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# EFFECTS OF CAERULEIN-RELATED PEPTIDES ON CHOLECYSTOKININ RECEPTOR BINDINGS IN BRAIN AND PANCREAS

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**Abstract**—A number of caerulein (CLN)-related peptides were synthesized and compared in terms of their affinities for cholecystokinin (CCK) receptors. We have found that these peptides can be classified into three types according to their relative affinities for the brain and pancreatic receptors. The first group (type A) of peptides includes CLN and analogs retaining the Tyr($SO_3H$)$^4$ residue and the COOH-terminal amide group. Type A peptides were as potent as CLN in inhibiting [$^{125}$I]BH-CCK-8 binding and showed almost the same affinities for pancreatic and brain receptors. When the Tyr($SO_3H$)$^4$ residue was either deleted or desulfated (type B), the affinities of the peptides decreased remarkably for the pancreatic receptors but much less for the brain receptors. The type C peptides were deamidated, oxidized, or shortened in the COOH-terminal region and exhibited greatly decreased affinities for both brain and pancreatic receptors but a much greater decrease for the brain receptor. These results indicate that, although the Tyr($SO_3H$)$^4$ residue and the COOH-terminal structure are both essential for CLN to bind to the CCK receptors, the former is of critical importance for the binding to the pancreas and the latter is rather important for the binding to the brain.

Cholecystokinin (CCK†), an important gastrointestinal hormone, may play an important role as a neurotransmitter or a neuromodulator [1, 2]. Recently, the occurrence of specific and saturable sites for the high-affinity binding of CCK has been demonstrated in mammalian pancreases and brains [3–5]. The properties of these binding sites in the peripheral and central tissues are remarkably similar in certain points. For example, both receptors‡ have almost the same $K_d$ values for CCK-33 or CCK-8, the binding is maximal at weakly acidic pH, and monovalent cations reduce the binding while divalent cations enhance the binding [3–5]. On the other hand, the most notable difference between the pancreas and the brain in the CCK binding is that CCK-4, pentagastrin, and desulfated CCK-8 are virtually inactive in the pancreas but rather potent in the brain [3, 5]. The potency of dibutyryl cyclic GMP for reducing the CCK binding in the brain was reported to be lower than in the pancreas [5]. However, little is known as to how these differences in the properties of receptor binding between the brain and the pancreas are related to the physiological functions of CCK in the central and peripheral tissues.

Caerulein (CLN) is a decapeptide isolated from frog skin and has been known to possess the same biological and pharmacological activities as CCK [6–8]. Moreover, this peptide shows almost the same affinities for brain and pancreatic CCK receptors as CCK-33 or CCK-8 [3]. To study the properties of the receptor binding of CCK in the central and peripheral organs in more detail, we synthesized CLN-related peptides and compared their affinities for the CCK receptors of both the brain and the pancreas.

## MATERIALS AND METHODS

*Materials*. CCK-8 was obtained from the Protein Research Foundation, Osaka, Japan. *N*-Succinimidyl 3-(4-hydroxy, 5-[$^{125}$I]indophenyl) propionate ([$^{125}$I]BH; 2000 Ci/mmole) was purchased from the New England Nuclear Corp., Boston, MA. Soybean trypsin inhibitor (SBTI), bacitracin, Tris, and EGTA were obtained from the Sigma Chemical Co., St. Louis, MO; *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (Hepes) from Calbiochem, La Jolla, CA; and bovine serum albumin type V (BSA) from

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† Abbreviations: CCK, cholecystokinin; CLN, caerulein; [$^{125}$I]BH, *N*-succinimidyl 3-(4-hydroxy, 5[$^{125}$I]indophenyl)propionate; SBTI, soybean trypsin inhibitor; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; BSA, bovine serum albumin type V; HPLC, high-performance liquid chromatography. In the paragraph on *Synthesis of peptides*, we used abbreviations recommended by the IUPAC-IUB Commission on Biochemical Nomenclature [*Biochem. J.* **126**, 773 (1972)] including Pyr, pyroglutamic acid; Z, benzyloxycarbonyl; Boc, *t*-butoxycarbonyl; Fmoc, 9-fluorenylmethoxycarbonyl; Ac, acetyl; Bzl, benzyl; Bu$^t$, *t*-butyl; HOSu, *N*-hydroxysuccinimide; TFA, trifluoroacetic acid; DMF, *N*,*N*-dimethylformamide; and EGTA, ethyleneglycolbis(amino-ethylether)tetraacetate.

‡ Although the relationship between the binding sites in the brain and the suggested CNS action of CCK has not been firmly established, we use the term "receptor" in the present work because some other published papers have already employed this term (see, for example, Refs. 3 and 5).

the Armour Pharmaceutical Co., Phoenix, AZ. Non-radioactive I-BH-CCK-8 was synthesized as described by Miller *et al.* [9].

*Synthesis of peptides.* CLN and related peptides were synthesized by the conventional methods in solution. Throughout the synthesis, the *N*-hydroxy-succinimide esters [10] and the azides [11] were used for the step-by-step coupling and for the fragment condensation respectively.

Pyr-Gln-Asp-Tyr-$NHNH_2$, derived from Z-Pyr-Gln-Asp(OBzl)-Tyr(Bzl)-NHNH-Z by catalytic hydrogenolysis ($H_2$/Pd), was coupled with H-Thr(Ac)-Gly-NHNH-Z to lead to the production of Pyr-Gln-Asp-Tyr-Thr(Ac)-Gly-$NHNH_2$ (I). This hexapeptide sequence was also synthesized by the step-by-step procedure starting from Boc-Thr-(Bzl)-Gly-NHNH-Z to give Z-Pyr-Gln-Asp(OBzl)-Tyr(Bzl)-Thr(Bzl)-Gly-NHNH-Z, from which Pyr-Gln-Asp-Tyr-Thr-Gly-$NHNH_2$ (II) was obtained by $H_2$/Pd. For production of the $Thr^5$-substituted analogues, Pyr-Gln-Asp-Tyr-X-Gly-$NHNH_2$ [X = Ala (IIIa), D-Ala (IIIb), Leu (IIIc), or D-Leu (IIId)] was synthesized stepwise with Z-X-Gly-NHNH-Boc as starting material. The resulting Z-Pyr-Gln-Asp-($OBu^t$)-Tyr-X-Gly-NHNH-Boc was converted into III by $H_2$/Pd followed by the TFA treatment. H-Trp-Met-Asp-Phe-R [R = $NH_2$ (IV) or OH (V)] was synthesized with Z-Asp($OBu^t$)-Phe-R′ (R′ = $NH_2$ or $OBu^t$), Boc-Met-Asp($OBu^t$)-Phe-R′ and Boc-Trp-Met-Asp-Phe-R as intermediates. H-Trp-Nle-Asp($OBu^t$)-Phe-$NH_2$ (VI) was synthesized stepwise starting from H-Phe-$NH_2$ and using the Z group for α-amino protection.

The hexapeptide azide derived from compound I was coupled with IV and the resulting decapeptide was sulfated with $SO_3$–pyridine complex in an anhydrous medium in the usual manner [12] to give Pyr-Gln-Asp-Tyr($SO_3H$)-Thr(Ac)-Gly-Trp-Met-Asp-Phe-$NH_2$ (VII). Compound VII was then treated for removal of the acetyl group with 1 M diethylamine at 25° for 30 min to produce CLN. The decapeptide derived from II and V, which has no protection on the Thr residue, was sulfated in an aqueous medium [13] (carbonate-hydrogencarbonate buffer, pH 10–11) by portionwise addition of $SO_3$–pyridine complex to give [$Phe\text{-}OH^{10}$]-CLN. The progress of the reaction was monitored by high-performance liquid chromatography (HPLC). Most of the CLN analogues and related peptides used in the present work (see Table 2) were synthesized in almost the same manner as described above with the exception of CLN-(4-10). CLN-(5-10), H-Thr-Gly-Trp-Met-Asp-Phe-$NH_2$, was allowed to react with Fmoc-Tyr($SO_3\frac{1}{2}Ba$)-OSu, which was derived from H-Tyr($SO_3\frac{1}{2}Ba$)-OH [14] by acylation with Fmoc-Cl [15] and subsequent esterification with HOSu, and the product was treated with 1 M diethylamine in DMF at 25° for 2 hr to yield CLN-(4-10).

The synthetic CLN and related peptides were chromatographed, if necessary for purification, on a column of either DEAE-Sephadex A-25 (Pharmacia) or DEAE-cellulose (DE-52, Whatman) with an ammonium bicarbonate buffer as eluant. The purified peptides showed the correct amino acid ratios (see Table 1) and were sufficiently pure according to reversed-phase HPLC.

*Binding studies.* CCK-8 was radiolabeled with [$^{125}$I]BH reagent as described by Miller *et al.* [9]. The labeled CCK-8 is referred to as [$^{125}$I]BH-CCK-8. Binding experiments were performed according to the method of Steigerwalt and Williams [4]. Briefly, rats and guinea pigs were killed by decapitation and the cerebral cortex or the pancreas removed from each animal was homogenized in 10 vol. of 50 mM Tris–HCl (pH 7.4) at 4° with a Teflon homogenizer. The homogenates were centrifuged twice at 48,000 *g* for 15 min with an intermediate rehomogenization in fresh buffer. The final pellets were resuspended in 5 vol. of an incubation medium, which contained 10 mM Hepes buffer, 118 mM NaCl, 4.7 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 5 mg/ml BSA, 0.2 mg/ml SBTI, and 1.0 mg/ml bacitracin and had been adjusted to pH 7.4 The suspensions were used for the binding experiments within 3 hr of their preparation or were immediately frozen and stored at −70°. In most assays, these membrane preparations (cortex, 400 μg protein; pancreas, 200 μg protein) were incubated with 23 pM [$^{125}$I]BH-CCK-8 at 27–28° in plastic microcentrifuge tubes containing a total volume of 300 μl of the incubation mixture. To determine the nonspecific binding, unlabeled CCK-8 was added at 1 μM. After incubation for 2 hr, the tubes were centrifuged at 10,000 *g* for 1 min in a Beckman Microfuge B. The supernatant fractions were

Table 1. Amino acid composition and optical rotation of synthetic caerulein and related peptides*

| Peptide | Asp | Thr | Glu | Gly | Ala | Met | Leu | Nle | Tyr | Phe | $[\alpha]_D$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Caerulein (CLN) | 2.00 | 0.92 | 1.91 | 1.03 | | 1.12 | | | 1.00 | 0.90 | |
| [$Glu^2$]-CLN | 1.96 | 0.90 | 1.94 | 0.98 | | 0.99 | | | 1.00 | 1.00 | −26.0 |
| [$Ala^5$, $Nle^8$]-CLN | 1.97 | | 1.96 | 0.98 | 1.00 | | | 0.96 | 1.00 | 1.00 | −28.7 |
| [D-$Ala^5$, $Nle^8$]-CLN | 1.96 | | 1.93 | 0.98 | 1.00 | | | 0.96 | 1.00 | 1.00 | −22.7 |
| [$Leu^5$, $Nle^8$]-CLN | 2.00 | | 1.92 | 1.01 | | | 0.98 | 1.03 | 1.00 | 1.00 | −30.6 |
| [D-$Leu^5$, $Nle^8$]-CLN | 2.00 | | 1.94 | 1.02 | | | 0.99 | 0.96 | 1.01 | 1.00 | −17.7 |
| Des-$Gln^2$-CLN | 2.20 | 0.96 | 0.97 | 0.98 | | 0.92 | | | 1.00 | 1.00 | −27.8 |
| [$Phe\text{-}OH^{10}$]-CLN | 1.94 | 0.93 | 1.93 | 0.97 | | 0.99 | | | 1.00 | 1.00 | −19.5 |
| [$Met(O)^8$]-CLN | 2.20 | 1.01 | 2.14 | 0.96 | | 1.00 | | | 0.99 | 1.00 | −21.4 |
| CLN-(1-9) | 2.00 | 0.99 | 1.97 | 1.00 | | 0.99 | | | 1.04 | | −23.6 |
| CLN-(1-8) | 1.00 | 0.98 | 1.91 | 1.02 | | 0.97 | | | 1.02 | | −27.2 |

* For amino acid analysis, the sample was hydrolyzed in 6 M HCl at 110° for 20 hr in the presence of small amounts of phenol and thioglycolic acid. Optical rotation was measured at 0.5% in 50 mM $NH_4HCO_3$ at 23°.

Table 2. Binding of [$^{125}$I]BH-CCK-8 to pancreatic and cerebral cortex membranes in rat and guinea pig*

| | [$^{125}$I]BH-CCK-8 bound (fmoles/mg protein) | | | |
|---|---|---|---|---|
| | Fresh membranes | N | Frozen membranes | N |
| Pancreas | | | | |
| Rat | 5.46 ± 0.37 | 3 | 6.74 ± 0.59 | 3 |
| Guinea pig | 1.41 ± 0.92 | 5 | 1.66 ± 1.48 | 5 |
| Cerebral cortex | | | | |
| Rat | 0.27 ± 0.03 | 4 | 0.15 ± 0.03 | 4 |
| Guinea pig | 1.01 ± 0.15 | 5 | 0.66 ± 0.06 | 5 |

* The preparation of membranes and the binding assays were performed as described in Materials and Methods. Values represent means ± S.E.M.

reserved for the analysis of degradation of [$^{125}$I]BH-CCK-8. The pellets were washed with the ice-cold incubation medium and recentrifuged for 15 sec. The radioactivity of the pellet was measured in a gamma counter. Nonspecific binding was subtracted from total binding to yield the specific binding. All assays were run in duplicate. When the radioactivity of the supernatant fractions was analyzed by HPLC under the conditions of Ref. 9, we found that enzymatic degradation of [$^{125}$I]BH-CCK-8 was negligible.

## RESULTS

As shown in Table 2, the [$^{125}$I]BH-CCK-8 binding capacity of rat cerebral cortex membranes was 1/20 that of rat pancreatic membranes, and thus too low for routine receptor binding assays. The brain CCK receptors of both rat and guinea pig were labile to freezing and thawing, with an approximately 30% decrease in binding capacity, while the pancreatic receptors were not affected. However, we found that the binding of [$^{125}$I]BH-CCK-8 to guinea pig pancreatic receptors varied from one preparation to another. We therefore used the rat as a source of pancreatic membrane receptors. These membranes were four times more active in binding than guinea pig pancreatic membranes and could be stored at −70° for at least 3 weeks without any loss of activity. For the brain receptors, the guinea pig cerebral cortex membranes were used. Since these membranes were relatively unstable at 4°, they were used within 3 hr after their preparation. We examined the validity of these selections of receptor origins and the results are described below.

Scatchard plots of the competitive inhibition of [$^{125}$I]BH-CCK-8 binding by unlabeled I-BH-CCK-8 were used to determine the number of binding sites and the affinities of the pancreatic and cortex receptors. The dissociation constant ($K_d$) and the total binding sites ($B_{max}$) of rat pancreatic receptors were calculated to be 0.56 ± 0.05 nM and 340 ± 70 fmoles/mg protein (N = 3), respectively, while guinea pig cortex receptors showed a little lower affinity ($K_d$ = 1.1 ± 0.2 nM, N = 3) and markedly fewer binding sites ($B_{max}$ = 42 ± 15 fmoles/mg protein) than rat pancreatic receptors.

Table 3 shows the affinities of the newly synthesized CLN-related compounds for the CCK receptors from the rat pancreas and the guinea pig cerebral cortex. The deletion or partial modification of three amino acid residues at the $NH_2$-terminal of the CLN molecule had practically no effect on the binding to both receptors. As has been reported by many investigators [3–5], removal of the sulfate ester or the sulfated tyrosine residue resulted in a great decrease in the affinities for CCK receptors, especially in the pancreas. For example, the affinities of [Tyr$^4$]-CLN for pancreatic and cortex receptors were reduced to 1/300 and 1/35, respectively, of those of CLN. CLN-(5-10) and CLN-(6-10) had affinities reduced to 1/17,000 and 1/69,000, respectively, for pancreatic receptors and reduced to 1/110 and 1/510, respectively, for cortex receptors. On the other hand, deamidation at the COOH-terminal of CLN caused almost total loss of binding activity. It is notable, however, that [Phe-OH$^{10}$]-CLN and CLN-(1-8) completely lost their binding abilities to brain receptors while retaining very low but significant binding activity for pancreatic receptors. Oxidation of Met$^8$ caused significant decreases in the binding ability to both pancreatic and cortex receptors, the $IC_{50}$ values being 60 and 400 times greater than those of CLN respectively. While substitution of Thr$^5$ by Ala or Leu caused little or no decrease in receptor binding, the same substitution by D-Ala or D-Leu reduced the affinity several hundred-fold towards pancreatic receptors and 10-fold towards cortex receptors.

To obtain more insight into the structure–affinity relationship of these peptides, we calculated the ratio of the $IC_{50}$ for pancreas to the $IC_{50}$ for cortex (P/C ratio) and found that the CLN-related peptides can be classified into the following three types: (1) the peptides which have P/C ratios of 0.44–1.0 and show high affinities for both receptors (type A), (2) the peptides which are desulfated ([Tyr$^4$]-CLN) or lack Tyr($SO_3H$)$^4$, and the peptides, in which Thr$^5$ is replaced by a D-amino acid, having P/C ratios larger than 5.5 (type B), and (3) [Met(O)$^8$]-CLN, [Phe(OH)$^{10}$]-CLN, and CLN-(1-8) possessing P/C ratios smaller than 0.22 (type C).

For these reasons, we used rat pancreatic and guinea pig cortex membranes in the present experiment. The validity of this selection was checked using receptor preparations from guinea pig pancreas and compounds selected from types A, B, and C respectively. The affinities to guinea pig pancreatic CCK receptors of CLN, CLN-(4-10), CLN-(5-10), [Tyr$^4$]-CLN, and [Met(O)$^8$]-CLN were 0.22, 0.22,

Table 3. Effects of CCK-8, I-BH-CCK-8 and CLN-related peptides on specific binding of [$^{125}$I]BH-CCK-8 to membranes from rat pancreas and guinea pig cerebral cortex

| Peptide | Structure (1 2 3 4 5 6 7 8 9 10) | Rat pancreas | $IC_{50}$* guinea pig cortex | P/C ratio† (Type) |
|---|---|---|---|---|
| CCK-8 | Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met——Asp-Phe-$NH_2$ | 0.40 | 0.76 | 0.53 (A) |
| I-BH-CCK-8 | I-BH-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met——Asp-Phe-$NH_2$ | 0.79 | 1.1 | 0.72 (A) |
| CLN derivative | | | | |
| CCK-4 | Trp-Met——Asp-Phe-$NH_2$ | 18,000 | 220 | 81 (B) |
| CLN-(5-10) | Thr-Gly-Trp-Met——Asp-Phe-$NH_2$ | 4,500 | 49 | 92 (B) |
| CLN-(4-10) | Tyr($SO_3H$)-Thr-Gly-Trp-Met——Asp-Phe-$NH_2$ | 0.32 | 0.72 | 0.44 (A) |
| des-Gln$^2$-CLN | Pyr‡-Asp-Tyr($SO_3H$)-Thr-Gly-Trp-Met——Asp-Phe-$NH_2$ | 0.22 | 0.38 | 0.56 (A) |
| CLN | Pyr-Gln-Asp-Tyr($SO_3H$)-Thr-Gly-Trp-Met——Asp-Phe-$NH_2$ | 0.26 | 0.43 | 0.60 (A) |
| [Tyr$^4$]-CLN | Pyr-Gln-Asp-Tyr———Thr-Gly-Trp-Met——Asp-Phe-$NH_2$ | 82 | 15 | 5.5 (B) |
| [Glu$^2$]-CLN | Pyr-Glu-Asp-Tyr($SO_3H$)-Thr-Gly-Trp-Met——Asp-Phe-$NH_2$ | 0.45 | 0.97 | 0.46 (A) |
| [Leu$^5$, Nle$^8$]-CLN | Pry-Gln-Asp-Tyr($SO_3H$)-Leu-Gly-Trp-Nle——Asp-Phe-$NH_2$ | 0.45 | 0.43 | 1.0 (A) |
| [D-Leu$^5$, Nle$^8$]-CLN | Pyr-Gln-Asp-Tyr($SO_3H$)-leu-Gly-Trp-Nle——Asp-Phe-$NH_2$ | 130 | 8.3 | 16 (B) |
| [Ala$^5$, Nle$^8$]-CLN | Pyr-Gln-Asp-Tyr($SO_3H$)-Ala-Gly-Trp-Nle——Asp-Phe-$NH_2$ | 0.85 | 1.2 | 0.71 (A) |
| [D-Ala$^5$, Nle$^8$]-CLN | Pyr-Gln-Asp-Tyr($SO_3H$)-ala-Gly-Trp-Nle——Asp-Phe-$NH_2$ | 74 | 5.9 | 13 (B) |
| [Met(O)$^8$]-CLN | Pyr-Gln-Asp-Tyr($SO_3H$)-Thr-Gly-Trp-Met(O)-Asp-Phe-$NH_2$ | 15 | 200 | 0.075 (C) |
| [Phe(OH)$^{10}$]-CLN | Pyr-Gln-Asp-Tyr($SO_3H$)-Thr-Gly-Trp-Met——Asp-Phe-OH | 1,600 | 7,200 | 0.22 (C) |
| CLN-(1-9) | Pyr-Gln-Asp-Tyr($SO_3H$)-Thr-Gly-Trp-Met——Asp-OH | >10,000 | >10,000 | |
| CLN-(1-8) | Pyr-Gln-Asp-Tyr($SO_3H$)-Thr-Gly-Trp-Met-OH | 1,600 | >10,000 | <0.16 (C) |

* $IC_{50}$: the concentration causing 50% inhibition of specific [$^{125}$I]BH-CCK-8 binding

† The peptides were classified into type A (0.44–1.0), type B (larger than 5.5) and type C (smaller than 0.22) according to the P/C ($IC_{50}$ for pancreatic receptors/$IC_{50}$ for cortex receptors) ratio.

‡ Pyr, leu, and ala denote pyroglutamic acid, D-leucine, and D-alanine respectively.

> 10,000, 63, and 18 nM, respectively, as expressed in $IC_{50}$, and these values were similar to those obtained with rat pancreatic membranes but not with guinea pig brain membranes. These results indicate that the above-mentioned difference between rat pancreas and guinea pig brain does not depend on species but organs.

## DISCUSSION

The present study revealed that the [$^{125}$I]BH-CCK-8 binding activity of the rat pancreas is higher than that of the guinea pig pancreas, but the guinea pig cerebral cortex was more potent than the rat cortex. Our results do not agree with those of Innis and Snyder [3] who reported that the binding levels of [$^{125}$I]BH-CCK-33 (CCK-33 radiolabeled with the BH reagent) to the rat cerebral cortex roughly approximated those to the guinea pig cortex. Although the reason for this discrepancy is not clear at the moment, one possible cause might be that we used [$^{125}$I]BH-CCK-8 while Innis and Snyder used [$^{125}$I] BH-CCK-33, which is known to be susceptible to enzymatic degradation.

The specificity of CCK receptors in the brain is suggested to be lower than that of pancreatic receptors, because the former receptors have substantial affinity for CCK-related peptides, such as desulfated CCK-8, CCK-4, or gastrin, while the latter receptors bind these peptides only weakly [3–5]. However, the peptides examined have been limited to those having the modified $NH_2$-terminal region or those retaining the COOH-terminal region unmodified. In this study we synthesized various types of peptides which include those modified at the COOH- and/or $NH_2$-terminal of the CLN molecule and compared their affinities for rat pancreatic and guinea pig cerebral cortex CCK receptors.

Brain receptors exhibited specificity comparable to, but different from, pancreatic receptors. Comparison of CLN and related peptides in terms of the P/C ratio led us to the elucidation of their structure–affinity relationship (Table 3).

The peptides examined in the present study were divided into three types. The peptides having binding activity similar to that of CLN showed almost the same affinity for both cortex and pancreatic receptors (type A, P/C ratio: 0.44–1.0). The compounds belonging to this type include those modified somewhere in the amino acid sequence of the CLN molecule, but retaining the Tyr($SO_3H$)$^4$ and the COOH-terminal amide group unmodified. The peptides with affinity lower than that of CLN were classified into two types, B and C. The type B peptides (P/C ratio: larger than 5.5) are those exhibiting a decrease in affinity which is more marked for pancreatic receptors than for brain receptors. The type C peptides (P/C ratio: smaller than 0.22) show affinities more markedly reduced for brain receptors than for pancreatic receptors. The type B peptides were those in which the Tyr($SO_3H$)$^4$ residue had been desulfated or deleted, while the type C peptides were those in which the COOH-terminal region had been deamidated or the chain had been shortened. The CLN analog having an oxidized Met residue also belongs to type C. One exception to this classification is that the peptides in which Thr$^5$ had been replaced by D-Ala or D-Leu, gave P/C ratios larger than 5.5. However, the substitution of Thr$^5$ with D-Ala or D-Leu may have caused some conformational change around the Tyr($SO_3H$)$^4$ residue adjacent to Thr$^5$. The present results suggest that, although the Tyr($SO_3H$)$^4$ residue and the COOH-terminal structure of CLN are both essential for the peptide to bind to CCK receptors, the sulfated tyrosine residue is more important for receptor binding in the pancreas than in brain and the inverse relationship exists for the N-terminal structure.

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