30 MΩ. CCK-B receptor-mediated responses were evoked with the CCK-B-selective agonist pentagastrin in the presence of the CCK-A receptor antagonist devazepide, because it has recently been reported that cells within the VMH contain CCK-A receptors (18). Pentagastrin, dissolved in the artificial cerebrospinal fluid, was added for 1-min periods. After determination of a control concentration-response curve for pentagastrin, antagonists were continuously perfused until the level of block reached equilibrium. For the lowest concentration of L-740,093 tested (0.3 nM), this took 2 hr. Once equilibrium with the antagonist was achieved the concentration-response curve for pentagastrin was repeated. The equilibrium dissociation constant ($K_b$) was determined from the rightward shift [concentration ratio (CR)] of the pentagastrin concentration-response curve produced by the antagonist, using the equation $K_b$ = [antagonist]/(CR − 1).

### Rat Gastric Acid Secretion

Gastric acid secretion was measured using a modification of the protocol described by Ghosh and Schild (19). Female Sprague-Dawley rats (approximately 180–210 g of body weight) that had been fed a liquid diet for 2 days and deprived of food overnight before experiments were anesthetized with urethane (1.5 g/kg, intraperitoneally). The trachea, carotid artery, and jugular vein were cannulated for artificial ventilation, blood pressure recording, and administration of pentagastrin, respectively. The body temperature of the rats was maintained by means of a heating blanket controlled via a rectal probe thermister.

After a high laparotomy, cannulae were inserted into the esophagus below the diaphragm (after which the esophagus was cut to ensure subdiaphragmatic vagotomization) and into the pyloric antrum for the perfusion (3 ml/min) of an aqueous glucose solution (5%, w/v) at 37°. The gastric glucose perfusate was administered through a Perspex funnel placed through the stomach wall into the nonsecretory lumen and was passed continuously over a pH electrode to record hydrogen ion concentration via a pH meter and antilogarithmic conversion unit, with output onto a flat-bed chart recorder.

After a 30-min stabilization period, during which glucose was perfused continuously through the stomach, drug or vehicle was given (dose volume, 4 ml/kg) by low intraperitoneal injection, and 30 min later a constant-rate intravenous infusion of pentagastrin (0.3 μg/kg/min) was started. Inhibition of pentagastrin-stimulated gastric acid secretion was then measured 30 min later. An $ID_{50}$ (dose producing 50% inhibition of pentagastrin-induced gastric hydrogen ion secretion) was determined. The vehicle for L-740,093 and L-365,260 was 10% ethanol/60% propylene glycol/30% saline.

### *Ex Vivo* Binding of CCK-B Antagonists

Male BKTO mice (25–30 g) were used in this study. Vehicle or drug was administered to mice either in a fixed volume of 0.1 ml (for the intravenous route) or dosed according to weight (1 ml/kg for the oral route). After 30 min, animals were anesthetized with isoflurane and transcardially perfused with 0.9% heparinized saline for 20 sec. Brains were removed and homogenized in assay buffer (20 mM HEPES, 1 mM EGTA, 5 mM $MgCl_2$, 150 mM NaCl, 0.025% bacitracin, pH 6.5), in a final dilution of 40 mg of wet weight/ml. For the binding assay, 100 μl of tissue homogenate were incubated with 50 pM $^{125}$I-BH-CCK-8S (50 μl), assay buffer (300 μl), and either saline, 1 μM CCK-8S (nonspecific binding), or drug (calibration curve) for 120 min at room temperature. The assay was terminated by rapid filtration and radioactivity was determined by counting of filters in an LKB γ counter. Results were expressed as percentage inhibition of specific binding of $^{125}$I-BH-CCK-8S for each dose. The potency ($ED_{50}$) for each compound was calculated graphically from the plot of percentage inhibition of specific binding versus log dose of each compound.

### Materials

$^{125}$I-BH-CCK-8S (specific activity, 2000 Ci/mmol) was obtained from Amersham and prepared as a 50 nM stock solution in 0.1% bovine serum albumin. Animal strains used were male Hartley guinea pigs and Sprague-Dawley rats (Bantin and Kingman, Hull, UK). 5′-Guanylylimidodiphosphate, bacitracin, soybean trypsin inhibitor type I-S, bovine serum albumin, HEPES, and EGTA were all obtained from Sigma.

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## Results

**Receptor binding profile.** The affinity of compounds for brain CCK-B receptors relative to pancreatic CCK-A receptors can be determined *in vitro* by their affinity in competing for the binding of radioligands in appropriate tissues. The present studies assessed the relative affinity and selectivity of the amidines L-737,415 and L-740,093, in comparison with L-365,260.

The homopiperidine L-737,415 (Fig. 1) was found to bind to the CCK-B receptor with high affinity and improved receptor selectivity, compared with the parent benzodiazepine L-365,260 (Table 1). Optimization of the CCK-B receptor affinity and selectivity within this series resulted in the azabicyclononane L-740,093 (Fig. 1), which bound with high affinity to CCK-B receptors from guinea pig cortex, with an $IC_{50}$ value of 0.1 nM (four experiments). The affinity for CCK-A receptors was >4 orders of magnitude lower ($IC_{50}$ of 1600 nM, four experiments) than that seen for the CCK-B receptor. This corresponded to a CCK-B receptor selectivity of 16,000-fold. In contrast, L-365,260 had 85-fold lower affinity and was 87-fold selective for CCK-B receptors (Table 1).

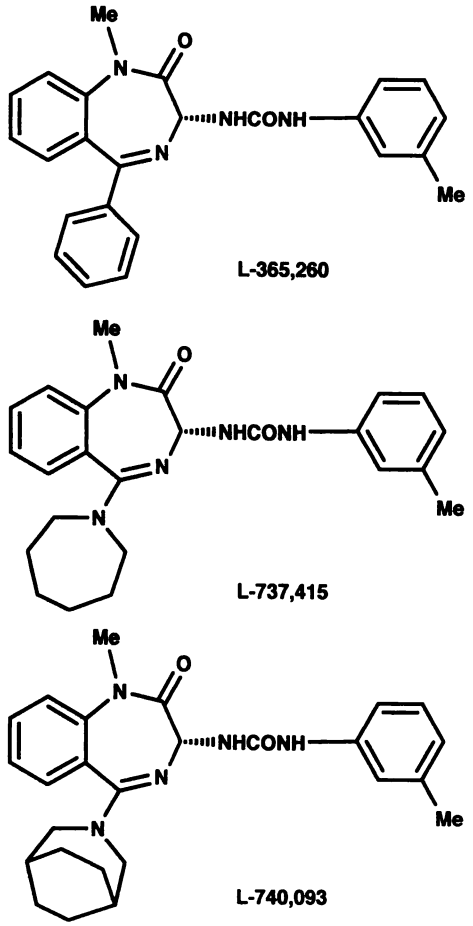


**Fig. 1.** Structures of L-365,260 and the benzodiazepine amidine CCK-B antagonists L-737,415 and L-740,093. *Me*, methyl.

TABLE 1

***In vitro* binding properties of benzodiazepine amidine CCK-B antagonists**

Binding results are expressed as the geometric means from a minimum of three independent determinations. The numbers in parentheses refer to the error range.

| | $IC_{50}$ | | CCK-A/CCK-B selectivity |
|---|---|---|---|
| | Guinea pig CCK-B | Rat CCK-A | |
| | *nM* | | |
| L-365,260 | 8.5 (6.5–11) | 740 (590–930) | 87 |
| L-737,415 | 1.2 (1.1–1.4) | 3,200 (3,100–3,300)* | 2,700 |
| L-740,093 | 0.10 (0.056–0.19) | 1,600 (1,400–1,800) | 16,000 |

* Displacement did not reach 50% in one of three experiments.

Earlier studies in the L-365,260 series established that the *R*-enantiomer (L-365,260) was selective for the CCK-B receptor and that the *S*-enantiomer (L-365,346) was selective for the CCK-A receptor. The binding characteristics of the enantiomers in the L-740,093 series were therefore examined. As expected, the *R*-enantiomer (L-740,093) was selective for the CCK-B receptor and the *S*-enantiomer (L-740,094) was CCK-A receptor selective (Table 2).

Pharmacological studies suggest that the brain CCK-B receptor is closely related to the stomach gastrin receptor. Recent cloning of the receptors from rats, dogs, and humans suggests that these receptors may be identical (20, 21). The gastrin receptor has a physiological role in the regulation of gastric acid secretion, and it is possible that this receptor is present within the CNS. We have compared the binding affinities of L-740,093 and L-365,260 for gastrin receptors in an *in vitro* receptor binding assay, to determine the relative affinities of these compounds for this receptor. L-740,093 bound with high affinity to guinea pig gastrin receptors, with an $IC_{50}$ of 0.04 nM (four experiments). This value was similar to the affinity of this compound in the CCK-B receptor binding assay. In contrast to its affinity for the CCK-B receptor, L-740,093 showed no activity at the benzodiazepine site on the γ-aminobutyric acid type A receptor complex labeled with [$^3$H]Ro 15–1788, at concentrations of up to 10 μM.

***In vitro* antagonism by L-740,093 of pentagastrin-induced excitation of rat VMH neurons.** The blockade of pentagastrin-induced excitation of single neurons in *in vitro* slice preparations of the rat VMH nucleus was used to determine the functional activity of L-737,415 and L-740,093 in an electrophysiological model of CCK-B receptor activation. Both L-737,415 and L-740,093 were very potent antagonists of CCK-B receptor-mediated excitatory responses and produced rightward shifts of the dose-response curve for administered pentagastrin (Fig. 2; Table 3). The response to L-740,093 was studied in some detail. At concentrations of 0.3 and 1.0 nM the block developed slowly, and at 0.3 nM it took approximately 2 hr to reach equilibrium. After this time the concentration-response curve for pentagastrin was flattened and there was a reduction in the maximum response (Fig. 2). This was probably due to the fact that, because of its extremely high affinity, the antagonist dissociated very slowly from the receptor and only brief (1-min) agonist applications were used, to overcome problems with receptor desensitization. Thus, true equilibrium between the agonist and antagonist was not achieved and the antagonist acted in a pseudoirreversible manner. In an attempt to alleviate this problem, when concentration ratios were estimated for the calculation of $K_b$ values these measurements were taken as close to the base of the concentration-response curve as possible. When calculated in this way, the mean $K_b$ values for L-740,093 were 0.06 ± 0.01 (six experiments) at 0.3 nM and 0.02 ± 0.006 (five experiments) at 1.0 nM. The apparent higher affinity of L-740,093 at 1 nM resulted from the more pronounced flattening of the concentration-response curve pro-

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TABLE 2

**Stereoselectivity of benzodiazepine amidine CCK-B antagonists**

Binding results are expressed as the geometric means from a minimum of three independent determinations. The numbers in parentheses refer to the error range.

| | C3 stereoselectivity | $IC_{50}$ | | CCK-A/CCK-B selectivity |
|---|---|---|---|---|
| | | Guinea pig CCK-B | Rat CCK-A | |
| | | *nM* | | |
| L-365,260 | *R* | 8.5 (6.5–11) | 740 (590–930) | 87 |
| L-365,346 | *S* | 340 (270–430) | 3.0 (1.8–4.9) | 0.0088 |
| L-740,093 | *R* | 0.10 (0.056–0.19) | 1,600 (1,400–1,800) | 16,000 |
| L-740,094 | *S* | 30 (27–35) | 7.9 (6.1–10) | 0.26 |

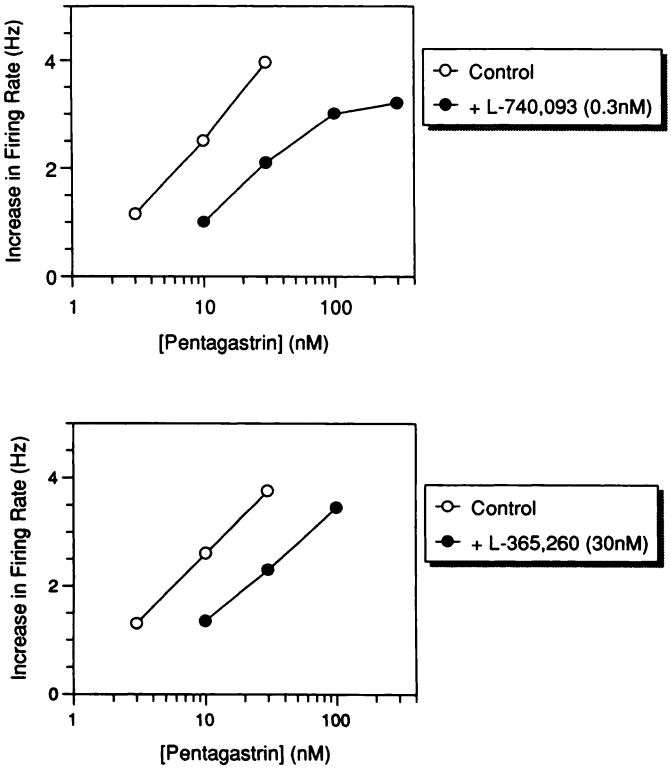


**Fig. 2.** Antagonist affinity of the benzodiazepine amidine CCK-B antagonist L-740,093 and the benzodiazepine L-365,260 in rat VMH slices. Extracellular recordings of action potential firing rate were made from single VMH neurons as described in Materials and Methods. CCK-B receptor-mediated responses were evoked with the CCK-B-selective agonist pentagastrin in the presence of the CCK-A receptor antagonist devazepide. Examples are from different cells in different slices.

TABLE 3

**Antagonist affinity of benzodiazepine amidine CCK-B antagonists**

Binding results are expressed as the geometric means from a minimum of four independent determinations. The numbers in parentheses refer to the error range. $K_b$ values were calculated as described in Results, using pentagastrin as the agonist.

| | Guinea pig CCK-B, $IC_{50}$ | Rat VMH neurons, $K_b$ |
|---|---|---|
| | *nM* | *nM* |
| L-365,260 | 8.5 (6.5–11) | 26 ± 8.6 |
| L-737,415 | 1.2 (1.1–1.4) | 0.067 ± 0.011 |
| L-740,093 | 0.10 (0.056–0.19) | 0.06 ± 0.01 |

duced by this concentration. For comparison, the antagonist potency of L-365,260 was determined in a similar manner. The $K_b$ for L-365,260 was 26 ± 8.6 nM (12 experiments), which is the same as the $K_b$ value for this compound (33 nM) previously determined using CCK-8S as the agonist (22).

**Gastric acid secretion in rats.** Because L-740,093 has similar affinities at CCK-B and gastrin receptors, the functional activity of L-740,093 *in vivo* could be determined using antagonism of pentagastrin-induced gastric acid secretion in anesthetized rats. Fig. 3 shows that L-740,093 caused a dose-related inhibition of the pentagastrin response, with an $ID_{50}$ of 0.01 mg/kg, intraperitoneally, and complete inhibition at 0.1 mg/kg, intraperitoenally. In this model L-365,260 also blocked pentagastrin-induced gastric acid secretion but was 100-fold weaker ($ID_{50}$ of 1.0 mg/kg).

***Ex vivo* binding studies.** An *ex vivo* binding assay was used to determine the CNS penetration of compounds interacting with central CCK binding sites after peripheral administration of unlabeled compound to mice. Brain membranes were incubated with $^{125}$I-BH-CCK *ex vivo*, and the presence of test compound was determined by measuring the amount of $^{125}$I-BH-CCK remaining specifically bound to the membranes. Cardiac perfusion was performed at the end of the experiment, to eliminate the potential contamination of brain tissue by drug in the residual blood volume. A comparison was made between L-737,415, L-740,093, and L-365,260.

The homopiperidine L-737,415 dose-dependently inhibited the *ex vivo* binding of $^{125}$I-BH-CCK-8S to mouse brain membranes after intravenous administration ($ED_{50}$ of 3.2 mg/kg). After intravenous administration of L-740,093, dose-dependent inhibition of *ex vivo* binding was also observed ($ED_{50}$ of 0.2

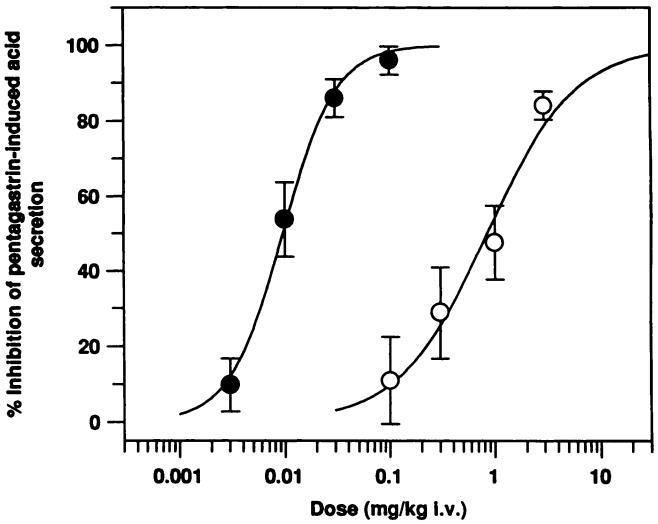


**Fig. 3.** Effects of L-740,093 (●) and L-365,260 (○) on pentagastrin-induced gastric acid secretion in rats. Gastric acid secretion was measured using a modification of the protocol described by Ghosh and Schild (19). Acid secretion was initiated with a constant-rate intravenous infusion of pentagastrin (0.3 μg/kg/min), as described in Materials and Methods. The vehicle for L-740,093 and L-365,260 was 10% ethanol/60% propylene glycol/30% saline. Each *point* is the arithmetic mean ± standard error for six animals.

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mg/kg, three experiments) (Fig. 4), corresponding to a 16-fold increase in potency. These values represented increases in potency of 4- and 65-fold, respectively, compared with L-365,260, which had an $ED_{50}$ of 13 mg/kg (three experiments) (Fig. 4).

In a second series of studies, the duration of inhibition by L-740,093 and L-365,260 was compared after intravenous administration of equivalent doses of the two compounds, calculated to produce 60–70% inhibition of specific binding in the *ex vivo* assay. These values were calculated to be 0.6 mg/kg for L-740,093 and 26 mg/kg for L-365,260. Results are shown in Fig. 5. L-740,093 showed inhibition of *ex vivo* binding for 1.5 hr after intravenous administration. A similar duration of action was observed for L-365,260.

## Discussion

In the past 20 years, a large number of biologically active peptides have been identified in the CNS (23). Many of these are present in discrete neuronal populations and fulfill the criteria to be considered potential neurotransmitters. Our understanding of their role in physiology and disease is often undermined by the lack of suitable potent and selective receptor antagonists. The discovery of the CCK receptor antagonist properties of the natural product asperlicin by Chang *et al.* (24) and the identification of benzodiazepines, such as devazepide and L-365,260, as selective CCK antagonists (6–8) have greatly facilitated our understanding of the functional role of CCK in both the CNS and the periphery.

Although L-365,260 is an important pharmacological tool for the discrimination of CCK-B receptors, it has certain properties that could limit its usefulness. The aqueous solubility of L-365,260 has been reported to be <0.002 mg/ml, which necessitates administration in nonaqueous vehicles for *in vivo* studies (25). The aim of this study was to identify compounds with

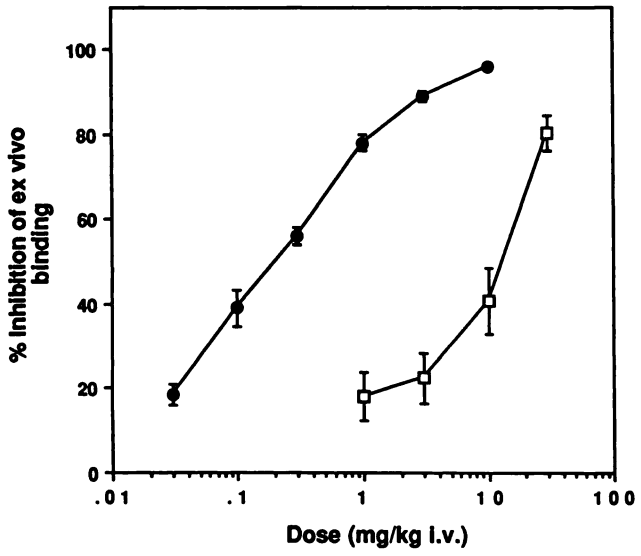


**Fig. 4.** Effects of L-740,093 (●) and L-365,260 (□) on inhibition of *ex vivo* binding in mice. Mice were pretreated with drug 30 min before sacrifice. *Ex vivo* binding of $^{125}$I-BH-CCK-8S was performed as described in Materials and Methods. The vehicle for L-740,093 and L-365,260 was 10% ethanol/60% propylene glycol/30% saline. The $ED_{50}$ values (dose required to inhibit binding by 50%) were 0.2 mg/kg, intravenously, for L-740,093 and 13 mg/kg, intravenously, for L-365,260. Each *point* is the arithmetic mean ± standard error for five animals.

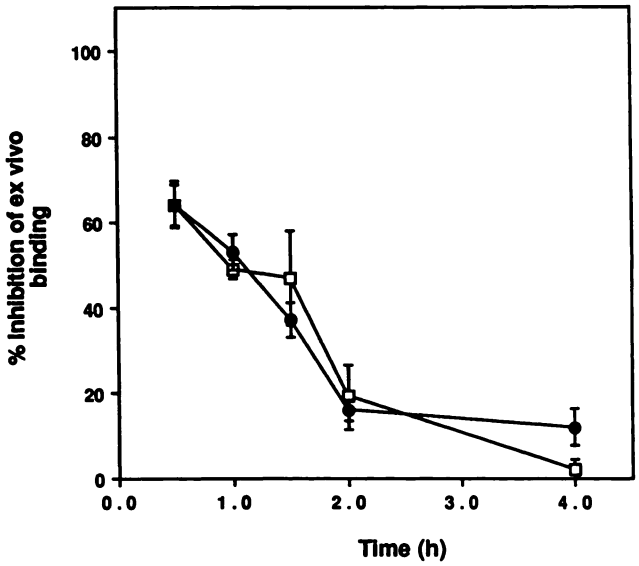


**Fig. 5.** Duration of action of L-740,093 (●) and L-365,260 (□) in an *ex vivo* binding assay in mice. Mice were administered 0.6 mg/kg L-740,093 intravenously or 26 mg/kg L-365,260 intravenously. These doses were predicted to produce 60–70% inhibition of the *ex vivo* binding of $^{125}$I-BH-CCK-8S. The vehicle for L-740,093 and L-365,260 was 10% ethanol/60% propylene glycol/30% saline. Each *point* is the arithmetic mean ± standard error for five animals.

improved CCK-B affinity and selectivity and, in particular, improved aqueous solubility.

A chemical series containing an amine-based functionality was sought, in which the $pK_a$ would be in a range to support salt formation but ensure that a significant proportion of the compound would be nonionized and presumably, therefore, brain-penetrating at physiological pH (15). Replacing the C-5 phenyl ring in L-365,260 with an amine generated a series of amidines whose $pK_a$ values were in the desired range (~7). The chemical development of the amidine series is described elsewhere (16).

The present studies describe the azabicyclononane derivative within the amidine series, i.e., L-740,093, which had extremely high affinity and selectivity for CCK-B receptors and is one of the most potent CCK-B ligands known. L-740,093 was found to have 210-fold higher affinity than L-365,260 and to be 3 orders of magnitude more selective than L-365,260 with respect to CCK-A receptors. In addition, the aqueous solubility of the crystalline HCl salts of L-737,415 and L-740,093 was improved by at least 2 orders of magnitude (0.6 mg/ml and 0.15 mg/ml, respectively). This will clearly offer an advantage when these compounds are used in whole-animal studies. In the present study, when the new antagonists were examined with L-365,260 identical vehicles were used, to enable direct comparisons to be made.

The VMH nucleus of rats contains high levels of CCK-B binding sites and CCK immunoreactivity. This region has been shown previously to be a useful preparation with which to study the pharmacology of brain CCK-B receptors and determine functional antagonist potency (18). Results from the present study indicated that L-740,093 was an antagonist with high affinity for functional CCK-B receptor-mediated responses in the CNS and had approximately 500 times higher affinity than did L-365,260. This increase in activity was somewhat larger than the relative increase in binding affinities of the two

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compounds for the CCK-B receptor in guinea pig cerebral cortex and may be related to the technical problems associated with receptor desensitization in the VMH slice preparation.

The antagonist properties of L-740,093 were confirmed in *in vivo* studies. When examined for functional activity in the rat gastric acid secretion model, L-740,093 was found to be 100-fold more potent than L-365,260 against pentagastrin-induced gastric acid secretion. Because there have not been any reports that known CCK-B antagonists, including the two amidines described in this study, are able to discriminate between CCK-B and gastrin binding, this remains a useful way of determining functional activity *in vivo*. Indeed, the recent cloning of the CCK and gastrin receptors from rats, dogs, and humans suggests that CCK-B and gastrin receptors may arise from a single gene product (20, 21).

The p$K_a$ values of the amidine groups in L-737,415 and L-740,093 are 7.5 and 7.1, respectively, and the estimated log *P* for both is >4.0. These values are within a reasonable range to promote CNS penetration, because it is estimated that about 50% of the molecules would be present in circulating plasma in their un-ionized form (15). To estimate the CNS potency of the two amidines, we have utilized an *ex vivo* binding assay that makes use of a cardiac perfusion step to remove potential contamination of the brain sample by the presence of test compound in residual plasma (26). Both L-737,415 and L-740,093 showed significant CNS activity in the *ex vivo* binding model after intravenous administration in mice. L-740,093 was the most potent compound in this assay that we have examined and showed a 60-fold increase in potency, compared with
L-365,260. These data therefore support the hypothesis that these compounds show excellent CNS penetration.

The present studies describe two amidine benzodiazepines, including one of the most potent and selective CCK-B antagonists yet described. Furthermore, these compounds have good aqueous solubility and excellent CNS penetration, providing an attractive profile with which to characterize the role of central CCK-B/gastrin receptors in physiology and disease.

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