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Cyclic Cholecystokinin Analogues That Are Highly Selective for Rat and Guinea Pig Central Cholecystokinin Receptors

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SUMMARY

Cholecystokinin (CCK) analogues (JMV310, JMV320, JMV328, and JMV332), obtained by side chain to side chain cyclization of a lysine residue in position 28 with a lysine residue in position 31, were found to be highly selective for the brain CCK receptor (CCK-B receptor), both in guinea pig and rat. In these analogues, the C-terminal tetrapeptide region of the molecule, which is the crucial determinant for binding to CCK-B receptors, has been constrained by cyclization. These analogues were highly potent in inhibiting binding of labeled CCK-8 to rat and guinea pig brain membranes (apparent affinity in the nanomolar range) but were poorly efficacious in inhibiting binding of labeled CCK-8 to rat or guinea pig pancreatic acini. In agreement with their low affinity for the pancreatic receptor, these CCK analogues were not very potent in stimulating amylase secretion. These cyclic CCK analogues were demonstrated to be highly selective for the brain CCK receptors.

CCK is a peptide hormone of 33 amino acid residues, which plays a major role both in the peripheral system (gall bladder contraction, pancreatic enzyme secretion, gut motility) (1) and in the central nervous system as a neurotransmitter and/or neuromodulator (2). The complete range of biological activities has been found in the carboxyl terminal octapeptide (CCK-8) of the entire molecule (3).

Extensive binding studies made in peripheral tissues such as pancreas have shown desulfated CCK-8, smaller C-terminal fragments including the C-terminal tetrapeptide (CCK-4), and gastrin to have low affinity for the CCK receptors. By contrast, CCK receptors found in most regions of the brain show relatively high affinity for these peptides and for CCK-8, with the C-terminal tetrapeptide Trp-Met-Asp-Phe-NH_2 being the minimal fragment exhibiting a high affinity for the central CCK receptor (4). On the basis of these differences, the classification of type A (peripheral, sulfate-dependent) and type B (like most brain, sulfate-independent) CCK receptors has been proposed (5). They were demonstrated to correspond to different biochemical entities (6). Moreover, it appears that CCK receptors in both the periphery and the central nervous system are heterogeneous. Potent ligands that are highly specific for either class of receptors are, therefore, essential for an understanding of the physiological role of CCK.

The availability of a number of potent nonpeptide ligand CCK antagonists (7), which have high selectivity for CCK receptors in peripheral tissues, has permitted a much clearer differentiation of CCK receptor types (8). Recently, nonpeptide compounds such as the benzodiazepine derivative L-365,260, have been proposed as potent and selective CCK-B receptor ligands (9, 10). Based on NMR studies, fluorescence measurements, and energy calculations, which have shown that CCK-8 exists preferentially in folded conformations characterized by both N- and C-terminal turns (11, 12), a cyclic analogue of the C-terminal octapeptide of CCK, i.e., Boc-γ-D-Glu-Tyr(SO_3H)-Nle-D-Lys-Trp-Nle-Asp-Phe-NH_2, bearing the intact C-terminal tetrapeptide that is the crucial determinant for binding to CCK-B receptors, was synthesized and found to be selective for CCK-B receptors (13). We would like to report in this paper the pharmacological properties of a series of cyclic analogues of the C-terminal hepta- and hexapeptides of CCK, mimicking the C-terminal turn of CCK-8, but modified in the C-terminal tetrapeptide region, that are highly selective for the central CCK receptor (CCK-B). These analogues (compounds JMV310, JMV320, JMV328, and JMV332) were obtained by side chain to side chain cyclization of a lysine residue in position 28 (replacing methionine) with a lysine residue in position 31 (replacing methionine) through a succinyl bridge (see scheme in Fig. 1). Simple molecular model examination showed that side chains of residues in positions 28 and 31 are good candidates for a possible cyclization with the help of succinic acid, stabilizing the peptide backbone-folded conformation of the parent molecule. These analogues are characterized by the fact that the C-terminal tetrapeptide of CCK, which is the crucial minimal structure able to bind to CCK-B receptors with high affinity, has been constrained by cyclization. They constitute, to our knowledge, the first example of CCK analogues modified in the C-terminal tetrapeptide region, exhibiting high and

ABBREVIATIONS: CCK, cholecystokinin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Boc, *t*-butyloxycarbonyl.

Boc-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (Boc-[Nle^{28}, Nle^{31}]-CCK-7)

Boc-Trp-Leu-Asp-Phe-NH_2 (JMV90)

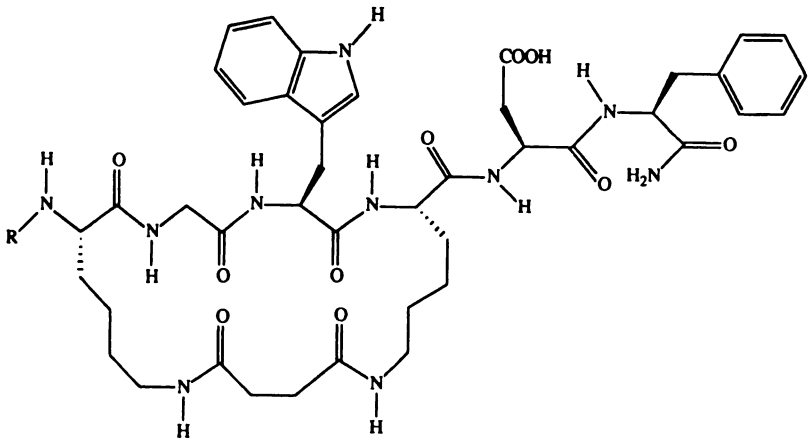

Fig. 1. Chemical structures of CCK and cyclic CCK analogues. JMV310, *R* = acetyl-Tyr(SO_3H)-; JMV320, *R* = acetyl-Tyr; JMV328, *R* = H; JMV332, *R* = Acetyl.

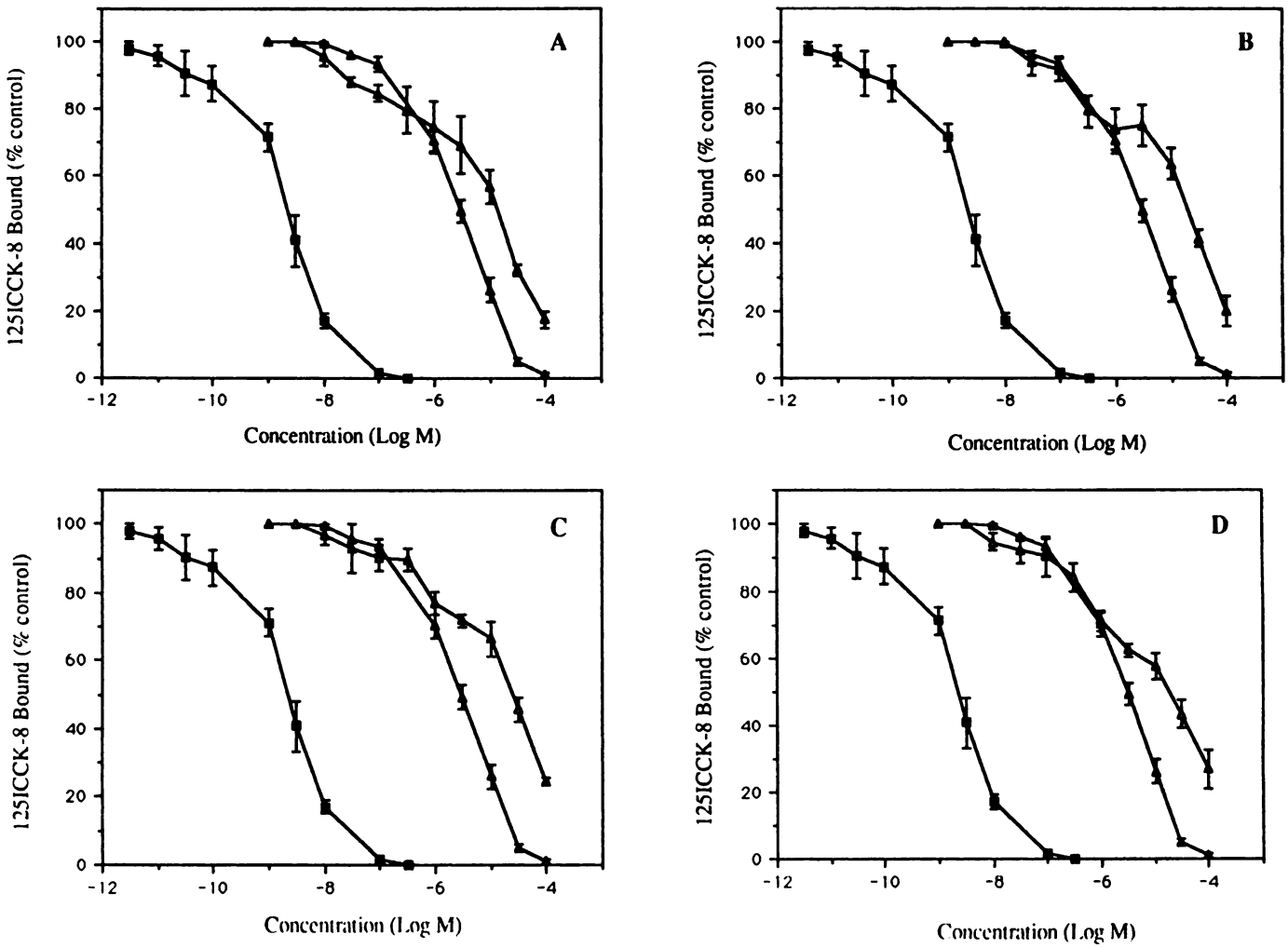

Fig. 2. Effects of Boc-[Nle^{28},Nle^{31}]-CCK-7, Boc-Trp-Leu-Asp-Phe-NH_2, and compounds JMV310, JMV320, JMV328, and JMV332 on inhibition of binding of labeled ^{125}I-CCK-8 to rat pancreatic acini. Isolated pancreatic acini were incubated for 30 min at 37° with 10 pM ^{125}I-CCK-8 plus various concentrations of Boc-[Nle^{28},Nle^{31}]-CCK-7 (■), Boc-Trp-Leu-Asp-Phe-NH_2 (▲), or cyclic CCK analogues (△) JMV310 (A), JMV320 (B), JMV328 (C), and JMV332 (D). Values are expressed as a percentage of the value obtained with labeled CCK-8 alone. In each experiment, each value was determined in duplicate, and the results given are the means from at least four separate experiments (± standard error). Specific binding was 13 ± 3% of the total radioactivity present in the sample.

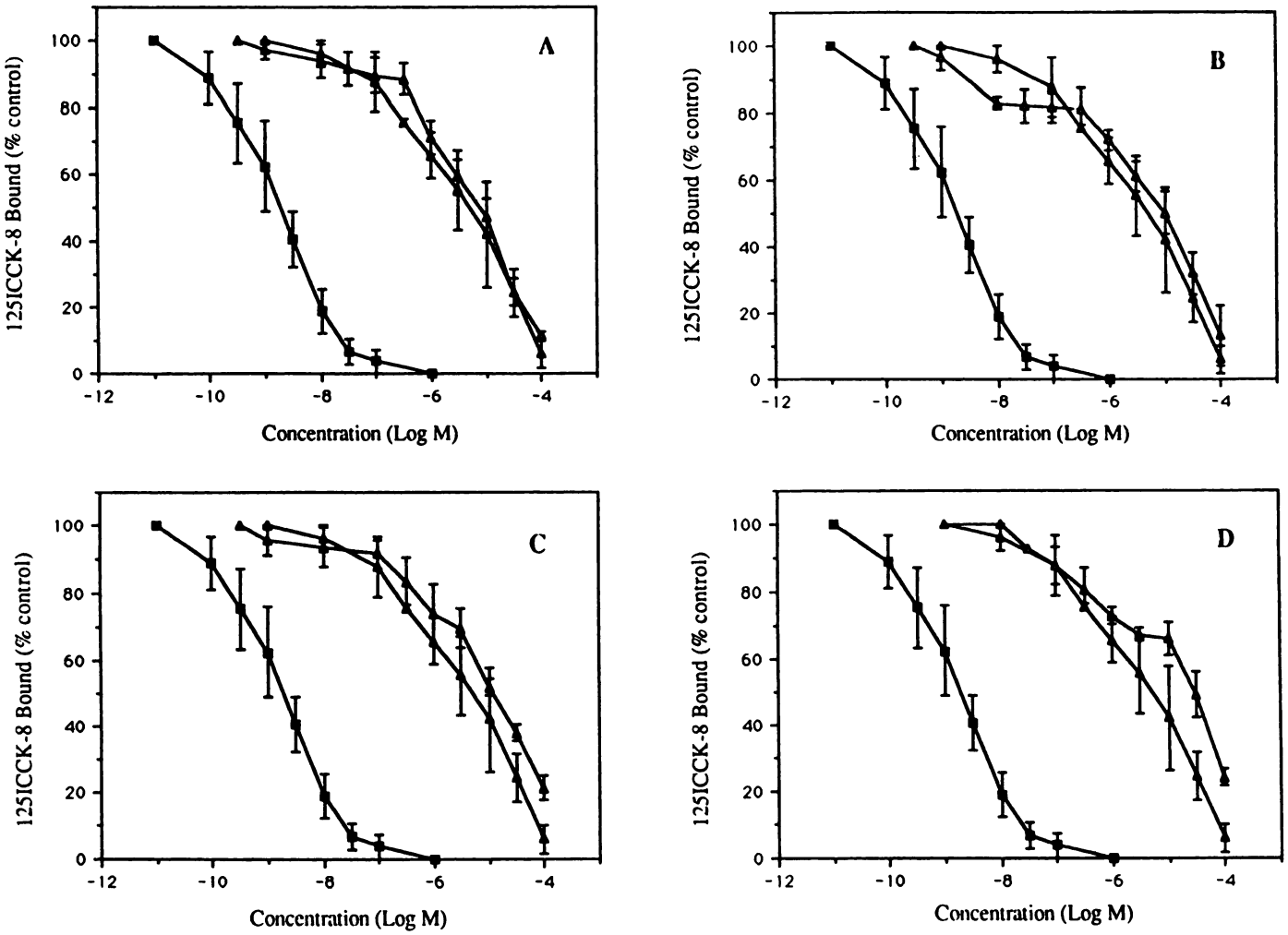

Fig. 3. Effects of Boc-[Nle^{28},Nle^{31}]-CCK-7, Boc-Trp-Leu-Asp-Phe-NH_2, and compounds JMV310, JMV320, JMV328, and JMV332 on inhibition of binding of labeled ^{125}I-CCK-8 to guinea pig pancreatic acini. Isolated pancreatic acini were incubated for 30 min at 37° with 25 pM ^{125}I-CCK-8 plus various concentrations of Boc-[Nle^{28},Nle^{31}]-CCK-7 (■), Boc-Trp-Leu-Asp-Phe-NH_2 (▲), or cyclic CCK analogues (△), JMV310 (A), JMV320 (B), JMV328 (C), and JMV332 (D). Values are expressed as a percentage of the value obtained with labeled CCK-8 alone. In each experiment, each value was determined in duplicate, and the results given are the means from at least four separate experiments (± standard error). Specific binding was 13 ± 3% of the total radioactivity present in the sample.

selective affinity for CCK-B receptors, and may be of great help in providing information about the active conformation of the CCK-B receptors.

Experimental Procedures

Materials

Male Wistar rats (180–200 g) were from Effa-Credo (Saint Germain l'Arbresle, France) and male guinea pigs (280–300 g) were obtained from le Centre d'Elevage d'Animaux de Laboratoire (Ardenay, France). HEPES was from Boehringer-Mannheim, purified collagenase was from Serva (Garden City Park, NY), soybean trypsin inhibitor was from Sigma (St. Louis, MO), Eagle's basal amino acid medium (100× concentrated) was from GIBCO (Grand Island, NY), essential vitamin mixture (100× concentrated) was from Microbiological Associates (Bethesda, MD), bovine plasma albumin (fraction V) was from Miles Laboratories Inc. (Elkhart, IN), Phadebas amylase test was from Pharmacia Diagnostics (Piscataway, NJ), and ^{125}I-labeled *N*-succinimidyl-3-(4-hydroxyphenyl)propionyl-CCK-8 was from Amersham Corp. (Buckinghamshire, UK). Boc-[Nle^{28}, Nle^{31}]-CCK-7, Boc-Trp-Leu-Asp-Phe-NH_2, and cyclic CCK derivatives were synthesized in our laboratory; their detailed syntheses have been described elsewhere (14). Unless otherwise stated, the standard incubation solutions contained 24.5 mM HEPES (pH 7.4), 98 mM NaCl, 6 mM KCl, 2.5 mM NaH_2PO_4, 5 mM sodium pyruvate, 5 mM sodium fumarate, 5 mM sodium glutamate, 2 mM glutamine, 11.5 mM glucose, 0.5 mM $CaCl_2$, 1 mM $MgCl_2$, 0.5 mg/ml bacitracin, 0.2% (w/v) albumin, 0.03% (w/v) soybean trypsin inhibitor, 1% (v/v) essential amino acid mixture, and 1% (v/v) essential vitamin mixture. The incubation solution was equilibrated with 95% O_2/5% CO_2 as the gas phase.

Methods

Tissue preparation. Dispersed acini from rat and guinea pig pancreas were prepared according to the previously described methods (15, 16). Rat and guinea pig brain membranes were prepared following the procedures of Pelaprat *et al.* (17).

Amylase secretion. Amylase release was measured using the procedure already described (15, 16). Briefly, acini were resuspended in the standard incubation solution supplemented with 1% bovine serum albumin and 1.3 mM calcium, containing about 1 mg of protein/ml, and samples (0.5 ml) were incubated at 37° for 30 min. Amylase activity was determined by the method of Ceska *et al.* (18), using the Phadebas reagent. Amylase release was measured as the difference in amylase

TABLE 1

Effects of Boc-[Nle^{28},Nle^{31}]-CCK-7, Boc-Trp-Leu-Asp-Phe-NH$_2$, and compounds JMV310, JMV320, JMV328, and JMV332 on specific binding of ^{125}I-CCK-8 in rat pancreatic acini and in rat brain membranes, as well as on amylase secretion from rat pancreatic acini

Compounds	Rat pancreatic acini		Rat brain membranes, binding, K_d
	Amylase release, ED	Binding, K_d	
	nM	nM	nM
Boc-[Nle^{28,31}]-CCK7	0.05 ± 0.01	0.21 ± 0.48 3.2 ± 0.5	0.40 ± 0.05
Boc-Trp-Leu-Asp-Phe-NH$_2$ (JMV90)	1,270 ± 279	2,600 ± 260	2.5 ± 0.8
JMV310	172 ± 51	230 ± 28 7,300 ± 560	4.0 ± 0.4
JMV320	140 ± 30	191 ± 32 11,400 ± 1,700	11.5 ± 3.5
JMV328	367 ± 93	1,320 ± 450 21,800 ± 2,500	13 ± 5.5
JMV332	265 ± 35	1,450 ± 450 11,700 ± 1,400	32 ± 9.5

TABLE 2

Effects of Boc-[Nle^{28},Nle^{31}]-CCK-7, Boc-Trp-Leu-Asp-Phe-NH$_2$, and compounds JMV310, JMV320, JMV328, and JMV332 on specific binding of ^{125}I-CCK-8 in guinea pig pancreatic acini and in guinea pig brain membranes, as well as on amylase secretion from guinea pig pancreatic acini

Compounds	Guinea pig pancreatic acini		Guinea pig brain membranes, binding, K_d
	Amylase release, ED_{50}	Binding, K_d	
	nM	nM	nM
Boc-[Nle^{28,31}]-CCK7	0.40 ± 0.08	0.11 ± 0.15 28 ± 6	0.26 ± 0.05
Boc-Trp-Leu-Asp-Phe-NH$_2$ (JMV90)	4,750 ± 750	700 ± 200 8,000 ± 1,250	1.6 ± 0.3 2,200 ± 540
JMV310	3,733 ± 674	603 ± 120 22,000 ± 2,800	0.6 ± 0.4 30 ± 15
JMV320	2,575 ± 814	45 ± 12 19,800 ± 2,500	1 ± 0.9 22 ± 8
JMV328	3,875 ± 268	610 ± 160 20,000 ± 3,500	5 ± 2 100 ± 65
JMV332	3,500 ± 845	830 ± 220 30,000 ± 7,500	2.5 ± 2 22 ± 15

activity, at the end of the incubation, that was released into the extracellular medium, with and without secretagogue, and is expressed as the percentage of maximal stimulation obtained with Boc-[Nle^{28},Nle^{31}]-CCK-7 (40 ± 5% of total amylase contained in the acini) minus the basal amylase secretion (10 ± 2% of total amylase contained in the acini) obtained without secretagogue.

Binding of labeled CCK-8 to rat or guinea pig pancreatic acini. Binding of ^{125}I-CCK-8 to rat and guinea pig pancreatic acini was performed as previously described (15, 16). Briefly, samples (0.5 ml containing ≈1 mg/ml protein) were incubated with the appropriate peptide concentrations for 30 min (equilibrium was reached) at 37° in the presence of 10 pM (rat) or 25 pM (guinea pig) ^{125}I-CCK-8, plus various concentrations of compounds to be tested. After washings and centrifugation at 10,000 × *g* for 10 min, the radioactivity associated with the acinar pellet was measured. Values are expressed as the percentage of the value obtained with labeled CCK-8 alone. The specific activities of the various preparations used in our experiments were about 2000 Ci/mmol. Binding in the absence of any unlabeled CCK peptide was 13 ± 3% of the total radioactivity present in the sample in both species. Nonspecific binding was determined in the presence of 1 μM Boc-[Nle^{28},Nle^{31}]-CCK-7 and was always less than 15% of the total binding in the case of rat pancreatic acini and less than 25% in the case of guinea pig pancreatic acini. All the analogues were stable under the incubation conditions, as checked by high pressure liquid chromatography.

Binding of ^{125}I-CCK-8 to rat or guinea pig brain membranes. Binding of ^{125}I-CCK-8 to guinea pig or rat brain membranes was performed according to previously described methods (17, 19). The buffer used was 50 mM Tris·HCl, 5 mM $MgCl_2$, 0.1 mg/ml bacitracin, pH 7.4 (Tris-$MgCl_2$-bacitracin buffer). Briefly, displacement experiments were performed by incubation of brain membranes (approximately 0.5 mg of protein) in the presence of 10 pM ^{125}I-CCK-8 (guinea pig) or 20 pM ^{125}I-CCK-8 (rat) plus various concentrations of compound to be tested, in a total volume of 1 ml, for 60 min (equilibrium was reached) at 25°. Nonspecific binding was determined in the presence of 1 μM Boc-[Nle^{28},Nle^{31}]-CCK-7 and was always less than 25% (guinea pig) and less than 40% (rat) of the total binding. Total binding was about 15% (guinea pig) and 12% (rat) of the total radioactivity contained in the sample. All the analogues were stable under the incubation conditions, as checked by high pressure liquid chromatography.

Results

The cyclic CCK analogues JMV310, JMV320, JMV328, and JMV332 were tested for their ability to displace the specific binding of ^{125}I-CCK-8 from CCK receptors on pancreatic acini and brain membranes from guinea pig and rat. They were also evaluated for their ability to stimulate amylase secretion from rat and guinea pig pancreatic acini. The cyclic CCK analogues were compared with Boc-[Nle^{28},Nle^{31}]-CCK-7, a very potent analogue of CCK-7 (20, 21), and with the potent CCK-4 analogue Boc-Trp-Leu-Asp-Phe-NH$_2$ (22, 23).

In rat or guinea pig pancreatic acini, Boc-[Nle^{28},Nle^{31}]-CCK-7, Boc-Trp-Leu-Asp-Phe-NH$_2$, and the cyclic analogues JMV310, JMV320, JMV328, and JMV332 were able to dose dependently inhibit binding of labeled CCK-8. Although Boc-[Nle^{28},Nle^{31}]-CCK-7 was very potent, neither compounds JMV310, JMV320, JMV328, and JMV332 nor the CCK-4 analogue Boc-Trp-Leu-Asp-Phe-NH$_2$ were highly efficient in inhibiting binding of labeled CCK-8 (Figs. 2 and 3). The apparent relative affinities of the peptides tested were Boc-[Nle^{28},Nle^{31}]-CCK-7 ≫ Boc-Trp-Leu-Asp-Phe-NH$_2$ ≈ JMV310 ≈ JMV320 ≈ JMV328 ≈ JMV332 for rat and Boc-[Nle^{28},Nle^{31}]-CCK-7 ≫ Boc-Trp-Leu-Asp-Phe-NH$_2$ ≈ JMV310 > JMV320 ≈ JMV328 ≈ JMV332 for guinea pig. Computer analysis of these data using the LIGAND program (24) best fit two classes of binding sites ($p < 0.01$) on guinea pig and rat pancreatic acini (Tables 1 and 2). Each CCK-related peptide tested was able to stimulate amylase secretion from rat or guinea pig pancreatic acini, and there was a close correlation between the ability of a fragment to stimulate amylase secretion and to inhibit binding of labeled CCK-8. In terms of the increase in amylase secretion caused by a maximally effective concentration, all CCK analogues tested had the same efficacies (Figs. 4 and 5). Although supramaximal concentrations of the CCK-4 analogue did not significantly alter the maximal response (16, 23), the cyclic CCK analogues, like Boc-[Nle^{28},Nle^{31}]-CCK-7, had a biphasic dose-response curve for stimulating amylase secretion from rat pancreatic acini (Fig. 4). In guinea pig pancreatic acini, the CCK cyclic analogues were as efficacious as Boc-[Nle^{28},Nle^{31}]-CCK-7 but, due to their very low potency, the biphasic character of the dose-response curve could not be demonstrated (Fig. 5).

In contrast to their weak interaction with peripheral CCK binding sites, compounds Boc-Trp-Leu-Asp-Phe-NH$_2$, JMV310,

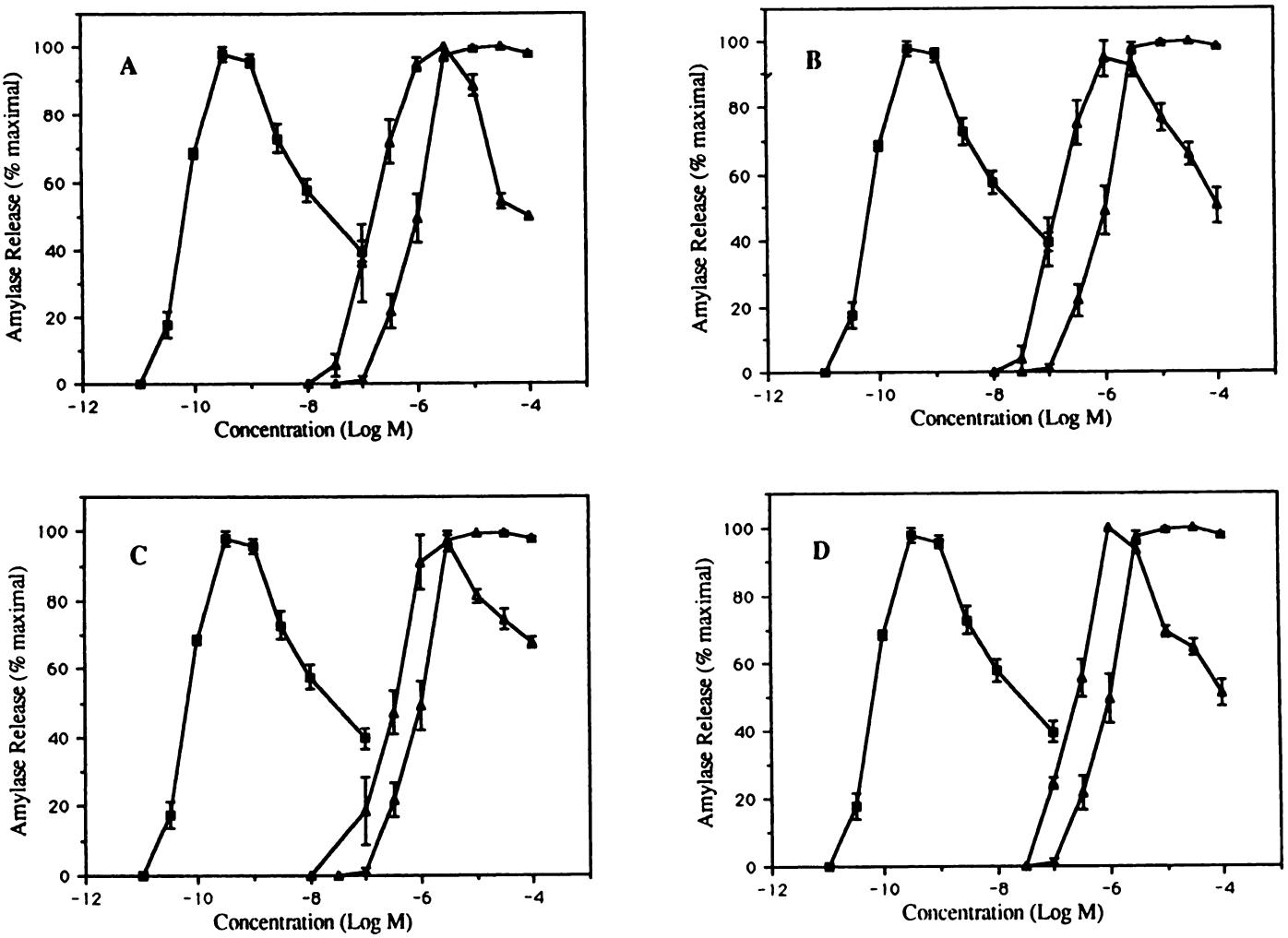

Fig. 4. Effects of Boc-[Nle^{28},Nle^{31}]-CCK-7 (■), Boc-Trp-Leu-Asp-Phe-NH_2 (▲), and cyclic CCK analogues (△) JMV310 (A), JMV320 (B), JMV328 (C), and JMV332 (D) on amylase release from rat pancreatic acini. Amylase release was measured as the difference of amylase activity, at the end of incubation, that was released into the extracellular medium with and without secretagogue and is expressed as percentage of maximal stimulation obtained with Boc-[Nle^{28},Nle^{31}]-CCK-7 (40 ± 5% of the total amylase contained in the acini) minus the basal amylase secretion (10 ± 2% of the total amylase contained in the acini). In each experiment, each value was determined in duplicate, and the results given are the means from at least four separate experiments (± standard error).

JMV320, JMV328, and JMV332 were strong ligands for central CCK receptors (Figs. 6 and 7). These CCK analogues were able to inhibit binding of ^{125}I-CCK-8 to rat and guinea pig brain membranes with high potencies (Tables 1 and 2). Computer analysis of displacement data was performed using a nonlinear, least-squares, curve-fitting computer program (LIGAND) (24). With each peptide tested, a two-site model gave a significantly better fit ($p < 0.01$) in rat brain membranes, whereas only a single affinity could be evidenced in guinea pig brain membranes (Tables 1 and 2). Boc-[Nle^{28},Nle^{31}]-CCK-7 and Boc-Trp-Leu-Asp-Phe-NH_2 possessed similar affinities for guinea pig and rat brain. In contrast, the cyclic CCK analogues were more potent on guinea pig than on rat brain membranes.

Discussion

Although comparison of intact cell binding data from acini with membrane binding data from brain should be interpreted carefully, the results of this study clearly show the high selectivity of the cyclic CCK analogues (compounds JMV310, JMV320, JMV328, and JMV332) for rat and guinea pig central CCK receptors versus peripheral CCK receptors. In fact, the selectivity of these derivatives is essentially due to a drastic loss of their affinity for peripheral binding sites. In agreement with their low affinity for the peripheral CCK receptor, compounds JMV310, JMV320, JMV328, and JMV332 were only weakly active in stimulating amylase secretion from rat or guinea pig pancreatic acini (Tables 1 and 2 and Figs. 4 and 5). They were slightly more potent than the CCK-4 analogue Boc-Trp-Leu-Asp-Phe-NH_2 in stimulating amylase secretion from rat pancreatic acini, whereas they were almost equipotent with Boc-Trp-Leu-Asp-Phe-NH_2 in guinea pig pancreatic acini. Nevertheless, the weak peripheral activity of compounds JMV310, JMV320, JMV328, and JMV332 was clearly lower, by a factor of approximately 10,000, than that of Boc-[Nle^{28},Nle^{31}]-CCK-7. As compared with the selective CCK-B nonpeptidic compounds described by Bock *et al.* (9), such as L-365,260, the cyclic CCK analogues described in this work are approximately 20 to 100 times less potent and 4 to 20 times less potent than the cyclic CCK analogues described by Charpentier *et al.* (13) on pancreatic receptors from guinea pigs. Although the CCK-4 analogue did not show a significant decrease in the response at supramaximal concentrations, a bi-

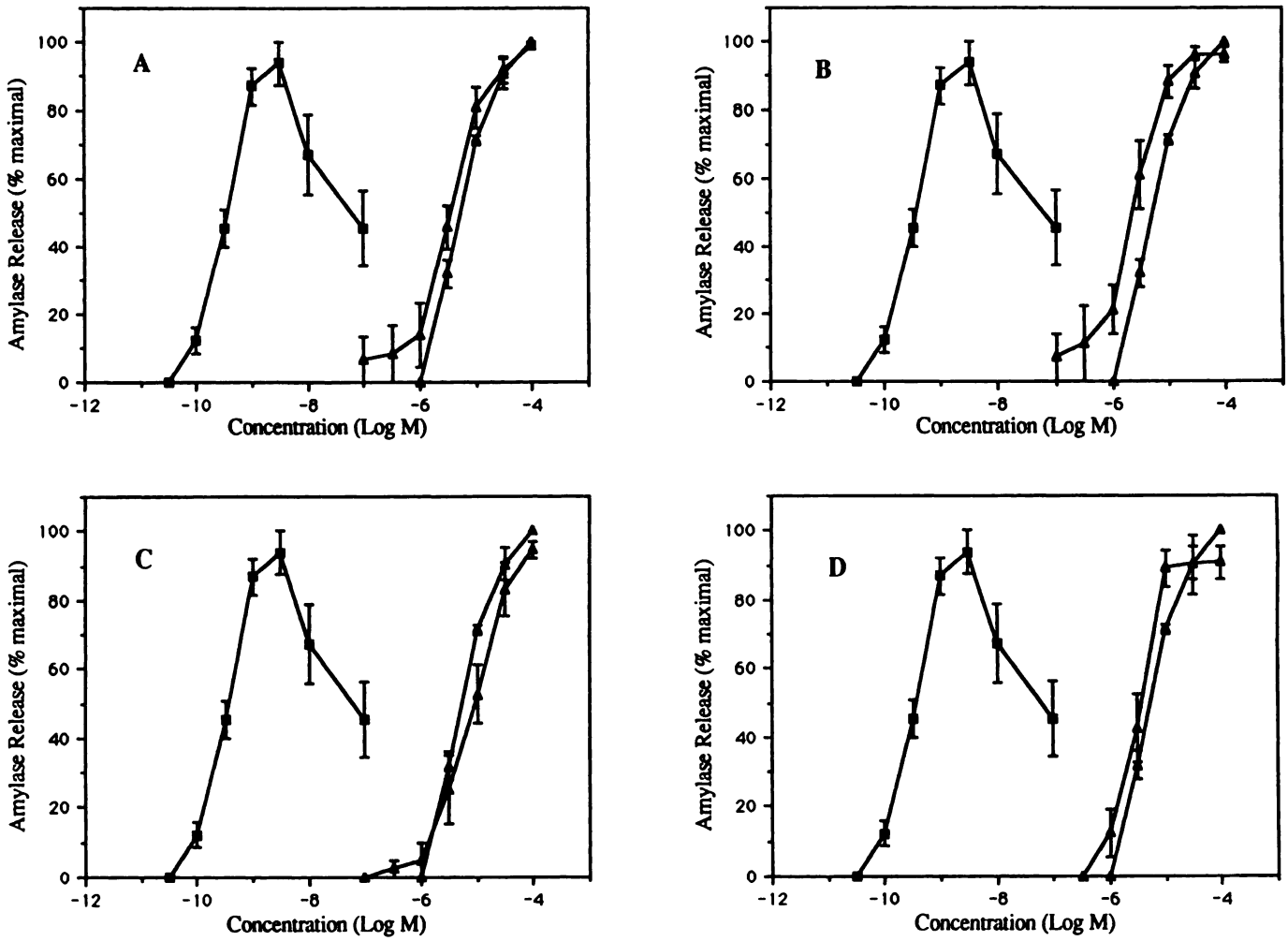

Fig. 5. Effects of Boc-[Nle^{28},Nle^{31}]-CCK-7 (■), Boc-Trp-Leu-Asp-Phe-NH_2 (▲), and cyclic CCK analogues (△) JMV310 (A), JMV320 (B), JMV328 (C), and JMV332 (D) on amylase release from guinea pig pancreatic acini. Amylase release was measured as the difference of amylase activity, at the end of incubation that was released into the extracellular medium with and without secretagogue and is expressed as a percentage of maximal stimulation obtained with Boc-[Nle^{28},Nle^{31}]-CCK-7 (40 ± 5% of the total amylase contained in the acini) minus the basal amylase secretion (10 ± 2% of the total amylase contained in the acini). In each experiment, each value was determined in duplicate, and the results given are the means from at least four separate experiments (± standard error).

phasic dose-response curve of amylase secretion could be demonstrated using compounds JMV310, JMV320, JMV328, and JMV332 on rat pancreatic acini, indicating that these cyclic compounds might act as agonists at both high and low affinity binding sites. Due to the extremely weak potency of these analogues in stimulating amylase secretion, such a pattern could not be observed using guinea pig pancreatic acini. In contrast to the results obtained previously showing the importance of the C-terminal heptapeptide of CCK for the complete response on pancreatic acini (16), neither the tyrosine residue, the sulfate ester of tyrosine, nor the $N\alpha$-protecting group appeared to be significant in the cyclic CCK analogues for the binding to peripheral CCK receptors and exhibition of complete biological activity on pancreatic acini.

A high affinity for central CCK receptors has been obtained for the cyclic CCK analogues JMV310, JMV320, JMV328, and JMV332 on the rat or guinea pig brain membranes, thus resulting in a high selectivity for the central CCK receptor versus the peripheral CCK receptor. Compounds JMV310 and JMV320 were only slightly less potent than the very potent CCK-7 analogue Boc-[Nle^{28},Nle^{31}]-CCK-7 in inhibiting the binding of ^{125}I-CCK-8 to guinea pig brain membranes, whereas they were, respectively, less potent on rat brain membranes. Compounds JMV328 and JMV332, lacking the sulfated tyrosine residue, were only slightly less potent than JMV310 and JMV320. By comparing the CCK receptors on guinea pig and mouse, Williams *et al.* (25) found significant differences in the ability of these binding sites to discriminate between unsulfated CCK-8 and CCK-4, with the receptor of brain cortex from guinea pig being more selective than those of mouse. More recently, Durieux *et al.* (19) showed a heterogeneity of central CCK receptors in guinea pig and rat brain cortex. Moreover, using [^{3}H]propionyl-CCK-8, the same group of investigators (26) demonstrated the presence of two binding sites in guinea pig brain cortex and one binding site in rat brain cortex. Our results with the cyclic CCK analogues that are selective for the central CCK receptor confirm the receptor heterogeneity between species and support the occurrence of large differences in the characteristics of high affinity binding sites of guinea pig and rat brain.

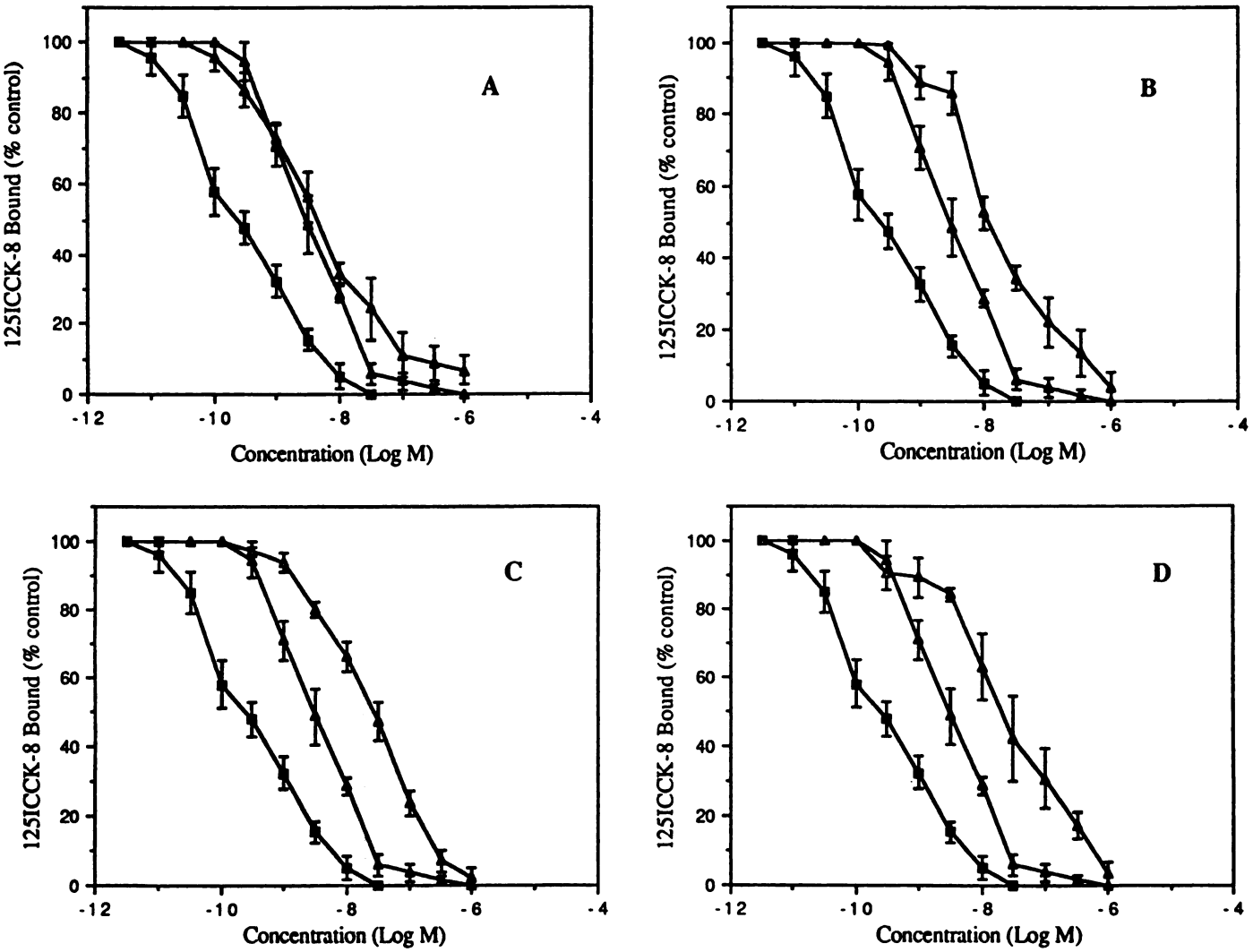

Fig. 6. Effects of Boc-[Nle^{28},Nle^{31}]-CCK-7, Boc-Trp-Leu-Asp-Phe-NH_2, and compounds JMV310, JMV320, JMV328, and JMV332 on inhibition of binding of labeled ^{125}I-CCK-8 to rat brain membranes. Rat brain membranes were incubated for 60 min at 25° with 20 pM ^{125}I-CCK-8 plus various concentrations of Boc-[Nle^{28},Nle^{31}]-CCK-7 (■), Boc-Trp-Leu-Asp-Phe-NH_2 (▲), or cyclic CCK analogues (△) JMV310 (A), JMV320 (B), JMV328 (C), and JMV332 (D). Values are expressed as a percentage of the value obtained with labeled CCK-8 alone. Nonspecific binding was determined in the presence of 1 μM Boc-[Nle^{28},Nle^{31}]-CCK-7. Nonspecific binding was always less than 40% of the total binding. Total binding was about 12% of the total radioactivity present in the sample. In each experiment, each value was determined in duplicate, and the results given are the means from at least five separate experiments (± standard error).

The cyclization of the C-terminal part of CCK between Lys^{28} and Lys^{31} residues by a succinyl moiety, including the C-terminal tetrapeptide portion in the cyclic structure that constrains the crucial part of the CCK molecule for binding to central CCK receptors, led to extremely selective synthetic ligands for guinea pig and rat brain CCK receptors. Examination of simple molecular models showed that, in these cyclic compounds, the Trp^{30} and Phe^{33}-NH_2 side chains can be positioned far from each other, a situation that seems to favor interactions with the central CCK receptor versus the peripheral CCK receptor (27). These compounds, which are to our knowledge the first cyclic CCK analogues that are highly selective for the central CCK receptor modified in the C-terminal part, will be useful in further elucidation of the roles of the CCK receptor subtypes and investigation of CCK functions in the central nervous system. Moreover, they will permit definition of the crucial requirements for interaction with the central CCK receptors and of the structural parameters responsible for receptor discrimination and they will allow rational design of pharmacologically active peptides or nonpeptide-specific CCK-like compounds that are highly selective for the central CCK receptors.

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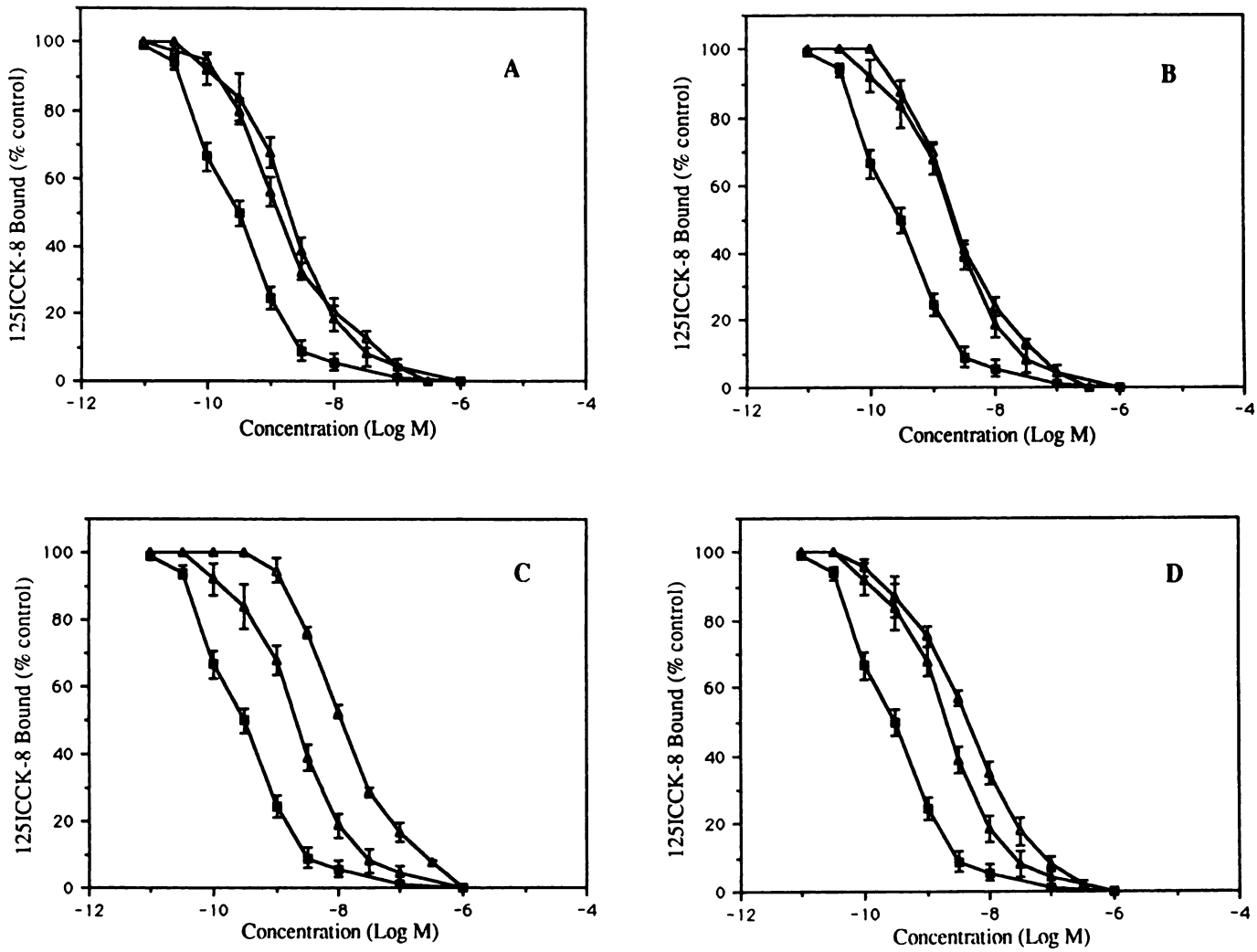

Fig. 7. Effects of Boc-[Nle^{28},Nle^{31}]-CCK-7, Boc-Trp-Leu-Asp-Phe-NH_2, and compounds JMV310, JMV320, JMV328, and JMV332 on inhibition of binding of labeled ^{125}I-CCK-8 to guinea pig brain membranes. Guinea pig brain membranes were incubated for 60 min at 25° with 10 pM ^{125}I-CCK-8 plus various concentrations of Boc-[Nle^{28},Nle^{31}]-CCK-7 (■), Boc-Trp-Leu-Asp-Phe-NH_2 (▲), or cyclic CCK analogues (△) JMV310 (A), JMV320 (B), JMV328 (C), and JMV332 (D). Values are expressed as a percentage of the value obtained with labeled CCK-8 alone. Nonspecific binding was determined in the presence of 1 μM Boc-[Nle^{28},Nle^{31}]-CCK-7. Nonspecific binding was always less than 25% of the total binding. Total binding was about 15% of the total radioactivity present in the sample. In each experiment, each value was determined in duplicate, and the results given are the means from at least five separate experiments (± standard error).

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