A Pseudopeptide That Is a Potent Cholecystokinin Agonist in the Peripheral System Is Able to Inhibit the Dopamine-like Effects of Cholecystokinin in the Striatum*

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There are no known specific effective cholecystokinin (CCK) receptor antagonists of both peripheral and central nervous systems. Here, we describe experiments which demonstrate that a synthetic pseudopeptide analogue of CCK-7 is a potent agonist in the peripheral system and behaves as a selective and highly potent inhibitor of the dopamine-like effects of CCK in the striatum. This compound, t-butyloxycarbonyl-Tyr[(SO,H),Nle][COCH,Gly]-Trp-Nle-Asp-Phe-NHz, is able to stimulate enzyme secretion from rat pancreatic acini, with high efficacy and potency. It is also very potent in inhibiting the binding of labeled CCK-8 to rat pancreatic acini (IC50 = 5 nM) and to guinea pig and mouse brain membranes (IC50 = 0.7 nM). However, this compound is able to antagonize the effects of intrastriatally injected t-butyloxycarbonyl-[Nle4,6,8,11]CCK-8 in mice, with high potency.

Cholecystokinin (CCK), a gastrointestinal hormone of 33 amino acid residues, was originally isolated from the gastrointestinal tract (1). It is a hormonal regulator of pancreatic and gastric secretions, contraction of the gall bladder, and intestine motility. The complete sequence of peripheral biological activities has been found in the carboxyl-terminal octapeptide (CCK-8) of the entire molecule (2). CCK-like peptides have since been detected in the brain, the sulfated octapeptide (CCK-8) being the most abundant form (3). They are believed to act as neurotransmitters or neuromodulators (4). Cholecystokinin appears to be involved in a wide variety of physiological functions, including satiety (5), respiration and thermoregulation (6), sedation (7), and analgesia (8). In addition, cumulative evidence indicates close functional inter-relationships between dopaminergic systems and cholecystokinin in certain brain regions, suggesting that CCK-8 may have a role in the pathogenesis of schizophrenia (9, 10) and in the therapeutic actions of antipsychotic drugs such as neuroleptics (11). The dopaminergic brain areas appear to be particularly rich in CCK-8 (12) and CCK-receptors (13). CCK-8 has been shown to closely interact with dopamine-mediated neurotransmission. CCK-8 appears to antagonize dopamine-induced effects in the mesolimbic system (14, 15), to modulate the sensitivity and the number of striatal and limbic dopamine receptors (16, 17), and to decrease dopamine turnover in the striatum (18). It became evident that, particularly to clarify further the role of CCK and dopamine in the central nervous system in general, it was necessary to have selective and potent central CCK-antagonists. Several CCK-receptor antagonists have been described recently, including amino acid (19), peptide (20), and nucleotide derivatives (21), as well as nonpeptidic compounds (22). All of them exhibited a significant degree of selectivity for peripheral CCK-receptors.

McDermott et al. (23) showed that a degradative activity present in synaptosomes and purified lysed synaptosomal membranes cleaves CCK-8 at the Met28-Gly29 bond, in vitro. Delineation of the degradative pathway was further reported (24), clearly indicating that brain synaptosomal membranes contain peptidases which cleave CCK-8, the initial endopeptidase cleavage being between Met28 and Gly29. Furthermore, our previous results concerning the importance of the peptide bonds in CCK-related peptides showed that only the bond between Met28 and Gly29 could be replaced without affecting either the binding to pancreatic or central CCK-receptors, or the biological activity (32). In order to obtain enzyme-resistant and potent analogues of CCK, we have synthesized the pseudopeptide Boc-Tyr(SO2H)-Nle-Ψ(COCH2)Gly-Trp-Nle-Asp-Phe-NHz (compound 1, Scheme 1) in which the peptide bond between Nle and Gly has been replaced by a ketomethylene bond (COCH2). We report here experiments which demonstrate that compound 1, an analogue of the carboxyl-terminal heptapeptide of CCK, is a potent CCK agonist in the peripheral system able to antagonize the action of CCK in the mouse striatum.

EXPERIMENTAL PROCEDURES

Materials

Male guinea pigs (280–300 g) were obtained from the Centre d’Elevage d’Animaux de Laboratoire (Ardenay, France); male Wistar rats (180–200 g) were from Effa-Credo (Saint Germain l’Arbresle, France); female Swiss mice (CD1 strain, Charles River, France) weighing 25–30 g were used. Heps was from Boehringer Mannheim; purified collagenase was from Serva (Varden City Park, NY); soybean trypsin inhibitor from Sigma (St. Louis, MO); Eagle’s basal amino acid medium (100 times concentrated) was from GIBCO (Grand Island, NY); essential vitamin mixture (100 times concentrated) was from Microbiological Associates (Bethesda, MD); bovine plasma albumin (fraction V) was from Miles Laboratories Inc. (Elkhart, IN); Phadebas™ analyte test was from Pharmacia LKB Biotechnology Inc. (Picataway, NJ); and 125I-labeled N-succinimidyl-3(4-hydroxyphenyl)propionoyl-CCK-8 was from Amersham (Buckinghamshire, UK). CCK-derivatives were synthesized in our laboratory. Detailed description of the synthesis of compound 1 will be published in a

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The abbreviations used are: CCK, cholecystokinin; BOC, t-butyloxycarbonyl; Heps, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid; BH-CCK-8, Bolton-Hunter-labeled CCK-8.
Cholecystokinin Antagonist

Scheme 1. Chemical structure of CCK-8 (a), Boc-[Nle<sub>28,31</sub>]CCK-8 (b), and Boc-Tyr(SO<sub>3</sub>)-Nle9(COCH<sub>3</sub>) Gly-Trp-Asp-Phe-NH<sub>2</sub> (c).

Results

Compound 1 was as potent and efficacious as Boc-[Nle<sub>28,31</sub>]CCK-8, in increasing amylase release from dispersed rat pancreatic acini (Fig. 1), maximal stimulation being obtained at a concentration of 0.3 nM. Compound 1 inhibited the binding of <sup>125</sup>I-BH-CCK-8 to rat isolated pancreatic acini with almost the same potency as Boc-[Nle<sub>28,31</sub>]CCK-8 (Fig. 2, I<sub>50</sub> = 5 nM). To directly investigate the ability of compound 1 to interact with central CCK-receptors, we measured its ability to inhibit the binding of <sup>125</sup>I-BH-CCK-8 to guinea pig and mouse brain membranes. Compound 1 was found almost as potent as Boc-[Nle<sub>28,31</sub>]CCK-8 in inhibiting the binding of labeled CCK-8 to guinea pig and mouse brain membranes (Fig. 3, A and B, I<sub>50</sub> = 0.7 nM). Results are summarized in Table I.

Given our previous observation that the natural CCK-8 exhibited dopaminomimetic properties following intrastriatal injection (25), we have investigated the behavioral effect of Boc-[Nle<sub>28,31</sub>]CCK-8 and compound 1 after their direct unilateral injection within the mouse striatum, which is a simple model designated for the screening of drugs, which gave the same effects as dopamine (26). As shown in Fig. 4, Boc-[Nle<sub>28,31</sub>]CCK-8 injected directly within the mouse striatum induced a strong contralateral turning behavior (terming...
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**Fig. 1.** The effect of Boc-[Nle\textsuperscript{28,31}]CCK-8 (■) and compound 1 (□) 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 expressed as percent of maximal stimulation obtained with Boc-[Nle\textsuperscript{28,31}]CCK-8 (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 five separate experiments.

**Fig. 2.** Effect of Boc-[Nle\textsuperscript{28,31}]CCK-8 and compound 1 on inhibiting binding of \textsuperscript{125}I-BH-CCK-8 to rat pancreatic acini. Acini were incubated for 30 min at 37 °C with 10 pm \textsuperscript{125}I-BH-CCK-8 plus various concentrations of Boc-[Nle\textsuperscript{28,31}]CCK-8 (■) or compound 1 (□). Values are expressed as the 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 five separate experiments. Specific binding in the absence of any unlabeled CCK-peptide was 13 ± 3% of the total radioactivity present in the sample.

**Fig. 3.** Effect of Boc-[Nle\textsuperscript{28,31}]CCK-8 and compound 1 on inhibiting binding of \textsuperscript{125}I-BH-CCK-8 to guinea pig (A) or mouse (B) brain membranes. Brain membranes were incubated for 60 min at 25 °C with 20 pm \textsuperscript{125}I-BH-CCK-8 in the presence of various concentrations of Boc-[Nle\textsuperscript{28,31}]CCK-8 (■) or compound 1 (□). Nonspecific binding was determined in the presence of 1 pm Boc-[Nle\textsuperscript{28,31}]CCK-8. Total binding was 10 ± 2% of the total radioactivity present in the sample, and nonspecific binding was always less than 2% of total binding. In each experiment, each value was determined in duplicate, and the results given are the means from five separate experiments.

**TABLE I**

| Boc-[Nle\textsuperscript{28,31}]CCK-8 | Compound 1 |
|-----------------------------------|------------|
| Amylase release (maximum activity) |            |
| Rat                               | 0.5        | 0.3 |
| Mouse                             | 0.4        | 0.4 |
| Binding (pancreatic acini) IC\textsubscript{50} |          |
| Rat                               | 1          | 5   |
| Mouse                             | 0.8        | 3   |
| Binding (brain membranes) IC\textsubscript{50} |      |
| Mouse                             | 0.5        | 0.7 |
| Guinea pig                        | 0.5        | 0.7 |

as −), this effect being maximal for 10 nm, and decreased thereafter [ANOVA, F (7,219) = 35.2; p < 0.001]. In fact, Boc-[Nle\textsuperscript{28,31}]CCK-8 was more potent than the natural CCK-8 peptide (H-Asp-Tyr(SO\textsubscript{3}H)-Met-Gly-Trp-Met-Asp-Phe-NH\textsubscript{2}), the maximal activity of which was found approximately at 500 nm (25). Buffer-injected mice exhibited ipsilateral turning (termed +). Compound 1 (1 nm to 1 \textmu M) did not induce a significant level of rotations in this model [ANOVA, F (4,115) = 1.38; p < 0.05] (Fig. 5). However, the contralateral turning induced by Boc-[Nle\textsuperscript{28,31}]CCK-8 (10 nm, dose corresponding to the maximal agonist activity) was dose dependently antagonized by co-injected compound 1 (1 nm to 1 \textmu M).
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FIG. 4. Contralateral turning induced by direct unilateral intrastratal injection of buffer (■) and of indicated concentrations of Boc-[Nle<sup>28,31</sup>]CCK-8 (▲). Intrastratal injections were made according to the method described by Worms et al. (25, 26). Boc-[Nle<sup>28,31</sup>]CCK-8 was dissolved in a pH 6 phosphate buffer. The injections were made free-hand in a volume of 1 µl, directly into the right striatum of conscious, nonrestrained mice. Control mice always received an injection of buffer (1 µl). Columns represent mean values obtained with 20 mice per dose; bars are S.E. * p < 0.05; ** p < 0.01; versus buffer controls (Student’s test).

FIG. 5. Turning induced by direct unilateral intrastratal injection of buffer (■) and of indicated concentrations of compound 1. Intrastratal injections were made according to the method described in Fig. 4 and under “Experimental Procedures.” Number of turns were counted according to the experiments described in Fig. 1. Columns represent mean values obtained with 20 mice per dose; bars are S.E.

[ANOVA, F(4,181) = 32.5; p < 0.001] (Fig. 6), a significant inhibition being already observed at a concentration as little as 0.01 µM. Compound 1 at different concentrations (10 nM to 1 µM) was able to produce a parallel rightward shift in the dose-response curve of the action of intrastrataly injected Boc-[Nle<sup>28,31</sup>]CCK-8 with no change in the maximal response (Fig. 7). With increasing concentrations of compound 1, the magnitude of the rightward shift was proportional to the concentration of the pseudopeptide derivative (Fig. 7).

DISCUSSION

In the present study, we investigated a synthetic pseudopeptide analogue of the carboxyl-terminal heptapeptide of CCK in which Met<sup>28</sup> and Met<sup>31</sup> were replaced by Nle (Scheme 1), a substitution that had proved in many cases, particularly in CCK-derived peptides, to affect neither the biological activities nor the binding to CCK-receptors (27). The amino terminus of the pseudopeptide was acylated by a Boc group, because it had been demonstrated that CCK-analogues with an N<sup>α</sup>-Boc protecting group were more potent than those bearing a free ω-amino group (28). In agreement with previous findings, with increasing concentrations of Boc-[Nle<sup>28,31</sup>]CCK-8 (34), amylase secretion increased, became maximal at 0.3 nM, and then decreased at supramaximal concentrations. The present results indicate that Boc-[Nle<sup>28,31</sup>]CCK-8 is a full and potent agonist of CCK in the peripheral system (amylose secretion from isolated rat pancreatic acini) with the same efficacy and potency as the natural agonist CCK-8. In the central nervous system, Boc-[Nle<sup>28,31</sup>]CCK-8, injected at very low doses directly into the mouse striatum, induced contra-
lateral turning (maximum of activity at approximately 0.01 ng/μl). This compound was approximately 50 times more potent than the natural agonist CCK–8 (maximum of activity at 0.5 ng/μl) when injected directly within mice striatum (25), indicating that acylation of the amino terminus by a Boc group and the replacement of both methionines by norleucines is of a beneficial effect. A similar pattern was obtained with compound Boc-Tyr(SO₃H)-NleΨ(COCH₃)Gly-Trp-Nle-Asp-Phe-NH₂ (1) which exhibited the same efficacy and potency as Boc-[Nle⁸,₁₃]CCK–8 in stimulating amylose release from isolated rat pancreatic acini. This compound has the same potency as Boc-[Nle⁸,₁₃]CCK–8 in inhibiting the binding of labeled CCK–8 to rat pancreatic acini and interestingly to mouse or guinea pig brain membranes. In contrast, compound 1 injected directly into the mouse striatum did not induce any clear rotatory behavior, but was able to dose dependently antagonize the effects of co-injected Boc-[Nle⁸,₁₃]CCK–8. Compound 1 produced a parallel rightward shift in the dose-response curve for the action of Boc-[Nle⁸,₁₃]CCK–8 on turning, indicating that this antagonism is competitive in nature (Fig. 7). Compound 1 is approximately 10,000 times more potent than the well known CCK-antagonist, proglumide, and about 10 to 100 times more potent than Z-CCK-27-32-NH₂ in inhibiting the dopamine-like effects induced by CCK in the striatum (25). However, neither proglumide nor Z-CCK-27-32-NH₂ were full agonists in the peripheral system. The present results indicate that compound 1 is able to competitively antagonize CCK in the mouse striatum. That is, compound 1 is devoid of agonist activity, inhibits the interaction of CCK with its cell membrane receptors in the mouse brain, and causes a parallel rightward shift in the dose-response curve for the induced turning caused by CCK. The present findings also indicate that the peptide bond between Nle and Gly is not an essential requirement for the binding to peripheral and central CCK-receptors, but is essential for intrinsic CCK-like biological activity in the central nervous system, at least in the striatum. Although the peptide bond between Nle and Gly is not essential for binding to CCK-receptors (32), it is interesting to notice that its replacement by a ketomethylene bond (COCH₃) does not influence at all the apparent affinity with which the pseudopeptide binds to its receptors, either in the peripheral system or in the central nervous system. The present data also indicate that it is possible to obtain very potent peripheral CCK-agonists by acylating the amino terminus in the carboxyl-terminal heptapeptide of CCK, by replacing the methionines by norleucines, and by modifying the peptide bond between norleucine and glycine by a ketomethylene bond in the heptapeptide; this modification led us to obtain a very potent selective central CCK-antagonist. To our knowledge, compound 1 is the only described potent selective central CCK-antagonist. It provides an interesting insight in the knowledge of structure-activity relationships of CCK-related peptides and a very useful tool for the understanding of CCK actions and the study of its pharmacology particularly in the central nervous system.

Considerable controversy exists in the literature as to whether CCK-peptides facilitate or decrease dopamine transmission in this brain area (9, 10, 15, 25). The results obtained in the present study are in accordance with a mediation of CCK-induced turning behavior by CCK-receptors which are present within the striatum and suggest that, under acute conditions, CCK exerts a facilitatory influence on dopamine transmission within the striatum.

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