Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 9254–9258, December 1986
Neurobiology

Octapeptides deduced from the neuropeptide receptor-like pattern of antigen T4 in brain potently inhibit human immunodeficiency virus receptor binding and T-cell infectivity

(acquired immunodeficiency syndrome/peptide T/neuropharmacology/autoradiography/hippocampus)

CANDACE B. PERT*†, JOANNA M. HILL*, MICHAEL R. RUFF‡, ROBERT M. BERMAN*, W. GERARD ROBEY§, LARRY O. ARTHUR¶, FRANCIS W. RUSCETTI||, AND WILLIAM L. FARRAR||

*Section on Brain Biochemistry, Clinical Neuroscience Branch, National Institute of Mental Health, ADAMHA; ‡Cellular Immunology Section, Laboratory of Microbiology and Immunology, National Institute of Dental Research; ||Laboratory of Molecular Immunoregulation, Biological Response Modifiers Program, Division of Cancer Treatment and §Office of the Director, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; and ¶Program Resources, Inc., Frederick Cancer Research Facility, Frederick, MD 21701-1240

Communicated by D. Carleton Gajdusek, August 18, 1986

ABSTRACT **The differentiation antigen T4, present on the helper/inducer subset of T lymphocytes, is thought to serve as the receptor for the human immunodeficiency virus (HIV). We find that a 60-kDa protein, immunoprecipitable by monoclonal antibody (mAb) OKT4, is present on membranes from human brain as well as human T cells. Furthermore, the radioiodinated HIV envelope glycoprotein [^{125}I-labeled gp120 (^{125}I-gp120)] can be specifically covalently affixed to a molecule present on rat, monkey, and human brain membranes to yield a complex that is indistinguishable from that formed on human T cells. T4 antigen has been studied on unfixed squirrel monkey, rat, and human brain sections by autoradiography using the mAb OKT4. A highly conserved neuroanatomical pattern has been demonstrated, suggesting an analogous organization in these three mammalian brains. Furthermore, the localization of ^{125}I-gp120 receptor binding appears similar to that of T4 and is highly reminiscent of patterns for many previously characterized neuropeptide receptors. A computer-assisted analysis of gp120 suggested that a previously unremarkable octapeptide sequence within the gp120 protein, which we have synthesized and termed "peptide T," may play an important role in HIV attachment. Thus, peptide T and three rationally designed peptide analogs, each with a systematic amino acid substitution, potently inhibit specific ^{125}I-gp120 binding to brain membranes. Additionally, when tested in a viral infectivity assay, these peptides show the same rank order and similar absolute potency to block HIV infection of human T cells. Thus, peptide T may provide a useful pharmacological or immunological basis for the control and treatment of AIDS.**

The central nervous and immune systems share a large number of highly conserved, specific cell-surface recognition molecules serving as receptors for neuropeptide-mediated intercellular communication (1, 2). Consequently, we explored the possibility that a variety of classical monoclonal antibodies (mAbs) raised to T-cell surface molecules would recognize receptors in brain. Our unpublished data on the presence of T4 on brain membranes was recalled with the discovery that T4 antigen is the human immunodeficiency virus (HIV) receptor (3–5). The clinical importance of this particular example of a shared receptor/antigen (6, 7) is that patients with acquired immunodeficiency syndrome (AIDS) show neuropsychological deficits (8, 9).

Receptors serving as specific entry proteins for viruses have been envisioned for some time (10, 11), and a demonstration of specific receptor-mediated vaccinia virus infectivity blocked by synthetic peptides has been reported (12). Since the same highly conserved neuropeptide informational substances integrate immune and brain function through receptors remarkably similar to those for HIV (1), we assumed that a shared amino acid sequence between the HIV gp120 and a short peptide previously identified in another context might indicate the core peptide essential for viral receptor binding. A computer-assisted comparison of all known protein sequences with HIV gp120 revealed that the octapeptide sequence Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr of the California HIV isolate (13) is almost identical to an envelope region of the Epstein–Barr virus, which has a glutamic acid residue instead of the third threonine (14). Spurred on by this improbable coincidence ($P = 1/20^7$), we synthesized this deduced octapeptide for further study and termed it "peptide T" because half of its amino acids are threonine, whose single-letter abbreviation is "T." Binding of ^{125}I-gp120 to T4 antigen in brain membranes was potently inhibited by peptide T and three synthetic analogs with systematic amino acid substitutions. Furthermore, HIV infection of human T cells was antagonized by low (0.1 nM) concentrations of peptide in the same rank order observed for virus receptor binding inhibition.

MATERIALS AND METHODS

Radiolabeling of gp120, Preparation of Brain Membranes, Binding and Crosslinking of gp120 to Receptor, and Immunoprecipitation of T4 Antigen. HTLV-IIIb isolate of HIV was propagated in H9 cells, and the gp120 was isolated by immunoaffinity chromatography and preparative NaDodSO$_4$/PAGE as described (15). Purified gp120 was labeled with ^{125}I by the chloramine-T method to a specific activity of >1500 Ci/mmol (1 Ci = 37 GBq). Fresh human, monkey, and rat hippocampus were quickly homogenized (Polytron, Brinkmann Instruments) in 100 vol of ice-cold 50 mM Hepes (pH 7.4). The membranes collected by centrifugation (15,000 × *g*) were washed in the original buffer volume and were used fresh or stored at −70°C. Before use, brain membranes and highly purified T cells (ref. 16; gift of Larry Wahl) were preincubated for 15–30 min in phosphate-buffered saline (PBS). Membranes derived from 2 mg (initial wet weight) of brain (≈100 μg of protein) were incubated with 28,000 cpm of

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Abbreviations: HIV, human immunodeficiency virus; mAb, monoclonal antibody; AIDS, acquired immunodeficiency syndrome; gp120, 120-kDa external HIV envelope glycoprotein; IL-2, interleukin 2.

†To whom reprint requests should be addressed at: Section on Brain Biochemistry, Clinical Neuroscience Branch, NIMH, Bldg. 10, Rm. 3N256, 9000 Rockville Pike, Bethesda, MD 20892.

^{125}I-gp120 for 1 hr at 37°C in 200 μl (final volume) of 50 mM Hepes containing 0.1% bovine serum albumin and the peptidase inhibitors bacitracin (0.005%), aprotinin (0.005%), leupeptin (0.001%), and chymostatin (0.001%). Incubations were rapidly vacuum-filtered and counted to determine the receptor-bound material (17). Alternatively, the ligand–receptor complex was covalently preserved with the rapid addition at 4°C of disuccinimidyl suberate to a final concentration of 0.5 mM. The crosslinked products were visualized on x-ray film after $NaDodSO_4$-solubilized membranes were subjected to PAGE.

Immunoprecipitation. Immunoprecipitates were prepared by incubation (overnight at 4°C) of 0.5% Triton X-100/PBS-solubilized, lactoperoxidase/glucose oxidase/^{125}I-iodinated brain membranes or intact T cells with indicated mAbs at 10 μg per reaction. A solid-phase immunoabsorbant (Immunobeads, Bio-Rad) was used to precipitate immune complexes prior to their resolution by $NaDodSO_4$/PAGE. Other details were as described for the immunoprecipitation of the transferrin receptor (9). Control incubations contained no primary mAb or a subclass control mAb (OKT8).

Chemical Neuroanatomy and Computer-Assisted Densitometry. Cryostat-cut 25-μm sections of fresh-frozen human, monkey, and rat brain were thaw-mounted and dried onto gelatin-coated slides, and receptors were visualized as described (18). Incubations, with or without antibodies (10 μg/ml) against T4, T4A, T8, and T11, were conducted overnight at 0°C in RPMI medium, crosslinked onto their antigens, and visualized with ^{125}I-labeled goat anti-mouse antibody as described (6). Incubations of slide-mounted tissue sections to label the antigen/receptor with ^{125}I-gp120 were conducted in 5-ml slide carriers with (1 μM) or without unlabeled gp120 or mAb OKT4A (10 μg/ml) (Ortho Diagnostics) exactly as described above for membranes.

Computer-assisted transformation of autoradiographic film opacity into quantitative color images was performed as described (19), with subsequent modifications of the palette choices by R.M.B. and Wayne Rasband. Coexposure of standards of known increments of radioactivity with the monkey brain sections generated a linear plot ($r > 0.99$) of logarithmic OD versus cpm, from which the relative concentration of radioactivity can be meaningfully extrapolated (20). Cell staining of brain sections with thionine was performed by classical methods, and visualization of receptors overlying the stained tissue was as described (18).

Peptide Synthesis. Peptides were synthesized by Peninsula Laboratories (Belmont, CA) by the solid-phase method, purified by preparative HPLC, and analyzed by TLC, high-voltage electrophoresis, and amino acid analysis.

Separation of T-Lymphocyte Subsets. Subsets of T cells were obtained by treatment of Percoll density-purified peripheral blood T cells with specific monoclonal antibodies (T4 or T8) at 10 μg/ml. The treated cells were then panned (21) on a plastic Petri dish that was coated with goat [$F(ab')_2$] anti-mouse immunoglobulin (Sero Lab, Westbury, MA) for 30 min at 4°C. The nonadherent cells were then removed, washed, and analyzed for reactivity by flow cytometry. The separated T4 and T8 cell populations have <5% contamination of other T-cell subsets. Cells were then cultured with phytohemagglutinin (1 μg/ml) for 72 hr and exposed to HIV as described below. Infected cells were phenotypically characterized when cytotoxicity assays were performed.

Virus Infection. The HIV virus used for infection was isolated from an interleukin 2 (IL-2)-dependent cultured T-cell line established from fresh AIDS patient material and passaged into HuT 78, a permissive IL-2-independent cell line. The protocols used for HIV infection were as follows (22). Cell-free supernatants (0.45 μl of filtrate) of this virus isolate were used to infect normal phytohemagglutinin-stimulated $T4^+$ lymphocytes. Supernatants containing >5 pmol of reverse transcriptase and ≈10^{10} particles per ml were incubated with 10^7 cells in a 1-ml volume for 30 min at 37°C with synthetic peptides in serum-free RPMI medium. Cells were thoroughly washed in peptide-containing RPMI medium, grown in IL-2-containing medium, and refed every 4–5 days in IL-2- and synthetic peptide-containing medium. Reverse transcriptase activity was assayed weekly from clarified cell culture supernatants as described (22). By 10–14 days after infection, reverse transcriptase activity was usually detected in supernatant fluids from the control cultures. The poly(rA)/oligo(dT) reverse transcriptase activity detected in these cocultivations had a Mg^{2+} preference. In the presence of Mn^{2+} or poly(dA)/oligo(dT), <5% of the Mg^{2+}-dependent activity was detected.

RESULTS

A single radiolabeled crosslinking product of ≈180 kDa was obtained after specific binding of ^{125}I-gp120 to membranes from either squirrel monkey or rat or to human brain membranes; this product was indistinguishable from that of human T cells (Fig. 1*A*). This result indicates that gp120 can be coupled to an ≈60-kDa protein; unreacted ^{125}I-gp120 ran adjacent to the no-membrane control (lane a). Immunoprecipitation of radioiodinated human brain membranes with OKT4 and OKT8 (10 μg/ml) (Fig. 1*B*) suggests that brain membranes contain a T4 antigen of ≈60 kDa, indistinguishable from that identified on human T lymphocytes (Fig. 1*C*); by contrast, OKT8 immunoprecipitated a low (≈30 kDa) molecular weight protein from human T lymphocytes (Fig. 1*C*) that was absent in brain membranes (Fig. 1*B*), indicating that brain T4 is not derived from resident lymphocytes. Similar results were observed with monkey (24) hippocampal membranes. These results, in agreement with previous work (3–5) and preliminary reports (23, 24), suggest that the T4 antigen serves as the viral receptor and is a highly conserved 60-kDa molecule shared by the immune and central nervous systems.

Fig. 2*A* shows the distribution of T4 antigen on a rostral-to-caudal series of coronal sections of squirrel monkey brain. OKT8, a T lymphocyte-directed mAb from the same subclass as OKT4 had no observable pattern (see last section). While there were detectable levels of T4 mAb binding to cytoarchitectonically meaningful areas of brain stem (e.g., the substantia nigra), the striking pattern of cortical enrichment was apparent at every level of the neuroaxis. Generally, within cerebral cortex the more superficial layers contained

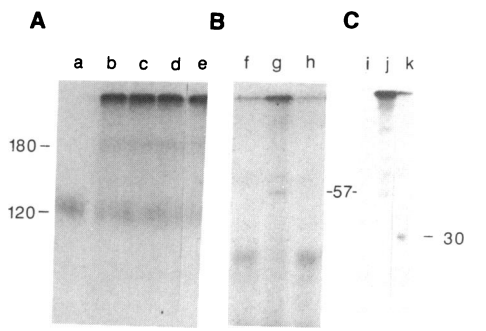

FIG. 1. Presence of the T4 antigen in brain tissue. (*A*) Crosslinking of ^{125}I-gp120 to brain membranes and T cells yields an identical 180-kDa product. Lanes: a, ^{125}I-gp120 only; b–e, ^{125}I-gp120 with monkey brain (lane b), rat brain (lane c), human brain (lane d), and human T cells (lane e). (*B* and *C*) Immunoprecipitation of ^{125}I-labeled monkey brain membranes (*B*) and human T cells (*C*). The following mAbs were used: no primary antibody control (lanes f and i), OKT4 (lanes g and j), and OKT8 (lanes h and k).

the densest concentrations of T4 antigen, and the frontal (Fig. 2*A*, 23 mm) and perilimbic cortex overlying the amygdala (Fig. 2*A*, 10.5–1.5 mm) (itself enriched with T4 antigen) were particularly receptor-rich throughout the deeper layers as well. The hippocampal formation had the densest concentration of receptors in monkey (Fig. 2*A*, 7.5–1.5 mm), rat (Fig. 2*B*, section a), and human brain (Fig. 2*C*, section a). Darkfield microscopy of squirrel monkey sections dipped in photographic emulsion (Fig. 2*C*, section b) revealed that the band of densest receptor labeling is located within the molecular layers of the dentate gyrus and hippocampus proper, which contain very few neurons. Thus, receptors appear to be richly distributed over neuropil (the neuronal extensions of dendrites and axons).

Evidence of the specificity of the chemical neuroanatomy and results suggesting that T4 and the viral envelope recognition molecule (gp120) are indistinguishable are shown in Fig. 2*B*. Coronal sections of rat brain revealed a similar

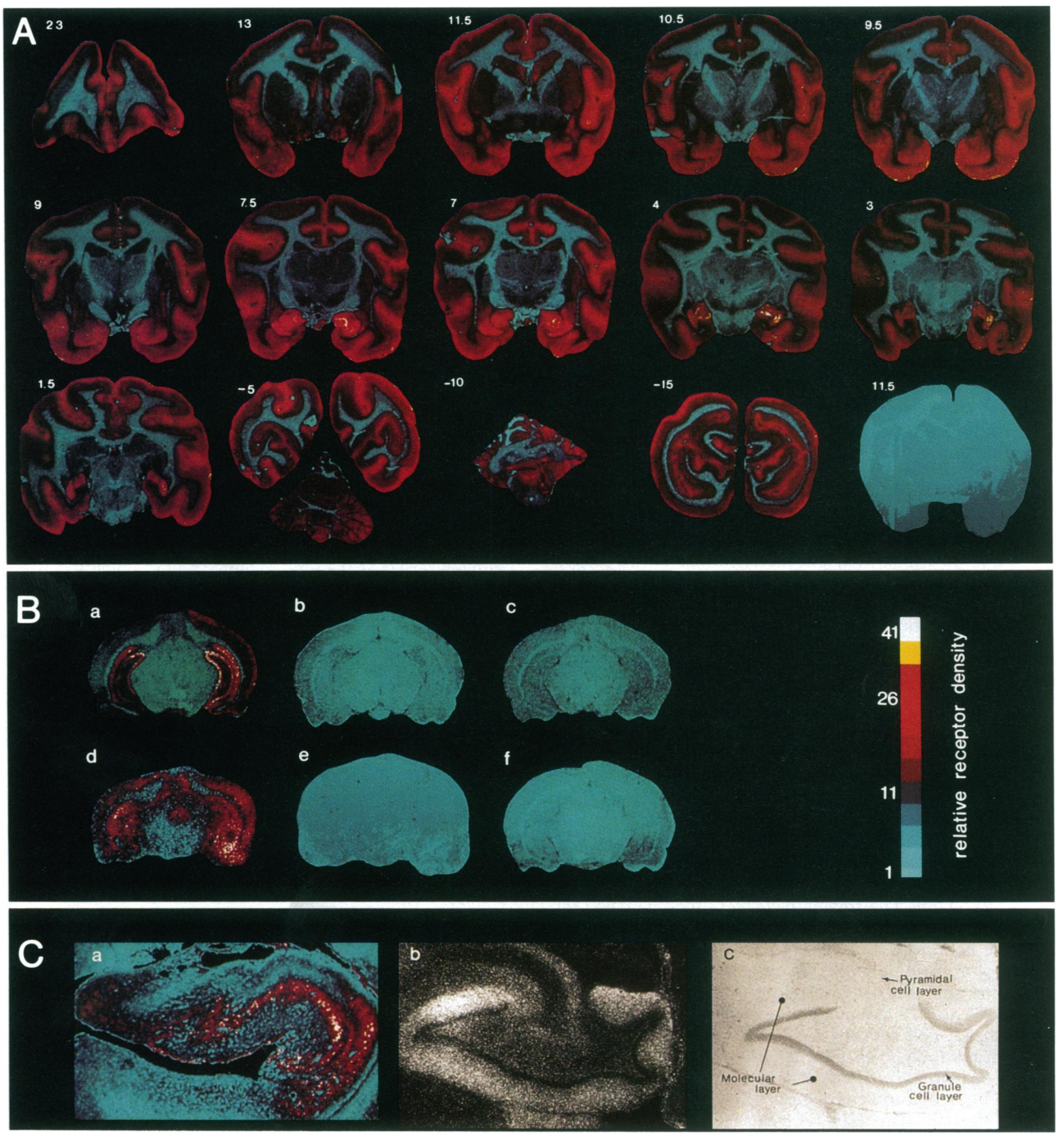

FIG. 2. (*A*) Computer-assisted autoradiographic visualization of T4 antigen on a series of coronal sections of squirrel monkey brain. Distance in millimeters anterior (+) and posterior (−) from the ear meatus is indicated on each section. Color bar labeling depicts relative receptor/antigen density. The last panel shows the absence of binding obtained in a simultaneously conducted incubation with OKT8 instead of OKT4. (*B*) Specificity and comparison of T4 antigen and ^{125}I-gp120 receptor patterns in rat brain. Sections: a, OKT4; b, secondary antibody only; c, OKT11; d, ^{125}I-gp120; e, ^{125}I-gp120 and gp120 (1 μM); f, gp120 and OKT4A (10 μg/ml). (*C*) Computer-assisted autoradiographic visualization of T4 antigen in human (section a) and monkey (section b) hippocampal formation; autoradiographic grains are visualized in the dark field as white areas over cell-stained tissue (section c).

cortex/hippocampus-rich pattern of receptor distribution whether OKT4 (Fig. 2*B*, section a) or ^{125}I-gp120 (Fig. 2*B*, section d) was used for visualization of sections separated by less than 1 mm. A similar pattern of the OKT4 binding and ^{125}I-gp120 binding was also evident at five other anatomical levels of rat brain (data not shown). Furthermore, this pattern was not apparent when incubation occurred in the presence of unlabeled gp120 (1 μM) (Fig. 2*B*, section e), OKT4A (10 μg/ml) (Fig. 2*B*, section f), or OKT4 (10 μg/ml) (not shown). Other mouse mAbs directed against other human T-cell surface antigens, including OKT8 (Fig. 2*B*, section c) and OKT11 (not shown), gave no detectable pattern on rat brain when visualized by ^{125}I-labeled goat anti-mouse IgG secondary antibody, just as there was no reproducible, detectable antigen/receptor with secondary antibody alone (Fig. 2*B*, section b).

Before undertaking viral infectivity experiments, we examined the properties of peptide T and three rationally designed peptide analogs in a viral envelope receptor binding assay. Fig. 3 shows the high (0.1 nM range) affinity and saturability (Fig. 3*A*) of ^{125}I-gp120 binding to freshly prepared rat brain membranes. Specificity (Fig. 3*B*) was demonstrated by blockade with OKT4 and OKT4A but not with OKT3 (0.1 μg/ml). Peptide T and two of its synthetic analogs [but not the irrelevant octapeptide substance P-(1–8)] significantly inhibited ^{125}I-gp120 binding in the 0.1 nM range (Fig. 3*C*). Substitution of a D-threonine amide in position 8 resulted in at least a 100-fold loss of receptor binding activity. The classical D-alanine substitution for L-alanine in position 1 of the octapeptide (25) resulted in a consistently more potent, presumably more peptidase-resistant (25), analog than peptide T; amidation of the C-terminal threonine also consistently produced somewhat greater potency.

The synthetic peptides then were tested independently for their ability to block viral infection of human T cells. At 100 nM the three peptides active in the binding assay were able to reduce detectable levels of reverse transcriptase activity by about 9-fold. The less-active binding displacer [D-Ala^1,D-Thr^8]peptide T amide was unable to block viral infection. Thus, as shown in Fig. 4, not only the rank order of potencies of the four peptides ([D-Ala^1]peptide T amide > [D-Ala^1]peptide T > peptide T > [D-Ala^1,D-Thr^8]peptide T amide) but also their absolute concentrations in inhibiting receptor binding and viral infectivity were closely correlated. No effect of peptides in the concentration range reported on lymphocyte viability could be detected.

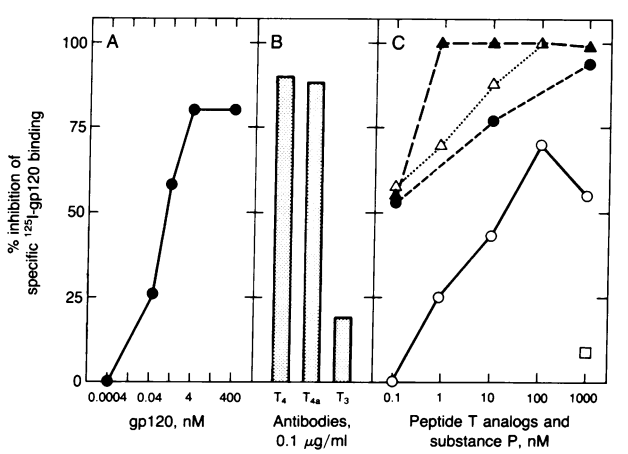

FIG. 3. (*A*) Displacement of specific ^{125}I-gp120 binding to fresh rat hippocampal membranes. (*B*) Specificity of OKT4 (bar labeled T4) and OKT4A (T4A) but not OKT3 (T3). (*C*) Inhibition of binding by peptide T (●) and analogs [D-Ala^1]peptide T (▲), [D-Ala^1]peptide T amide (△), and [D-Ala^1,D-Thr^8]peptide T amide (○) compared with that of substance P-(1–8) octapeptide (□). Each determination was performed in triplicate; the results of one experiment, which was performed three times with similar results, are shown. Specific binding displaceable by 10 μg of OKT4 and OKT4A per ml ranged between 27% and 38% of total binding, which was 2201 ± 74 cpm in the experiment shown.

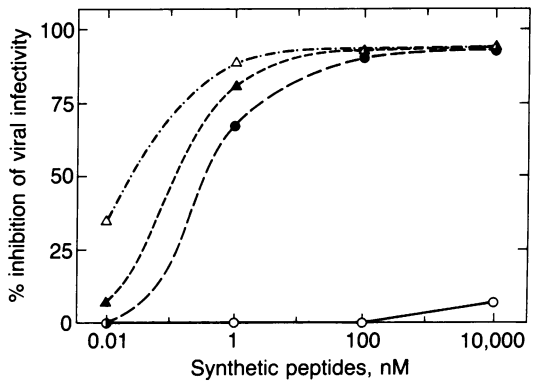

FIG. 4. Viral infectivity (T-cell reverse transcriptase activity) is blocked by peptide T (●) and its synthetic analogs [D-Ala^1]peptide T (▲), [D-Ala^1]peptide T amide (△), and [D-Ala^1,D-Thr^8]peptide T amide (○). Each determination was performed in duplicate. Results represent a single experiment, which was repeated three times with similar results.

DISCUSSION

From one perspective, the apparent conservation of the HIV receptor structure across three mammalian species seems surprising when AIDS has yet to be successfully transmitted to nonhuman primates, let alone rodents. However, possession of a recognition molecule on cell surfaces for viral entry seems to be necessary but is clearly not sufficient to support viral replication. It is relevant that neuropeptides (26, 27) are distributed throughout the brain and body and show profound evolutionary stability, being present in largely unaltered form in unicellular organisms as well as higher animals. Their receptors, also highly conserved (28, 29), are shared components in an intercellular communication network where specificity is conveyed by cell-surface recognition molecules (1, 30). In this context, our observations concerning the apparent conservation of the HIV receptor suggest that the antigen known as T4 may similarly subserve a function as a receptor for some perhaps-as-yet-undescribed peptide informational substance. Presumably, the synthetic "peptide T" that we describe is a close analog of an endogenous peptide informational substance, a readily testable notion.

In addition to similarity of molecular weights of receptors, we demonstrate profound conservation of regional distribution pattern [i.e., high in hippocampal neuropil and recently expanded cortical regions mediating higher functions (6)] in three mammals, suggesting some analogous function, which may be altered by viral infection in AIDS patients. For example, since the hippocampus is classically considered the locus of memory formation, the demonstration of memory loss early in the disease process in some AIDS patients (7) might be a result of interference with information processing in the receptor-rich hippocampus. We have emphasized that the brain distribution pattern of HIV receptors suggests merely potential targets for virus infection (31). However, recent evidence** suggests that the yield of viruses from various brain regions dissected from AIDS victims is strikingly similar to the receptor regional pattern reported here.

**Shaw, G. M., Cobbs, C. S., Hahn, B. H., Price, R. W., Navia, B. A., O'Hara, C. J. & Groopman, J. E., Second International Conference on AIDS, June 23–25, 1986, Paris (abstr.).

We do not know the relevance, if any, of the shared presence of peptide T in the Epstein–Barr virus envelope. While HIV and Epstein–Barr virus are clearly both lymphotropic viruses, the Epstein–Barr virus receptor (32) is considered unrelated to T4. While new experiments on the effect of peptide T on Epstein–Barr virus infection might prove interesting, the shared sequence may be of historical interest only. An analysis of additional isolates suggests that the core sequence required for HIV attachment may be an even shorter pentapeptide. Both classical HIV isolates HTLV-IIIb (33) and LAV (34) contain the sequence Thr-Thr-Ser-Tyr-Thr, which differs from peptide T by only the one amino acid (serine instead of asparagine) and is quite potent (35).

The most potent of the synthetic peptides, [D-Ala^1]peptide T amide, inhibits receptor binding and T-cell infectivity in the 0.1 nM range, thus deserving further study for therapeutic potential. Certainly, the sensitivity of other strains of HIV to these peptides should be thoroughly explored. The several-orders-of-magnitude loss in apparent receptor affinity accomplished by a single amino acid enantiomeric substitution (D-Thr in position 8 of [D-Ala^1]peptide T amide) is typical of neuropeptides, for which extensive structure–activity analyses have been performed, particularly for opiate peptides (36). The "threshold effect," whereby activity is lost in the bioassay but is still detectable in the receptor binding assay, is also not unusual.

The synthesis of this peptide must be viewed as the beginning of binding assay-assisted rational peptide drug design, with bioavailability in the central nervous system as well as further increases in potency and stability as important goals. Although it would not be unprecedented if the HIV virus had additional receptors for cellular entry, the similar brain distribution of viral envelope receptor and T4 antigen are compatible with a single entry protein. Our identification of the highly sought-after attachment portion of the viral envelope (e.g., glycoprotein 120 residues 196–200 in ref. 13) should help the production of neutralizing antibodies and vaccine development. Perhaps even more important, peptide T, or a derivative, might be useful clinically to halt or attenuate the spread of the virus in infected individuals.

Dr. Frederick Goodwin's unlimited support and encouragement through all phases of this work was critical to its accomplishment. We gratefully acknowledge the personal attention of Dr. Jaw-Kang Chang in the peptide syntheses and Mr. Mark Gunnell for utilizing the WYLBUR program for protein sequences. This project has been funded at least in part with federal funds from the Department of Health and Human Services, under Contract N01-C0-23910 with Program Resources, Inc.

1. Pert, C. B., Ruff, M. R., Weber, R. J. & Herkenham, M. (1985) *J. Immunol.* **135,** 820S–826S.
2. Ruff, M. R. & Pert, C. B. (1984) *Science* **225,** 1034–1036.
3. Dalgleish, A. G., Beverley, P. C. L., Clapham, P. R., Crawford, D. H., Greaves, M. F. & Weiss, R. A. (1984) *Nature (London)* **312,** 763–768.
4. Klatzmann, D., Champagne, E., Chamaret, S., Gruest, J., Guetard, D., Hercend, T., Cluckman, J.-C. & Montagnier, L. (1985) *Nature (London)* **312,** 767–770.
5. McDougal, J. S., Mawle, A., Cort, S., Nicholson, J., Cross, D., Scheppler, J., Hicks, D. & Sligh, J. (1985) *J. Immunol.* **135,** 3151–3157.
6. Hill, J. M., Farrar, W. L. & Pert, C. B. (1986) *Psychopharmacol. Bull.* **22,** 689–694.
7. Hill, J. M., Ruff, M. R., Weber, R. J. & Pert, C. B. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4553–4557.
8. Snider, W. D., Block, B. E. & Letterman, G. (1983) *Ann. Neurol.* **14,** 403–418.
9. Johnson, R. T. & McArthur, J. C. (1986) *Trends Neurosci.* **9,** 91–94.
10. Crowell, R. L. (1976) *Cell Membrane Receptors for Viruses, Antigens and Antibodies, Polypeptide Hormones and Small Molecules* (Raven, New York), pp. 202–212.
11. Tignor, G. H., Smith, A. L. & Shope, R. E. (1984) in *Concepts in Viral Pathogenesis*, eds. Notkins, A. & Oldstone, M. (Springer, Berlin), pp. 157–182.
12. Epstein, D. A., Marsh, Y. V., Schreifer, A. B., Newman, S. R., Todaro, G. J. & Nestor, J. J., Jr. (1985) *Nature (London)* **318,** 663–667.
13. Sanchez-Pescador, R., Power, M. D., Barr, P. J., Steimer, K. S., Stempien, M. M., Brown-Shimer, S. L., Gee, W. W., Renard, A., Randolph, A., Levy, J. A., Dina, D. & Luciw, P. A. (1985) *Science* **227,** 484–492.
14. Baer, R., Bankier, A. T., Biggin, M. D., Deininger, P. L., Farrell, P. J., Gibson, T. J., Hatfull, G., Hudson, G. S., Satchwell, S. C., Deguin, C., Tuffnell, P. S. & Barrell, B. G. (1984) *Nature (London)* **310,** 207–211.
15. Robey, W. G., Arthur, L. O., Matthews, T. J., Langolis, A., Copeland, T. D., Oroszlan, S., Bolognesi, D. P., Gilden, R. V. & Fischinger, P. J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 702–707.
16. Wahl, L., Kotona, I. M., Wilder, R., Winter, C. C., Haroui, B., Sher, I. & Wahl, S. M. (1984) *Cell. Immunol.* **85,** 373–378.
17. Pert, C. B. & Snyder, S. H. (1973) *Science* **179,** 1011–1014.
18. Herkenham, M. & Pert, C. B. (1982) *J. Neurosci.* **2,** 1129–1149.
19. Goochee, C., Rasband, W. & Sokoloff, L. (1980) *Ann. Neurol.* **7,** 359–370.
20. Herkenham, M. (1985) *NIDA Res. Monogr.* **62,** 13–29.
21. Mage, M., Mathieson, B., Sharrow, S., McHugh, L., Hammerling, U., Kanellopoulos-Langevin, C., Brideau, D. & Thomas, C. (1982) *Eur. J. Immunol.* **11,** 228–233.
22. Ruscetti, F. W., Kalyanaraman, V. S., Overton, R., Mikovits, J., Stevenson, H., Stromberg, K., Herberman, R. B., Farrar, W. L. & Ortaldo, J. R. (1986) *J. Immunol.*, in press.
23. Ruscetti, F., Farrar, W. L., Hill, J. M. & Pert, C. B. (1986) *Peptides*, in press.
24. Farrar, W. L., Hill, J. M., Ruff, M. R. & Pert, C. B. *Lymphokine Res.*, in press.
25. Pert, C. B., Pert, A., Chang, J.-K. & Fong, B. T. W. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3729–3733.
26. LeRoith, D., Shiloach, J., Roth, J. & Lesniak, M. A. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 6184–6189.
27. LeRoith, D., Liotta, A. S., Roth, J., Shiloach, J., Lewis, M. E., Pert, C. B. & Krieger, D. T. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2086–2091.
28. Venter, J. C., Eddy, B., Hall, L. & Fraser, C. M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 272–276.
29. Zipser, B., Ruff, M. R., Higgins, W. & Pert, C. B. (1985) *15th Annu. Mtg. Soc. Neurosci.* **11,** 308.
30. Schmitt, F. D. (1984) *Neuroscience* **13,** 991–1011.
31. Hill, J. M., Farrar, W. L. & Pert, C. B., *Int. J. Neurosci.*, in press.
32. Fingeroth, J. D., Weis, J. J., Tedder, T. F., Strominger, J. L., Biro, P. A. & Fearon, D. T. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4510–4514.
33. Ratner, L., Haseltine, W., Patarca, R., Livak, K. J., Starcich, B., Josephs, S. F., Doran, E. R., Rafalski, J. A., Whitehorn, E. A., Baumesiter, K., Ivanoff, L., Petteway, S. R., Jr., Pearson, M. L., Lautenberger, J. A., Papas, T. S., Ghrayeb, J., Chang, N. T., Gallo, R. C. & Wong-Staal, F. (1985) *Nature (London)* **313,** 277–284.
34. Wain-Hobson, S., Sonigo, P., Danos, O., Cole, S. & Alizon, M. (1985) *Cell* **40,** 9–17.
35. Pert, C. B. & Ruff, M. R. (1986) *Clin. Neuropharmacol.* **9,** Suppl. 4, 5198.
36. Kosterlitz, H. (1976) *Opiates and Endogenous Opioid Peptides* (North Holland, Amsterdam).