0026-895X/91/050615-06$03.00/0

MOLECULAR PHARMACOLOGY, 39:615-620

ACCELERATED COMMUNICATION

Pharmacological Characterization of Type B Cholecystokinin Binding Sites on the Human JURKAT T Lymphocyte Cell Line

MARIE-FRANCOISE LIGNON, NICOLE BERNAD, and JEAN MARTINEZ

Centre CNRS-INSERM de Pharmacologie-Endocrinologie, Faculté de Pharmacie, 34060 Montpellier, Cédex 5, France

Received December 27, 1990; Accepted February 18, 1991

SUMMARY

Recent studies have demonstrated the presence and the regulatory function of some neuropeptides in the immune system. In the present study, we have used labeled cholecystokinin (26–33) amide to characterize high affinity cholecystokinin (CCK) binding sites on a human JURKAT lymphoma cell line. Binding was temperature dependent, saturable, and specific. Analysis of the data demonstrated a single class of binding sites with high affinity for the ligand ($K_d \approx 3.2 \pm 0.5 \times 10^{-11}$ M) and a binding capacity of 0.42 fmol/10^6 cells (≈300 sites/cell). These CCK binding sites displayed a typical CCK-B pharmacological profile, established by use of several agonists and antagonists selective for the CCK receptor types, namely compound L-364,718, the Merck CCK antagonist selective for the peripheral CCK receptor (CCK-A), and compound L-365,260, the Merck CCK antagonist selective for the central CCK receptor (CCK-B). The CCK cyclic analogue recently developed in our laboratory that is highly selective for the CCK-B receptor (i.e., JMV320) also showed high affinity for the CCK receptor on the JURKAT cell line. The presence of CCK-B-like binding sites on a lymphoid cell line could provide a useful model for pharmacological characterization of CCK-B binding sites and could contribute to a better understanding of their regulation.

Recent advances in neuroimmunoendocrinology have led to the conclusion that the central nervous system is involved in immune responses and that the immune system can regulate neuroendocrine functions (1). Cells of the immune system have been shown to harbor specific binding sites for several neuroendocrine hormones, including ACTH, VIP, growth hormone, substance P, insulin, enkephalin, and β-endorphin (2, 3).

CCK is a peptide found in the gastrointestinal tract and is also a neuropeptide that is highly concentrated in specific areas of the brain. It was reported several years ago that human lymphocytes produce CCK-like immunoreactivity, or prepro-CCK, CCK-39, CCK-33, and CCK-8, in fentomole amounts (4, 5).

The regulatory function of CCK in the immune system has been suggested very recently by several studies. Ferrara *et al.* (6) have demonstrated that CCK-8 elicits an increase in intracellular calcium in peripheral blood mononuclear cells. These authors also demonstrated that CCK-8 acts as a co-mitogen for anti-CD3 monoclonal antibody-mediated mitogenesis (7). Moreover, the chemoattractant property of CCK-8 has been reported by Ruff *et al.* (8). Therefore, experiments have been carried out in order to characterize CCK binding sites on T lymphocytes. Because continuous cell lines that express binding sites are extremely useful tools, we have performed such experiments on a human T lymphocyte leukemic line, JURKAT, and on a murine thymoma cell line, NOB. The present study demonstrates the presence of high affinity CCK binding sites on the human JURKAT lymphoblastic cell line. ^{125}I-BH-CCK-8 bound to an apparently homogeneous population of binding sites, displaying a typical pharmacological CCK-B-like profile. To our knowledge, this is the first report characterizing CCK binding sites on cells of the immune system.

Experimental Procedures

Materials. ^{125}I-BH-CCK-8 (2000 Ci/mmol) was purchased from Amersham (Les Ulis, France). Compounds L-364,718 and L-365,260 were gifts from Dr Anderson, Merck Sharp & Dohme (West Point, PA). CCK derivatives were synthesized in our laboratory. Other drugs were from commercial sources.

Cell culture. JURKAT cells (T cell leukemia), $T3^+$ or $T3^-$, and NOB cells (murine thymoma) were obtained from Dr. Jacques Dornand (Université des Sciences et Techniques du Languedoc, Montpellier, France). Cell lines were grown in suspension culture, using RPMI 1640 medium supplemented with 10% fetal calf serum and antibiotics (50 units/ml penicillin and 50 μg/ml streptomycin), in a humidified incubator at 37° under an atmosphere of 5% CO_2 in air. However, JURKAT cells have been passaged continuously for 8 months with little change in CCK radioligand binding.

ABBREVIATIONS: CCK, cholecystokinin; ^{125}I-BH-CCK-8, Bolton Hunter cholecystokinin(26–33)amide; Boc, *tert*-butyloxycarbonyl; HEPES, 4 (2-hydroxyethyl)-1-piperazineethanesulfonic acid; ACTH, corticotropin; VIP, vasoactive intestinal peptide.

^{125}I-BH-CCK-8 binding assays. Cells were harvested by centrifugation at 1500 × *g* for 5 min and washed twice with binding incubation medium consisting of 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, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, 0.5 mg/ml bacitracin, 0.01% (w/v) soybean trypsin inhibitor, 1% (v/v) essential amino acid mixture, and 1% (v/v) vitamin mixture. Binding studies were carried out at 37° for 60 min, using 10 pM ^{125}I-BH-CCK-8 plus appropriate drug concentrations, in a final volume of 500 μl containing 4 × 10^6 cells/ml. The reaction was stopped by addition of fresh standard medium plus 2% bovine serum albumin and centrifugation for 10 min at 10,000 × *g*. Nonspecific binding was measured in the presence of 1 μM CCK-8 and was always less than 10% of total binding. Saturation experiments were performed with ligand concentrations varying from 1 to 200 pM. Dissociation kinetics were studied by incubating cells with ^{125}I-BH-CCK-8 until equilibrium; then, a saturable concentration of unlabeled peptide was added and the residual binding was measured at various times.

Data analysis. Data were analyzed using a nonlinear, least squares, curve-fitting computer program (LIGAND) (9). Data from saturation experiments were analyzed according to the Scatchard representation; regression lines were used for computation, by the least squares method, of the affinity constant, K_d, and the number of sites, B_{max}. Data from competition studies were analyzed by linear regression of Hill plots of the inhibition curve, to obtain IC_{50}.

Results

The ability of ^{125}I-BH-CCK-8 to bind to isolated human T lymphocytes, to the human leukemia JURKAT cell line, lacking or not the CD3 antigen ($T3^-$ or $T3^+$), and to the murine thymoma cell line NOB was investigated. Specific binding of ^{125}I-BH-CCK-8 was detected in the two JURKAT cell lines, $T3^-$ and $T3^+$, with both binding radiolabeled CCK-8 with the same high affinity. In contrast, the murine cell line did not bind labeled CCK-8 under the same experimental conditions. Further experiments to characterize the CCK binding sites on JURKAT cells were performed using the $T3^-$ cell subclass. Fig. 1 shows that ^{125}I-BH-CCK-8 binding to JURKAT cells was time- and temperature dependent, with the maximum specific

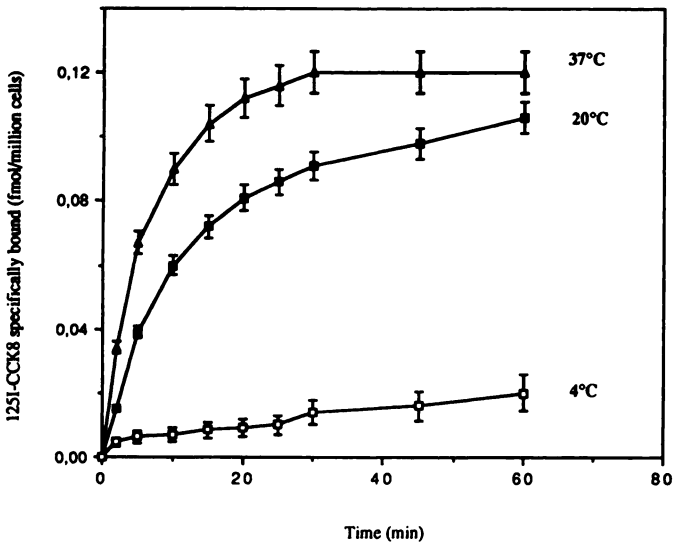


Fig. 1. Time and temperature dependence of ^{125}I-BH-CCK-8 binding to human JURKAT cells. Cells (4 × 10^6/ml) were incubated with 10 pM ^{125}I-BH-CCK-8 for various times at 4° (□), 20° (■), and 37° (▲), in the presence or absence of 1 μM CCK-8. Data points are means of duplicate samples and are representative of three separate experiments.

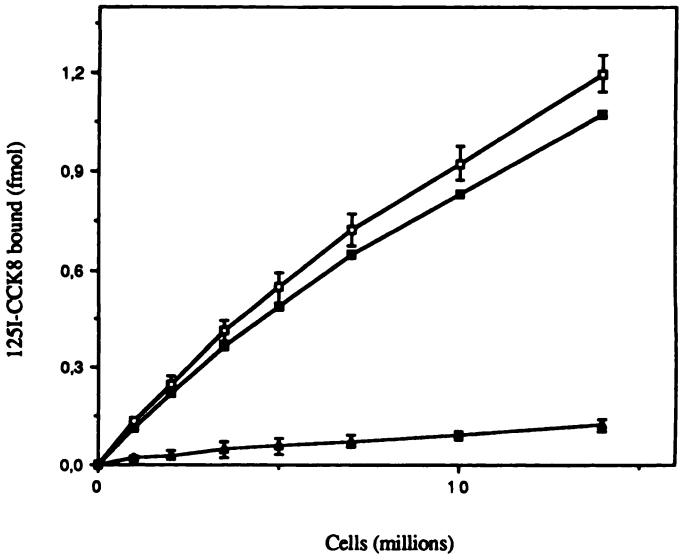


Fig. 2. ^{125}I-BH-CCK-8 binding as a function of cell number. Increasing amounts of human JURKAT cells were incubated with ^{125}I-BH-CCK-8 (10 pM). Total (□) and nonspecific (▲) binding were determined. The difference between the two represents specific binding (■). The means ± standard errors of three determinations are indicated.

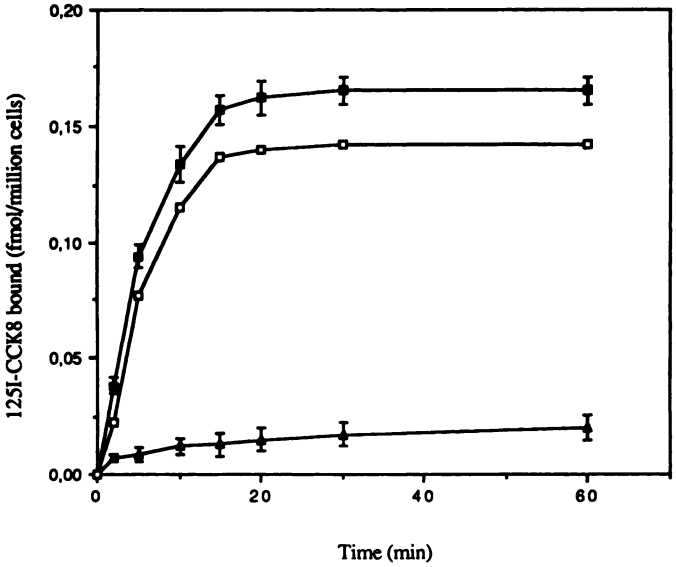


Fig. 3. Association of ^{125}I-BH-CCK-8. Human JURKAT cells (4 × 10^6 cells/ml) were incubated at 37° with 10 pM ^{125}I-BH-CCK-8, in the absence (■) and in the presence (▲) of 1 μM CCK-8. The difference between the two represents specific binding (□). The mean values ± standard errors of six determinations are indicated.

binding appearing at 37°. At this temperature, the steady state was achieved in about 30 min and lasted until 1 hr. Fig. 2 shows that binding was a linear function of cell number. The kinetics of association were investigated. Fig. 3 shows that ^{125}I-BH-CCK-8 equilibrated slowly with the JURKAT cell line. Specific binding of labeled CCK was half-maximal after about 7 min and maximal after a 30-min incubation. The dissociation of ^{125}I-BH-CCK-8 from JURKAT cells was also studied (Fig. 4). Specific binding decreased 50% by 10 min after addition of 1 μM unlabeled CCK-8. Even after 2 hr, however, 10% of the specific binding remained.

Saturation experiments using ^{125}I-BH-CCK-8 showed that specific binding was saturable, whereas nonspecific binding was

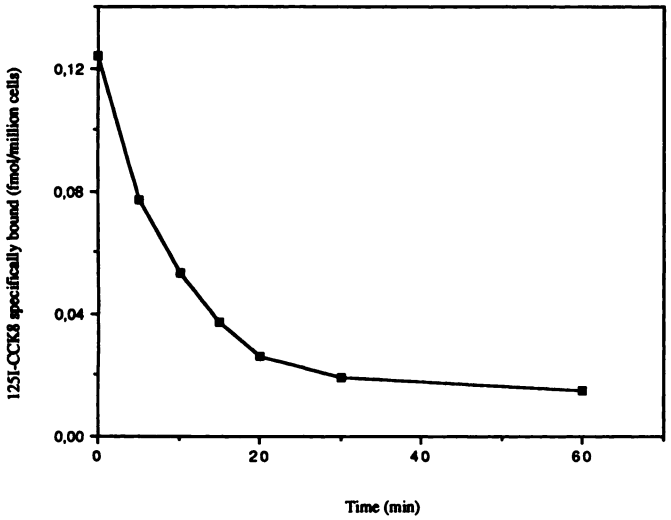
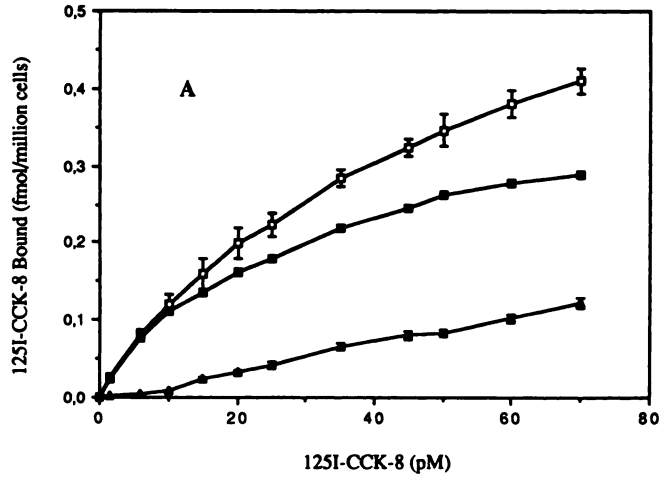


Fig. 4. Dissociation of ^{125}I-BH-CCK-8. Human JURKAT cells (4 × 10^6 cells/ml) were incubated at 37° for 60 min with 10 pM ^{125}I-BH-CCK-8. Then, CCK-8 (1 μM) was added, and the amount of specifically bound ^{125}I-BH-CCK-8 was determined as a function of time. Values are the means of six separate experiments.

a linear function of radiolabeled CCK-8 concentration (Fig. 5A). Nonspecific binding, as assessed in the presence of 1 μM CCK-8, represented less than 10% of total binding. Scatchard analysis of data showed linear plots (Hill number = 0.99 ± 0.08) (Fig. 5B). Computerized analysis of the binding isotherms using the LIGAND program indicated that there was no evidence for binding site heterogeneity for ^{125}I-BH-CCK-8 (six experiments). ^{125}I-BH-CCK-8 bound with high affinity (K_d = 0.032 ± 0.005 nM) to a single class of sites (B_{max} ≈ 300/cell).

In order to characterize the ligand specificity of CCK binding sites present in JURKAT cells, CCK-related compounds and nonpeptide CCK antagonists that are known to discriminate between CCK-A and CCK-B binding sites were tested for their potency to inhibit specific binding of ^{125}I-BH-CCK-8 (Fig. 6). Fig. 6 shows that specific binding of ^{125}I-BH-CCK-8 was inhibited by numerous CCK analogues, in a dose-dependent manner. The concentrations of unlabeled peptide or nonpeptide antagonist required to inhibit half of the specific binding (IC_{50}) for CCK-8, Boc-[Nle^{28}, Nle^{31}]-CCK-7 (**1**) (10), Boc-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (**2**), Boc-Trp-Nle-Asp-Phe-NH_2 (**3**), cyclic analogue JMV320 (11, 12), and compounds L-364,718 (13) and L-365,260 (14) are reported in Fig. 7 and Table 1, the order of potency being compound **1** > CCK-8 > compound JMV320 > compound **3** ≥ compound **2** > L-365,260 > L-364,718. For comparison, the potency of these compounds in inhibiting ^{125}I-BH-CCK-8 binding to rat pancreatic acini and guinea pig brain membranes is also reported in Table 1. The order of potency of the compounds is very similar in the JURKAT cells and in brain membranes and clearly differs from the relative potencies of these compounds in inhibiting ^{125}I-BH-CCK-8 binding in pancreatic acini. Particularly, compound L-365,260, a specific CCK-B antagonist, is about 30 times more potent in inhibiting binding of labeled CCK-8 on JURKAT cells than is the specific CCK-A antagonist L-364,718. Similarly, compound JMV320, a cyclic CCK analogue that is highly specific for CCK-B binding sites, is very potent on JURKAT cells. These results clearly indicate that CCK binding sites in the JURKAT cells can be classified as CCK-B binding sites.



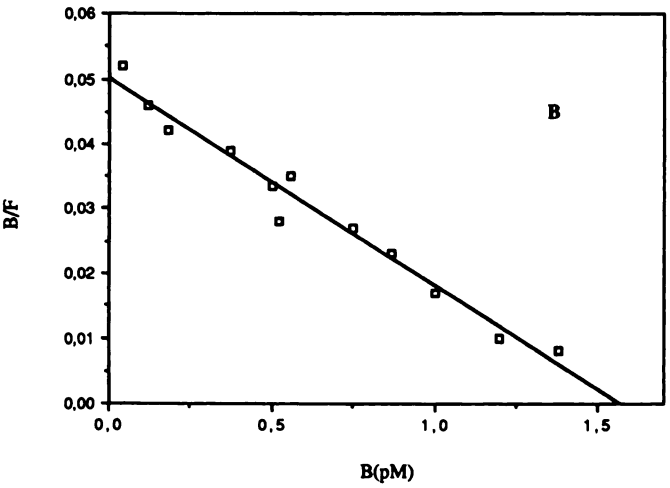


Fig. 5. ^{125}I-BH-CCK-8 binding on human JURKAT cells as a function of radiolabeled peptide concentration. A, Human JURKAT cells (4 × 10^6 cells/ml) were incubated at 37° for 60 min with various concentrations of ^{125}I-BH-CCK-8, in the presence (△) (nonspecific binding) or absence (□) (total binding) of 1 μM CCK-8. Specific binding was calculated (■) and the mean values ± standard errors of three determinations, each repeated in duplicate, are indicated. B, Scatchard replot of the specific binding data. *B*, bound; *B/F*, bound/free.

Discussion

Interactions between the central nervous system and the immune system, particularly through neuropeptides, are of growing interest (15), and a number of reports suggest that brain/gut mediators affect the immune response (3, 16). Lymphocytes and monocytes have recently been shown either to respond to or to produce a variety of peptides, including ACTH, β-endorphin, preproenkephalin, VIP, substance P, somatostatin, growth hormone, and CCK (7, 17). Continuous cell lines that express binding sites of interest have proven to be extremely useful tools for studying cellular events associated with receptor activation. Recently, type A CCK binding sites were demonstrated in CHP212 neuroblastoma cells (18), followed by their biochemical characterization (19). In the present study, we report the presence on JURKAT cells of high affinity binding sites for the peptide hormone CCK. The cell line bound radiolabeled CCK-8 to a single class of binding sites, in a time- and temperature-dependent manner, and the binding appeared

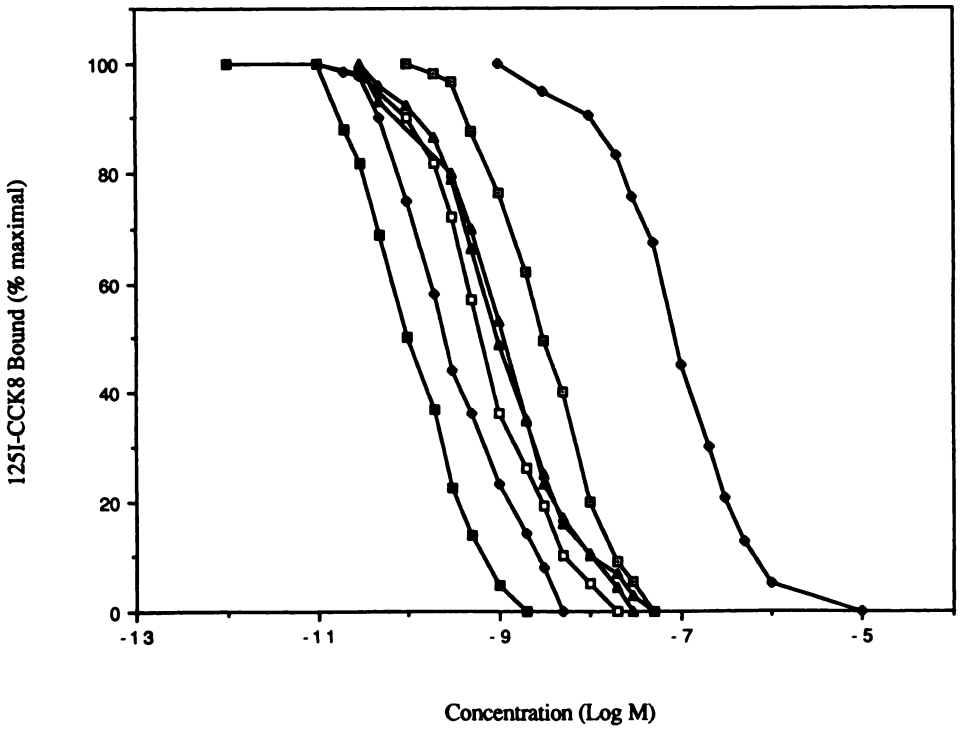


Fig. 6. Competitive binding of ^{125}I-BH-CCK-8 on human JURKAT cells. JURKAT cells were incubated at 37° for 60 min with various concentrations of CCK-8 (◇), Boc-[Nle^{28},Nle^{31}]-CCK-7 (**1**) (■), Boc-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (**2**) (▲), Boc-Trp-Nle-Asp-Phe-NH_2 (**3**) (△), JMV320 (□), L-364,718 (♦), or L-365,260 (⊡), with 10 pM ^{125}I-BH-CCK-8. Each *point* represents the mean of five separate experiments, each repeated in duplicate. The standard error was less than 10% of the mean value.

Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (CCK-8)

Boc-Tyr(SO_3H)-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (Boc-[Nle^{28}, Nle^{31}]-CCK-7, **1**)

Boc-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (**2**)

Boc-Trp-Nle-Asp-Phe-NH_2 (**3**)

Ac-Tyr-Lys-Gly-Trp-Lys-Asp-Phe-NH_2 (JMV320)

$CO-(CH_2)_2-CO$

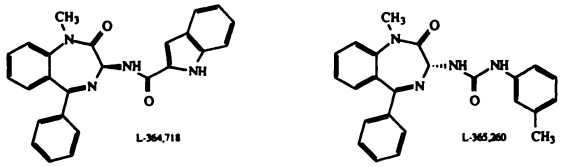


Fig. 7. Chemical structures of CCK analogues and nonpeptide CCK antagonists.

to be reversible. Because 10% of the specific CCK-8 binding remained even 1 hr after the addition of the competitor, it is possible that some of the CCK-8 may be internalized.

Two types of CCK binding sites have been identified, with the ligand binding specificity differing for CCK binding sites in the pancreas (and gall bladder) and in the brain. According to the classification of Moran *et al.* (20), CCK-A binding sites are found predominantly in the peripheral system, and desulfated CCK-8 and smaller C-terminal fragments have low affinity for these binding sites (21). In contrast, CCK-B binding sites, found in most regions of the brain, show relatively high affinity for these peptides and for CCK-8, with the C-terminal tetrapeptide being the minimal fragment exhibiting a high affinity for the CCK-B receptor (22, 23). However, in terms of binding selectivity, CCK-B receptors and gastrin receptors show almost the same characteristics, with the main difference residing in the fact that at CCK-B receptors CCK-8 is about 10 times more potent than gastrin, whereas at gastrin receptors CCK-8 and gastrin show equal affinity (24). We have shown that CCK binding sites in JURKAT cells are of the CCK-B subclass. Boc-[Nle^{28},Nle^{31}]-CCK-7 (**1**) and CCK-8 were the most potent compounds in inhibiting binding of labeled CCK-8 to JURKAT cells. The desulfated CCK-7 analogue Boc-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (**2**), the tetragastrin analogue Boc-Trp-Nle-Asp-Phe-NH_2 (**3**), the CCK-B receptor-selective cyclic analogue JMV320 (10–12), and the CCK-B receptor-selective nonpeptide antagonist L-365,260 (14) were able to inhibit binding of labeled CCK-8 with high potency, suggesting a CCK-B-like binding site. This was also suggested by the lower affinity of the CCK-A receptor-selective nonpeptide antagonist L-364,718 (13). Interestingly, the CCK analogue Boc-

TABLE 1

Abilities of different CCK agonists and antagonists to inhibit binding of ^{125}I-BH-CCK-8 to rat pancreatic acini (CCK-A), to guinea pig brain membranes (CCK-B), and to human JURKAT cells

Results are expressed as IC_{50} values and are means ± standard errors of at least six separate experiments.

	IC_{50}					
	CCK-8	1	3	JMV320	L-364,718	L-365,260
	nM					
Rat pancreatic acini	3 ± 0.6	2.3 ± 0.5	4,000 ± 800	21,800 ± 2,700	1.5 ± 0.3	370 ± 60
Guinea pig brain membranes	0.20 ± 0.03	0.20 ± 0.02	2.6 ± 0.4	3.2 ± 0.7	280 ± 100	4 ± 1.8
JURKAT cells	0.27 ± 0.04	0.1 ± 0.1	1.0 ± 0.1	0.7 ± 0.1	100 ± 6	3.3 ± 0.5

[Nle^{28},Nle^{31}]-CCK-7 (**1**) was about 2.5 times more potent than CCK-8. This difference may be accounted for by the presence of an aminopeptidase activity in JURKAT cells that is able to degrade CCK-8.

We have characterized high affinity CCK binding sites on JURKAT cells; these sites exhibit a CCK-B profile. Some differences in the affinity of compounds for ^{125}I-BH-CCK-8 binding sites were noted. In general, all the tested CCK analogues [CCK-8, Boc-[Nle^{28},Nle^{31}]-CCK-7 (**1**), Boc-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (**2**), Boc-Trp-Nle-Asp-Phe-NH_2 (**3**), and the cyclic compound JMV320] were about 2–3 times more potent in JURKAT cells than in guinea pig brain membranes. The nonselective CCK analogues [CCK-8 and Boc-[Nle^{28},Nle^{31}]-CCK-7 (**1**)] were about 10 times more potent in JURKAT cells than in rat pancreatic acini. The CCK-A receptor-selective antagonist L-364,718 was about 3 times more potent in JURKAT cells than in guinea pig brain membranes and about 60 times less potent in JURKAT cells than in rat pancreatic acini. The CCK-B receptor-selective antagonist L-365,260 was equipotent in guinea pig brain membranes and in JURKAT cells and about 100 times more potent in JURKAT cells than in pancreatic acini (Table 1). However, the rank order of potency of the tested compounds was similar in JURKAT cells and in guinea pig brain membranes, indicating a CCK-B-like profile for CCK binding sites in JURKAT cells. An important question is whether CCK-B-type binding sites in JURKAT cells are different from CCK-B binding sites in the brain and from gastrin binding sites. Until more compounds are tested, we believe that it is premature to draw conclusions about whether CCK-B binding sites in JURKAT cells differ from CCK-B binding sites in terms of ligand-binding specificity, and further studies are clearly necessary to identify these binding sites.

Carbachol, histamine, and VIP were unable to specifically and efficiently inhibit binding of labeled CCK-8 to JURKAT cells, whereas gastrin-13 was very potent ($IC_{50} \approx 0.5$ nM).

Different research groups have demonstrated that lymphocytes produce different forms of CCK in low amounts (4, 5). It was recently reported that CCK-8 mediates an increase in intracellular calcium in human fura-2-loaded peripheral blood mononuclear cells and that this effect could be blocked by high concentrations of the CCK-A-selective nonpeptide antagonist L-364,718 (6, 25). These authors showed that neither gastrin-17 nor pentagastrin was able to exhibit the CCK-8 actions. They also demonstrated that CCK-8 acted as a co-mitogen for anti-CD3 monoclonal antibody-mediated mitogenesis and, again, that compound L-364,718 at high concentrations blocked this effect (7). These studies suggest that CCK-A binding sites might mediate these biological actions, because gastrin and gastrin analogues are without effect, and that these actions are inhibited, although at high doses, by the CCK-A-selective nonpeptide antagonist. However, no CCK receptor was characterized, and the role of CCK-8 as a critical lymphokine remains to be confirmed. Under the same incubation conditions, we were reliably unable to detect ^{125}I-BH-CCK-8 specific binding sites in isolated normal human T lymphocytes, even after activation by phytohemagglutinin or concanavalin A.

In summary, we have demonstrated that JURKAT cells bound ^{125}I-BH-CCK-8 in a saturable and specific manner and expressed a single class of high affinity CCK binding sites ($K_d = 3.2\ 10^{-11}$ M, $B_{max} = 1.73\ 10^{-12}$ M). The ligand-binding properties of these binding sites are in good agreement with those found in brain membranes (CCK-B sites) and differ from the predominant type of CCK binding site found in the pancreas (CCK-A type). To our knowledge, no human T lymphocyte cell line has been shown as yet to bear CCK binding sites. Future investigations with this cell line may prove useful in establishing cellular events associated with CCK-B binding sites, because very little is known about the second messengers or signaling systems associated with these binding sites. Additional studies will be necessary to confirm that these binding sites constitute a functional receptor and to elucidate their physiological significance in these malignant lymphoid cell lines.

References

1. Solomon, G. F. Psychoneuroimmunology: interactions between central nervous system and immune system. *J. Neurosci. Res.* **18**:1–9 (1987).
2. Blalock, J. E. A molecular basis for bidirectional communication between the immune and neuroendocrine systems. *Physiol. Rev.* **69**:1–32 (1989).
3. Weigent, D. A., and J. E. Blalock. Interactions between the neuroendocrine and immune systems: common hormones and receptors. *Immunol. Rev.* **100**:79–108 (1987).
4. Okahata, H., Y. Nishi, K. Muraki, K. Sumii, Y. Miyachi, and T. Usui. Gastrin or cholecystokinin like immunoreactivity in human blood cells. *Life Sci.* **36**:369–373 (1985).
5. Donabedian, R. K., N. Odum, A. Soljganard, B. K. Jacobsen, and J. Rehfeld. The active neuropeptide cholecystokinin and its inactive precursors are present in human peripheral mononuclear cells. *Clin. Res.* **37**:848A (1989).
6. Ferrara, A., M. A. McMillen, H. C. Schaefer, K. A. Zucker, J. R. Goldenring, and I. M. Modlin. Cholecystokinin mediated calcium signals in human peripheral blood mononuclear cells. *FASEB J.* **3**:A998 (1989).
7. Ferrara, A., M. A. McMillen, H. C. Schaefer, K. A. Zucker, and I. M. Modlin. Effect of cholecystokinin receptor blockade on human lymphocyte proliferation. *J. Surg. Res.* **48**:354–357 (1990).
8. Ruff, M. R., P. Sacerdote, C. J. Wiedermann, and C. B. Pert. *Hans Selye Symposium on Neuroendocrinology and Stress*. Springer Verlag, New York (1987).
9. Munson, P. J., and D. Rodbard. LIGAND characterization of binding systems: a versatile computerized approach. *Anal. Biochem.* **107**:220–239 (1980).
10. Lignon, M. F., M. C. Galas, M. Rodriguez, and J. Martinez. Correlation between phospholipid breakdown, intracellular calcium mobilization and enzyme secretion in rat pancreatic acini treated with Boc-[Nle^{28},Nle^{31}]-CCK-7 and JMV180, two cholecystokinin analogues. *Cell. Signalling* **2**:339–346 (1990).
11. Rodriguez, M., M. Amblard, M. C. Galas, M. F. Lignon, A. Aumelas, and J. Martinez. Synthesis of cyclic analogues of cholecystokinin highly selective for central receptors. *Int. J. Peptide Protein Res.* **35**:441–451 (1990).
12. Rodriguez, M., M. F. Lignon, M. C. Galas, M. Amblard, and J. Martinez. Cyclic cholecystokinin analogues that are highly selective for rat and guinea pig central cholecystokinin receptors. *Mol. Pharmacol.* **38**:333–341 (1990).
13. Chang, R. S. L., and V. J. Lotti. Biochemical and pharmacological characterization of a new extremely potent and selective nonpeptide cholecystokinin antagonist. *Proc. Natl. Acad. Sci. USA* **83**:4923–4926 (1986).
14. Lotti, V. J., and R. S. L. Chang. A new potent and selective non-peptide gastrin antagonist and brain cholecystokinin receptor (CCK-B) ligand: L-365,260. *Eur. J. Pharmacol.* **162**:273–280 (1989).
15. Morley, J. E., N. E. Kay, G. F. Solomon, and N. P. Plotnikoff. Neuropeptides: conductors of the immune orchestra. *Life Sci.* **41**:527–544 (1987).
16. O'Dorisio, M. S., and A. Paneira. Neuropeptides and immunopeptides: messengers in the neuroimmune axis. *Ann. N. Y. Acad. Sci.* **594**:1–499 (1990).
17. Payan, D. G., J. P. McGillis, and E. J. Goetzl. Neuroimmunology. *Adv. Immunol.* **39**:299–323 (1986).
18. Barret, R. W., M. E. Steffey, and C. A. W. Wolfram. Type A cholecystokinin receptors in CHP212 neuroblastoma cells: evidence for association with G protein and activation of phosphoinositide hydrolysis. *Mol. Pharmacol.* **35**:394–400 (1990).
19. Klueppelberg, U. G., X. Molero, R. W. Barret, and L. J. Miller. Biochemical characterization of the cholecystokinin receptor on CHP212 human neuroblastoma cells. *Mol. Pharmacol.* **38**:159–163 (1990).
20. Moran, T. H., P. H. Robinson, M. S. Goldrich, and P. R. McHugh. Two brain

cholecystokinin receptors: implications for behavioral actions. *Brain Res.* **362**:175–179 (1986).
21. Villanueva, M. L. S., M. Collins, R. T. Jensen, and J. D. Gardner. Structural requirements for action of cholecystokinin on enzyme secretion from pancreatic acini. *Am. J. Physiol.* **242**:G416–G422 (1982).
22. Knight, M., C. A. Tamminga, L. Steardo, M. E. Beck, P. Barone, and T. N. Chase. Cholecystokinin-octapeptide fragments: binding to brain cholecystokinin receptors. *Eur. J. Pharmacol.* **105**:49–55 (1984).
23. Innis, R. B., and S. H. Snyder. Distinct cholecystokinin receptors in brain and pancreas. *Proc. Natl. Acad. Sci. USA* **77**:6917–6921 (1980).
24. Menozzi, B., J. D. Gardner, and P. N. Maton. Properties of receptors for gastrin and CCK on gastric smooth muscle cells. *Am. J. Physiol.* **257**:G73–G79 (1989).
25. McMillen, M. A., A. Ferrara, H. C. Schaefer, J. R. Goldenring, K. A. Zucker, and I. M. Modlin. Cholecystokinin mediates a calcium signal in human peripheral blood mononuclear cells and is a co-mitogen. *Ann. N. Y. Acad. Sci.* **594**:399–402 (1990).

Send reprint requests to: Jean Martinez, Centre CNRS-INSERM de Pharmacologie-Endocrinologie, Faculté de Pharmacie, 15 Av. C. Flahault, 34060 Montpellier, Cédex France.