JOURNAL OF VIROLOGY, Feb. 1991, p. 961–967
0022-538X/91/020961-07$02.00/0

Vol. 65, No. 2

Evidence for Neurotoxic Activity of *tat* from Human Immunodeficiency Virus Type 1

JEAN-MARC SABATIER,[1] ERIC VIVES,[1] KAMEL MABROUK,[1] ABDELAZIZ BENJOUAD,[2] HERVÉ ROCHAT,[1] ALAIN DUVAL,[3] BERNARD HUE,[3] AND ELMOSTAFA BAHRAOUI[1]*

Laboratoire de Biochimie, Centre National de la Recherche Scientifique URA 1179, Faculté de Médecine, Secteur Nord, Boulevard P. Dramard, 13326 Marseille Cédex 15,[1] Laboratoire de Biologie et Génétique des Déficits Immunitaires, CERVI, Hôpital Pitié-Salpétrière, 75651 Paris Cédex 13,[2] and Laboratoire de Neurophysiologie, Centre National de la Recherche Scientifique URA 611, Université d'Angers, 49045 Angers Cédex,[3] France

Received 13 July 1990/Accepted 15 October 1990

The human immunodeficiency virus (HIV) genome codes for a *trans*-activating regulatory protein, *tat*. Using chemically synthesized *tat*, it was found that ^{125}I-*tat* and ^{125}I-tat_{38-86} specifically bound to rat brain synaptosomal membranes with moderate affinity ($K_{0.5}$ = 3 μM). Interaction of *tat* with nerve cells was also revealed by flow cytometry, which showed its binding to rat glioma and murine neuroblastoma cells, using both direct fluorescence with fluorescein isothiocyanate-labeled *tat* and indirect immunofluorescence assays. This interaction was investigated with electrophysiology using isolated excitable frog muscle fibers and cockroach giant interneuron synapses. *tat* acted on the cell membrane and induced a large depolarization, accompanied by a decrease in membrane resistance, thereby modifying cell permeability. The neurotoxicity of *tat* was further demonstrated in vitro, on glioma and neuroblastoma cell growth, as well as by a ^{51}Cr release assay in both tumor cell lines. Interestingly, no hemolytic activity of *tat* for human erythrocytes was found even when *tat* was tested at its highly neurotoxic concentration. Experiments in vivo showed that synthetic *tat* is a potent and lethal neurotoxic agent in mice. The use of *tat* peptide derivatives showed that basic region from 49 to 57 is necessary and sufficient for binding to cell membranes and toxicity.

It is now established that infection with human immunodeficiency virus type 1 (HIV-1) is often complicated by neurological syndromes that include dementia, subacute encephalitis, and vacuolar degeneration of the spinal cord (9, 19, 28, 32). The identification and isolation of HIV-1 from the brain suggests that the retrovirus itself is responsible for the neurological disorders observed in HIV-infected patients. Other lentiviruses, including visna virus (15) and simian immunodeficiency virus (22), are also associated with brain infections. Among central nervous system (CNS) cells, monocyte and macrophage lines are preferentially infected by HIV, but infection of other neural cell types has also been discussed (20, 35).

The pathogenic mechanism by which the virus causes encephalopathy remains unknown. It was reported recently that the HIV envelope glycoprotein manifests neurotoxic activity by increasing free Ca^{2+} in rat neurons, thus causing cellular damage (4, 7). This effect can be prevented by Ca^{2+} channel antagonists.

As an approach to another possible cause of neurological dysfunction, we investigated whether other HIV proteins could be implicated in this pathology. For this study, numerous peptides were chemically synthesized on an Applied Biosystems peptide synthesizer (model 430A) with the stepwise solid-phase method (25, 29).

By testing the neurotoxicity of synthetic fragments of various lengths, derived from gp160, p25, *nef*, and *tat* proteins, we discovered that the intracerebroventricular injection of *tat* or some *tat* fragments caused toxic and lethal effects in mice. The 86-residue *tat* protein from HIV-1 has been previously reported to be critical for virus replication through its role in viral *trans* activation (1, 11, 12, 14, 18, 33).

We have further investigated *tat* neurotoxicity by structure-activity relationships, using binding experiments and electrophysiology. We first investigated the capacity of radiolabeled tat_{38-86} from HIV-1, LAV_{Bru} isolate, to bind to rat brain synaptic nerve ending particles (synaptosomal membranes) prepared by the method of Gray and Whittaker (13). Protein was measured by a modified Lowry method (24). ^{125}I-tat_{38-86} (>10^{-8} M), the most active peptide in vivo (Table 1), bound to synaptosomal membranes in a dose-dependent manner and with a saturable effect (Fig. 1A and B). Binding was specific, as shown by the capacity of increasing amounts of unlabeled tat_{38-86} to fully inhibit this interaction (Fig. 1C). The concentration of unlabeled tat_{38-86} inhibiting 50% of ^{125}I-tat_{38-86} binding ($K_{0.5}$) was about 3 μM. The interaction between tat_{38-86} and synaptosomal membranes thus has a medium-range affinity. Specificity of interaction was further demonstrated by the capacity of full-length synthetic tat_{2-86}, tat_{2-86} CM (*S*-carboxamide methylcysteine derivative of tat_{2-86}), tat_{21-86}, tat_{21-86} CM, tat_{47-72}, and tat_{48-86} to fully inhibit the interaction of ^{125}I-tat_{38-86} with synaptosomal membranes in a binding assay. The $K_{0.5}$ values obtained with these *tat* peptides were 2.5 to 4.5 μM, comparable to that of tat_{38-86}, indicating comparable affinities for the synaptosomal binding site. In addition, a binding assay with full-length ^{125}I-tat_{2-86} CM gave results similar to those obtained with ^{125}I-tat_{38-86} (data not shown). Interestingly, peptide tat_{67-86} did not compete with ^{125}I-tat_{38-86} for binding, while tat_{48-86} fully inhibited this interaction, indicating that region from 48 to 66 includes the *tat* binding site. This region contains a highly basic domain from 49 to 57 previously found critical for efficient *tat trans* activation, which was presumed to be a nuclear targeting signal or a nucleic acid binding site (18). Also, the basic domain possesses sequence homology with HIV-1 *tev* and human T-cell leukemia virus type 1 *rex* proteins (2, 31). The

* Corresponding author.

TABLE 1. Specific neurotoxicity of synthetic tat_{2-86}, tat_{2-86} CM, and peptides mimicking various regions of *tat* (*tat* peptides) in mice[a]

Peptide	LD_{50} (μg)
tat_{2-24}	Inactive
tat_{2-86}	180 (18.7)
tat_{2-86} CM	81 (8.1)
tat_{2-86} CM after chymotryptic digestion	81 (8.1)
tat_{11-24}	Inactive
tat_{13-48}	Inactive
tat_{21-86}	180 (24.1)
tat_{21-86} CM	90 (11.5)
tat_{21-86} CM after chymotryptic digestion	90 (11.5)
tat_{36-50}	Inactive
tat_{38-60}	130 (46.4)
tat_{38-72}	36 (8.8)
tat_{38-86}	13.5 (2.4)
tat_{38-86} after tryptic digestion	Inactive
tat_{46-60}	90 (45.7)
tat_{47-72}	180 (56.9)
tat_{48-86}	18 (4.1)
tat_{56-70}	Inactive
tat_{57-86}	Inactive
tat_{65-80}	Inactive
tat_{67-86}	Inactive

[a] *tat* peptides were tested in vivo for toxicity by determining the LD_{50} after intracerebroventricular injection in 20-g C57/BL6 mice. Injections were carried out in groups of eight mice per dose, using 5 μl of solutions containing peptides in 0.1% (wt/vol) bovine serum albumin and 0.9% (wt/vol) sodium chloride. The LD_{50} is expressed in both micrograms and nanomoles (in parentheses) of inoculated peptides. Peptides were considered inactive when no lethal effect was observed after injection of 200 μg. At this dose, 30 various-length (M_r = 1,500 to 8,000) control peptides, either derived from HIV proteins or unrelated to HIV, were found to be inactive (data not shown). These control peptides include highly basic peptides: fragment 1-34 of human spleen H1b histone, full-length histone from calf thymus or its fragments 1-25 or 21-38 from H2b and 1-29 from H4, peptide 1419-1444 from *M. genitalium* adhesin, and poly-L-lysine (M_r = 3,800). Proteolytic digestions of *tat* peptides were controlled by Edman sequencing.

data suggest that the interaction of *tat* with synaptosomal membranes is mediated primarily by its basic domain. As a negative control, a highly basic peptide, but unrelated to the *tat* protein (fragment 1-34 from human spleen H1b histone) (27), was tested as a competitor for ^{125}I-tat_{38-86}, and no binding inhibition was obtained (Fig. 1C). These results demonstrate a specific binding of *tat* and argue for the presence of *tat* binding sites in rat CNS membranes.

To further investigate this interaction with nervous system cells, fluorescence was used to analyze the binding of fluorescein isothiocyanate (FITC)-labeled tat_{2-86} with murine neuroblastoma and rat glioma cells. Figure 1D clearly shows specific direct binding of FITC-labeled tat_{2-86} ($>10^{-7}$ M) on cell membranes, in a dose-dependent manner. Similar results were obtained when *tat*-cell binding was stained by rabbit anti-*tat* antibodies in an indirect immunofluorescence assay. FITC-labeled *tat* peptides including the basic domain (tat_{38-60}, tat_{38-72}, and tat_{47-72}) bound to both cell types, while FITC-labeled tat_{2-24} and tat_{57-86} were inactive for binding (data not shown).

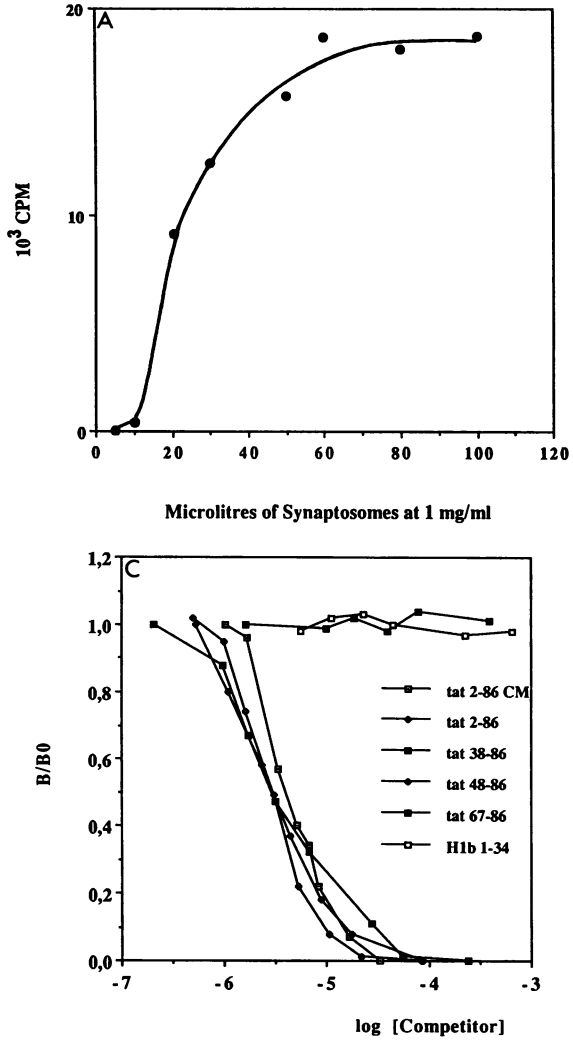
At the electrophysiological level, we tested the effects of these peptides on the polarization of excitable frog muscle fibers (8) and the postsynaptic membrane of the giant interneuron on the sixth abdominal ganglion of the cockroach *Periplaneta americana* (5, 17). The effects of tat_{2-86} CM in isolated frog muscle fibers (Fig. 2A) were determined by studying the modification of membrane potentials measured under current clamp conditions with the double mannitol-gap technique (8). The application of 5 μM tat_{2-86} CM induced a rapid and large membrane depolarization of 66.2 ± 2.1 mV (mean ± standard error of the mean; n = 6). Consequently, the amplitude of the action potential elicited with a brief depolarization under current clamp conditions decreased and disappeared without any modification in its shape. Depolarization was accompanied by a decrease of membrane resistance. Tetrodotoxin at 1 μM, which blocks Na^+ entry through the Na^+ channel (26), did not antagonize this effect. Similarly, Cd^{2+} ions (10^{-3} M), which block Ca^{2+} permeability (16), did not inhibit the depolarization effect induced by tat_{2-86} CM. A depolarization effect similar to that of tat_{2-86} CM was obtained with tat_{38-86}. In accordance with binding assay results, peptides tat_{67-86} and fragment 1-34 of human spleen H1b histone at 10^{-4} M had no effect on membrane polarization or on the action potential.

In addition, tat_{38-86} was tested on the cercal-afferent giant interneuron synapses in the CNS of the cockroach *P. americana*. The electrophysiological method used was the single-fiber oil-gap method (5). The *tat* peptide was applied close to the synapses with a micropressure ejection system. Synaptic events were monitored on an oscilloscope, digitized, and stored with a signal analyzer. The most striking effect of tat_{38-86} (10^{-6} to 10^{-5} M) was a sudden depolarization with firing of the postsynaptic membrane of the giant interneuron under test (Fig. 2B). This effect was accompanied by a decrease of postsynaptic membrane resistance (about 50%) and a resulting decrease in the composite excitatory postsynaptic potential evoked by electrical presynaptic stimulation. The amplitude of the AP triggered by block stimulation was somewhat modified. tat_{38-86} at 10^{-5} M produced a mean amplitude depolarization of 10 mV, varying with the localization of the tip of the injecting micropipette. This depolarization was totally reversible within 30 min after *tat* action.

In summary, peptides tat_{2-86} CM and tat_{38-86} dramatically modify membrane polarization, probably by creating a non-ion-selective membrane permeability.

The modification of cell membrane permeability was also shown by the capacity of tat_{2-86} to induce ^{51}Cr release from chromated murine neuroblastoma and rat glioma cells (5×10^4 cells at 53,000 cpm). Significant ^{51}Cr release from these labeled cells was obtained with about 3 and 7 μg of tat_{2-86} (2.8 and 6.6 μM); 50% release of ^{51}Cr from these cells was obtained with 60 to 70 μg of tat_{2-86} (5.7×10^{-5} and 6.6×10^{-5} M), in which the 100% effect corresponds to cellular ^{51}Cr release in the presence of 1% (vol/vol) Triton X-100. As expected, a similar neurotoxic effect of tat_{2-86} on these cell lines was observed when propidium iodine-treated cells were analyzed for viability by using flow cytometry (data not shown).

When murine neuroblastoma and rat glioma cells were cultured in the presence of tat_{2-86}, there was obvious damage and growth inhibition of tumor cell lines. Culture of rat glioma cells in the presence of various concentrations of tat_{2-86} (0.7 to 70 μg; 1.3×10^{-7} to 1.3×10^{-5} M) induced cell death after 72 h (Fig. 3). Similar neurotoxicity was observed in cell culture with *tat* peptides including the basic domain (data not shown). The mode of action of tat_{2-86} apparently differs from that of mellitin, a cytolytic neurotoxic from *Apis mellifera* venom (34), since no hemolytic activity was found when human erythrocytes were incubated with a highly neurotoxic concentration of tat_{2-86} (4×10^{-5} M). The analysis of the tropism of tat_{2-86} and its peptide derivatives for phospholipids, using the monomolecular film technique, demonstrated a direct interaction of the *tat* basic domain



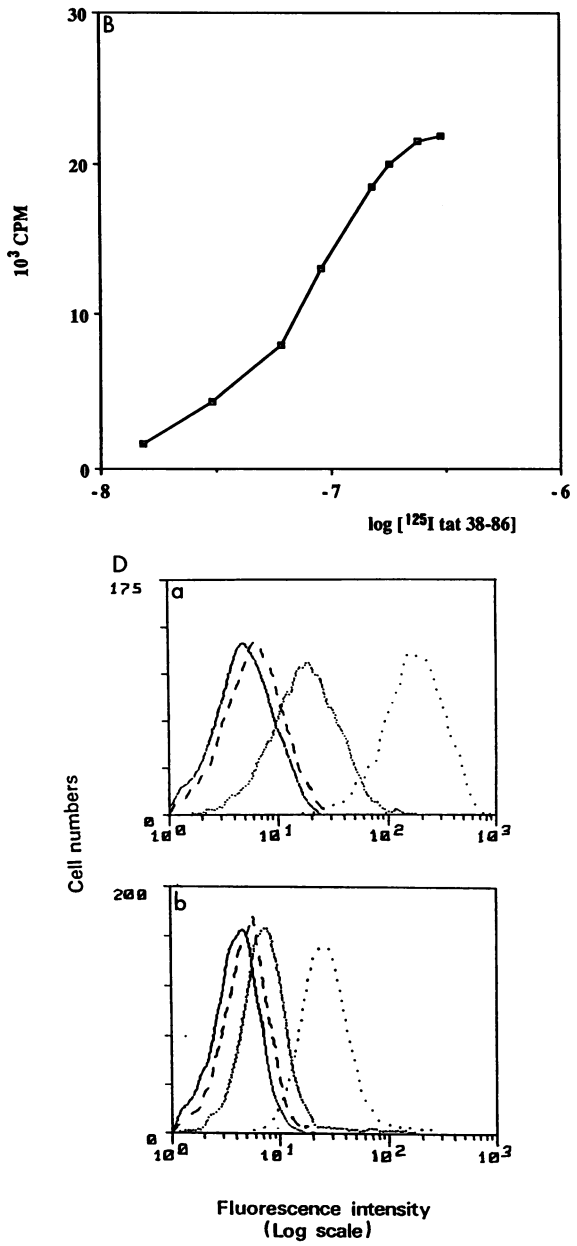


FIG. 1. (A) Titration of ^{125}I-tat_{38-86} binding to increasing amounts of rat brain synaptosomal membranes. Synaptosomes (0 to 100 μl at 1 mg of protein per ml) were incubated for 1 h at 37°C with 50 μl of ^{125}I-tat_{38-86} at 7.5×10^{-8} M (2.5×10^5 cpm) in a final volume of 150 μl of phosphate-buffered saline (PBS; pH 7.4) containing 0.5% (wt/vol) bovine serum albumin and 0.05% (wt/vol) NaN_3. After three washes and centrifugations (5 min at 3,000 × *g*), the radioactivity in the pellet was counted in a gamma counter (Kontron). (B) Saturable binding of ^{125}I-tat_{38-86} to rat brain synaptosomes. Synaptosomes (30 μl at 1 mg of protein per ml) were incubated with variable concentrations of ^{125}I-tat_{38-86} (3×10^{-8} to 3×10^{-7} M) with the protocol described for panel A. (C) Inhibition of binding of ^{125}I-tat_{38-86} to rat brain synaptosomes by unlabeled tat_{2-86}, tat_{2-86} CM, tat_{38-86}, and tat_{48-86}. ^{125}I-tat_{38-86} (50 μl) at 1.2×10^{-8} M and 50 μl of synaptosomes at 1 mg of protein per ml were incubated with increasing concentrations of competitors (10^{-8} to 10^{-3} M) in a final volume of 150 μl. The pellets were washed three times in 1 ml of the same buffer, and radioactivity was determined. B0 is the binding of ^{125}I-tat_{38-86} in the absence of competitors and, B is the binding in the presence of the indicated concentrations of unlabeled peptides. The values are means of four identical experiments. Nonspecific binding, less than 10% of the total binding, was determined in the presence of a large excess (10^{-4} M) of unlabeled tat_{38-86}. (D) Neural cell surface binding of synthetic FITC-labeled tat_{2-86}. For FITC labeling, tat_{2-86} (5 mg) was incubated in darkness with FITC (125 μg) for 2 h at 25°C in 250 μl of 100 mM sodium bicarbonate buffer (pH 9.5). After purification of the fluorescent derivatives, various amounts (0.1 to 10 μg; 2×10^{-7} to 2×10^{-5} M) of FITC-labeled tat_{2-86} were incubated for 1 h at 37°C with 10^6 murine neuroblastoma NB2 Ag (histogram a) or rat glioma C6 BU1 (histogram b) cells in 50 μl of PBS (pH 7.4) containing 0.5% (wt/vol) bovine serum albumin and 0.05% (wt/vol) NaN_3. After two washes, cells were resuspended in 500 μl of PBS containing 1% (wt/vol) paraformaldehyde. Membrane fluorescence intensity was measured by flow cytometry, using the FACS analyzer (Becton-Dickinson). Shown is cell fluorescence in the absence (——) or in the presence of 0.1 μg (- - -), 1 μg (·····), and 10 μg (· · · ·) of FITC-labeled tat_{2-86}.

(sequence 49 to 57) with only negatively charged phospholipids, as assessed by increased surface pressure ($\Delta\pi$ = 5.63 dynes/cm) when 4.5 μM of tat_{47-72} was applied on a dilauryl phosphatidylserine monolayer (initial film pressure, π = 11.25 dynes/cm). These results indicate the capacity of *tat* to bind to the membrane lipid bilayer by its basic domain and to penetrate. In view of this mechanism, the nonhemolytic activity of *tat* is in good agreement with the asymmetrical

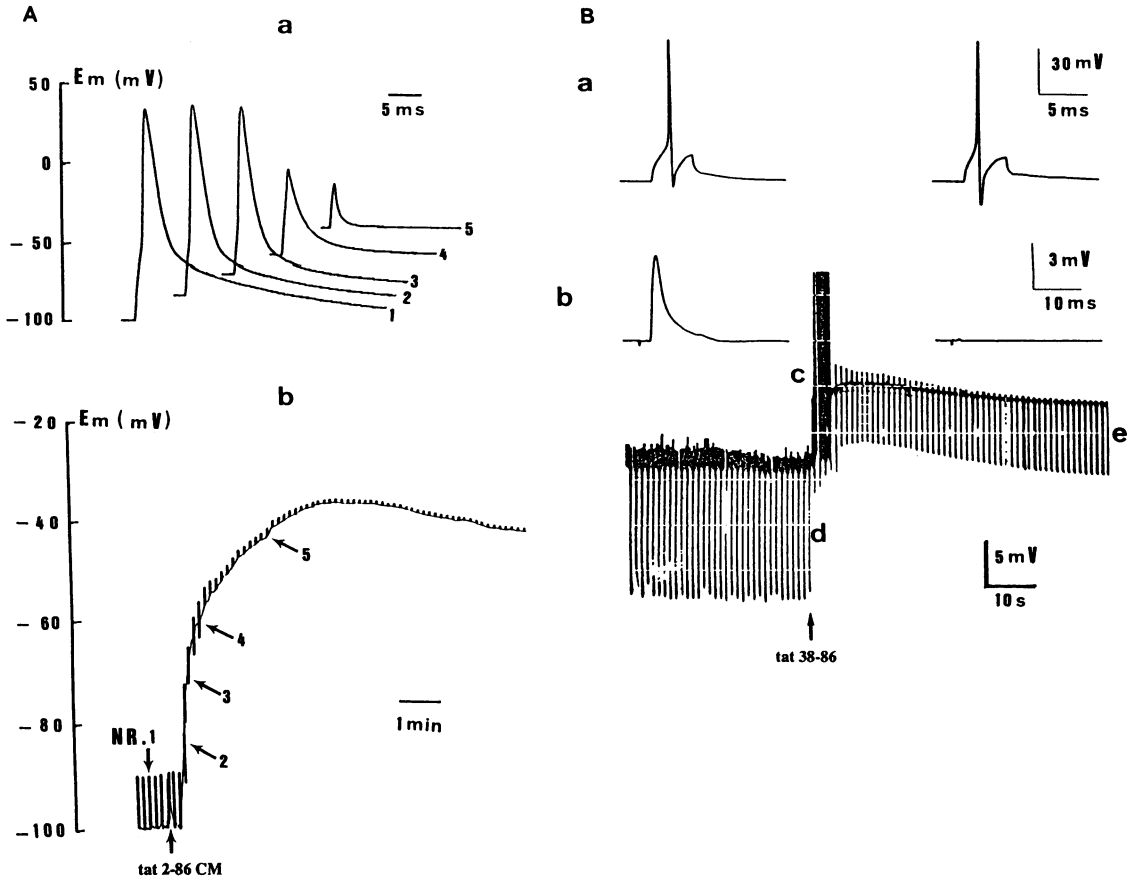


FIG. 2. (A) Evolution of action potential (a) and resting potential (b) in frog muscle fiber during the application of 5 μM tat_{2-86} CM. The action potentials evoked under current clamp conditions in graph a were recorded on a digital oscilloscope, and the resting potential in graph b was recorded on a chart recorder. In graph b, the small vertical deflections correspond to the lower part of the action potential. The experiments were performed at room temperature (18°C) on skeletal muscle fibers isolated from the semitendinosus muscle of the frog *Rana esculanta*. Em, Membrane potential; NR, normal Ringer solution. Normal physiological solution contained 12 mM NaCl, 2.5 mM KCl, 1.2 mM $CaCl_2$, and 5 mM glucose. The solution was buffered with 6.5 mM Tris hydrochloride, and pH was adjusted to 7.2 with Tris base. The mannitol solution was isotonic with the physiological solution. (B) Effect of tat_{38-86} on the cercal-afferent giant interneuron synapses in the neuropil of the sixth abdominal ganglion of the cockroach *P. americana*. Shown are changes of the action potential (a), excitatory postsynaptic potential (b), resting postsynaptic potential (c), and membrane resistance (d) during microinjection of 1 μl of 10^{-5} M of tat_{38-86} in the sixth abdominal ganglion. The peptide was solubilized in 208 mM NaCl–3.1 mM KCl–10 mM $CaCl_2$ buffer, pH 7.4. Arrow indicates the injection of tat_{38-86}. The membrane resistance is measured as the downward deflection of hyperpolarizing pulse (e).

lipid composition of the human erythrocyte plasma membrane. The negatively charged phosphatidylserine is located in the inner half of the lipid bilayer and is presumably not accessible to *tat* action. Such interactions with negatively charged phospholipids are also thought to be responsible for pharmacological activities of some snake toxins (3).

The neurotoxicity of *tat* peptides in vivo was studied by intracerebroventricular injection in mice. tat_{2-86} was lethal to mice (Table 1), with clinical effects resembling the behavioral neurotoxic symptoms induced by some snake toxins such as cardiotoxins: apathy followed by preliminary muscular tremors (about 10 min postinoculation), convulsions, wasting, and spastic paralysis just before death generally occurring between 15 min and 2 h, depending on the dose injected. Moreover, the 50% lethal doses (LD_{50}) of tat_{2-86} and its active peptide derivatives were in the same range as those obtained with these snake toxins (3). This neurotoxic activity is specific, since a number of control peptides (derived from gp160, *nef*, and p25 or unrelated to HIV proteins) of different lengths (M_r = 1,500 to 8,000) injected at higher doses did not induce neurotoxic symptoms and lethality.

The use of a panel of *tat* peptides (Table 1) delimited the minimal neurotoxic region of *tat*. tat_{2-86} neurotoxicity in vivo depends on its basic domain from 49 to 57, since tat_{46-60}, which mimics this region, or all peptides containing the entire basic domain are lethal to mice whereas other *tat* peptides are inactive (regions 2 to 24, 11 to 24, 13 to 48, 36 to 50, 56 to 70, 57 to 86, 65 to 80, and 67 to 86). Neurotoxic activity is further demonstrated to be specifically related to the basic domain of *tat* by the fact that tryptic digestion within this arginine/lysine-rich region completely abolished neurotoxicity, while chymotryptic cleavage of tat_{2-86} CM (at specific positions 8, 11, 26, 32, 38, 43, 47, and 69), which preserved the integrity of the basic domain, did not affect *tat* lethality. Controls consisting of highly basic peptides such as poly-L-lysine were inactive at the dose of 200 μg injected in mice (see footnote to Table 1). The data show that both oxidized tat_{2-86} and tat_{21-86}, containing a region of cystines, are about twofold less neurotoxic than their homologous

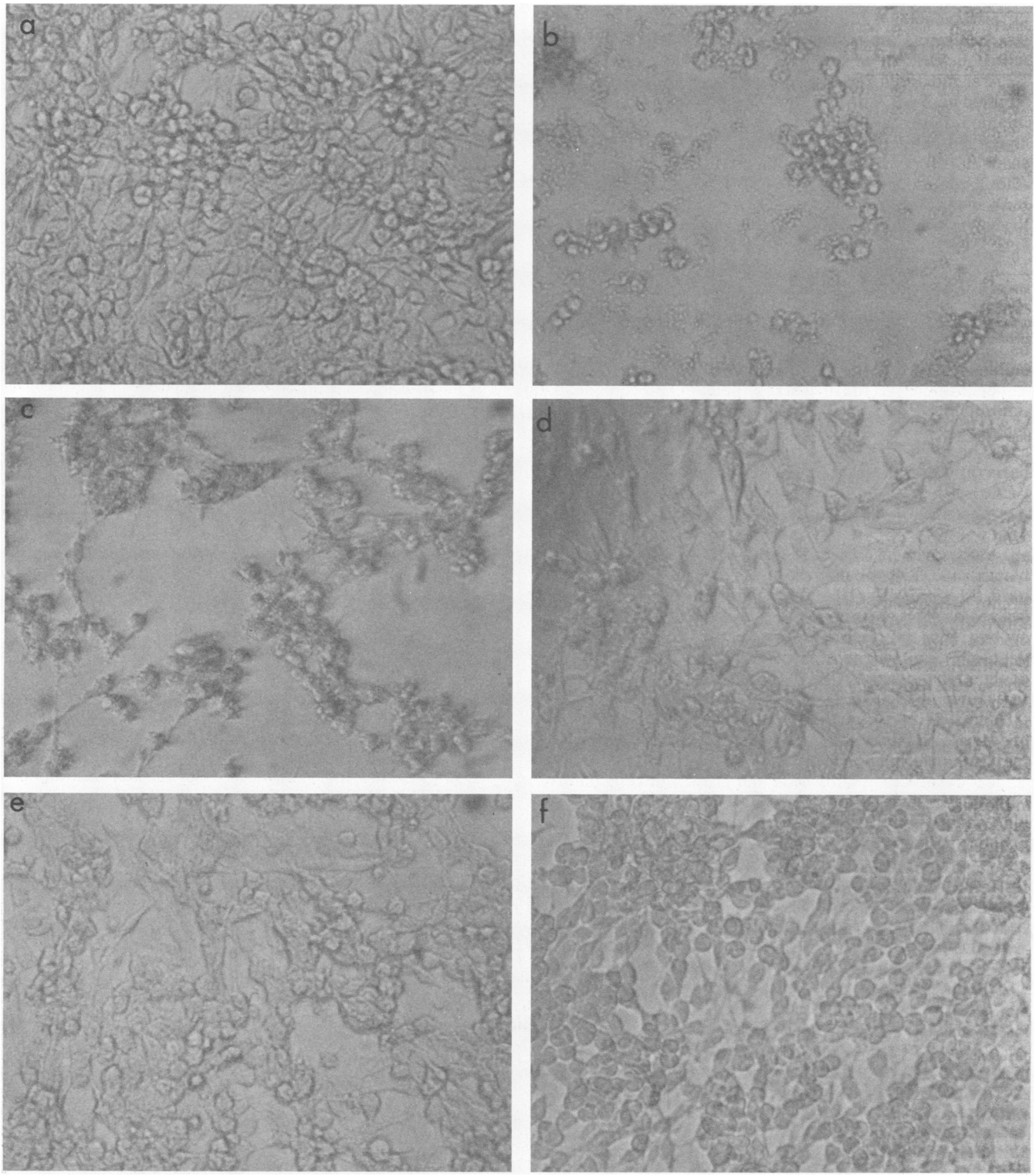


FIG. 3. Effects of various concentrations (0.13 to 13 μM) of tat_{2-86} on rat glioma cell line (C6 BU1) growth. Cells (10^6) were cultured with tat_{2-86} in 400 μl of RPMI containing 10% fetal calf serum, 1% glutamine, and 1% antibiotics (48-well plates from Nunc, Roskilde, Denmark). Photographs (magnification, ×172) were taken after 72 h of cell culture in the absence of tat_{2-86} (a) or in the presence of tat_{2-86} at 13 μM (b), 6.3 μM (c), 1.3 μM (d), and 0.13 μM (e). Cell growth with 10^{-4} M poly-L-lysine (M_r = 3,800) is shown in panel f.

reduced and S-carboxamidated derivatives (tat_{2-86} CM and tat_{21-86} CM). This decreased neurotoxicity could be explained by conformational constraints or variations in accessibility of the basic domain within the intact molecule. The conformational importance of the basic region for lethal activity is suggested by the different neurotoxic potencies (LD_{50}) of active *tat* peptides: the presence of adjacent but per se inactive *tat* regions 38 to 46 and 73 to 86 was found to increase the neurotoxicity of the basic domain about 6- and 14-fold respectively. Interestingly, neurotoxic peptides were inactive when injected subcutaneously or intravenously in mice at doses as high as $40 \times LD_{50}$.

In summary, tat_{2-86} and its peptide derivatives containing the basic domain can (i) specifically bind to rat brain synaptosomal membranes, rat glioma cells, and murine neuroblastoma cells, (ii) generate a membrane depolarization by modifying cell membrane permeability in excitable biological systems in both vertebrates and invertebrates, (iii) manifest potential cytotoxic activity in nerve cell lines, and (iv) induce lethal neurotoxicity in mice.

The neurotoxic effects are seen at relatively high tat_{2-86} concentrations (1 to 10 μM), although there are no data supporting the existence of free tat_{2-86} at such a concentration in the plasma, the CNS, or cerebral spinal fluid from HIV-infected individuals. Nevertheless, it cannot be ruled out that the same concentration range of tat_{2-86} could be attainable locally during HIV infection within close proximity of some acutely replicating HIV cells. Moreover, it has been shown recently that the protein can be released by HIV-infected cells into the supernatant (10) and taken up by other cells (12).

While ^{125}I-tat_{2-86} and FITC-labeled tat_{2-86} bound to the cell membrane at concentrations greater than 10^{-8} and 10^{-7} M, respectively, the neural cell permeability changes and toxicity on cell growth are mainly observed at tat_{2-86} concentrations of 10^{-6} to 10^{-5} M. Thus, it is possible that tat_{2-86} interacts with the cell membrane at concentrations lower than that causing cytotoxic cell changes. One can speculate that tat_{2-86} binding can directly provoke some biological effects such as neural cell stimulation, promoting a neurological dysfunction, activation and growth of cells involved in the formation of Kaposi's sarcoma (10, 37), and inhibition of antigen-induced lymphocyte proliferation (36). In addition, tat_{2-86} might act synergistically with other viral or cellular factors in vivo to bring about increased cell lysis. Such synergistic cytotoxicity has previously been described for human neutrophil defensin (23), a highly basic peptide exhibiting cytolytic activity at concentrations of 1 to 50 μM. Many basic cytotoxic proteins and peptides, implicated in contact-dependent antibody-mediated cytotoxicity and antimicrobial activity, are active at micromolar concentrations (6, 21, 30, 38, 39). The common mechanism by which all of these basic peptides damage target cell membrane is reported to be pore formation, leading to non-ion-selective membrane permeability; tat_{2-86} may belong to this class of cationic proteins. However, more studies are required to investigate the involvement of *tat* in the pathogenicity of virus-induced encephalopathy observed in patients with AIDS.

We thank L. Montagnier and J. C. Gluckman, J. Van Rietschoten, M. Pelhate, R. Verger, and S. Muller for helpful discussions and B. Zalk for the gift of tumor cell lines. M. Alvitre and M. Yagello are acknowledged for assistance, and S. Illand is acknowledged for expert typing.

This work was supported by funds from Agence Nationale de Recherche sur le SIDA and from Fondation de l'Avenir (Paris, France).

REFERENCES

1. **Arya, S. K., S. Guo, S. F. Josephs, and F. Wong-Staal.** 1985. *trans*-activator gene of human T-lymphotropic virus type III (HTLV-III). Science **229**:69–73.
2. **Benko, D. M., S. Schwartz, G. N. Pavlakis, and B. K. Felber.** 1990. A novel human immunodeficiency virus type 1 protein, *tev*, shares sequences with *tat*, *env*, and *rev* proteins. J. Virol. **64**:2505–2518.
3. **Bougis, P., M. Tessier, J. Van Rietschoten, H. Rochat, J. F. Faucon, and J. Dufourcq.** 1983. Are interactions with phospholipids responsible for pharmacological activities of cardiotoxins? Mol. Cell Biochem. **555**:49–64.
4. **Brenneman, D. E., G. L. Westbrook, S. P. Fitzgerald, D. L. Ennist, K. L. Elkins, M. R. Ruff, and C. B. Pert.** 1989. Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide. Nature (London) **335**:639–642.
5. **Callec, J. J.** 1974. *In* J. E. Treherne (ed.), Insect neurobiology, p. 119–178. North Holland, Amsterdam.
6. **Christensen, B., J. Fink, R. B. Merrifield, and D. Mauzerall.** 1988. Channel-forming properties of cecropins and related model compounds incorporated into planar lipid membranes. Proc. Natl. Acad. Sci. USA **85**:5072–5076.
7. **Dreyer, E. B., P. K. Kaiser, J. T. Offermann, and S. A. Lipton.** 1990. HIV-1 coat protein neurotoxicity prevented by calcium channel antagonists. Science. **248**:364–367.
8. **Duval, A., C. O. Malecot, M. Pelhate, and H. Rochat.** 1989. Changes in Na^+ channel properties of frog and rat skeletal muscles induced by the AaH II toxin from the scorpion Androctonus australis. Pfluegers Arch. **415**:361–371.
9. **Eilbott, D. J., N. Peress, H. Burger, D. La Neve, J. Orenstein, H. E. Gendelman, R. Seidman, and B. Weiser.** 1989. Human immunodeficiency virus type 1 in spinal cords of acquired immunodeficiency syndrome patients with myelopathy: expression and replication in macrophages. Proc. Natl. Acad. Sci. USA **86**:3337–3341.
10. **Ensoli, B., G. Barillari, S. Z. Salahuddin, R. C. Gallo, and F. Wong-Staal.** 1990. *tat* protein of HIV-1 stimulates growth of cells derived from Kaposi's sarcoma lesions of AIDS patients. Nature (London) **345**:84–86.
11. **Fisher, A. G., M. B. Feinberg, S. F. Josephs, M. E. Harper, L. M. Marselle, G. Reyes, M. A. Gonda, A. Aldovini, C. Debouk, R. C. Gallo, and F. Wong-Staal.** 1986. The *trans*-activator gene of HTLV-III is essential for virus replication. Nature (London) **320**:367–371.
12. **Frankel, A. D., and C. O. Pabo.** 1988. Cellular uptake of the *tat* protein from human immunodeficiency virus. Cell **55**:1189–1193.
13. **Gray, E. G., and V. P. Whittaker.** 1962. The isolation of nerve endings from brain: an electron microscopic study of cell fragments derived by homogenization centrifugation. J. Anat. **96**:79–88.
14. **Green, M., M. Ishino, and P. M. Loewenstein.** 1989. Mutational analysis of HIV-1 *tat* minimal domain peptides: identification of *trans*-dominant mutants that suppress HIV-LTR driven gene expression. Cell **58**:215–223.
15. **Haase, A. T.** 1986. Pathogenesis of lentivirus infections. Nature (London) **322**:130–136.
16. **Hagiwara, S., and L. Byerly.** 1983. The calcium channel. Trends Neurosci. **6**:189–193.
17. **Harrow, I. D., B. Hue, M. Pelhate, and D. B. Sattelle.** 1980. Cockroach giant interneurons stained by colbalt-backfilling of dissected axons. J. Exp. Biol. **84**:341–343.
18. **Hauber, J., M. H. Malim, and B. R. Cullen.** 1989. Mutational analysis of the conserved basic domain of human immunodeficiency virus *tat* protein. J. Virol. **63**:1181–1187.
19. **Ho, D. D., T. R. Rota, R. T. Schooley, J. C. Kaplan, J. D. Allan, J. E. Groopman, L. Resnick, D. Felsenstein, C. A. Andrews, and M. S. Hirsch.** 1985. Isolation of HTLV-III from cerebrospinal fluid and neuronal tissues of patients with neurologic syndromes related to the acquired immunodeficiency syndrome. N. Engl. J.

Med. **313**:1493–1497.

20. **Koenig, S., H. E. Gendelman, J. M. Orenstein, M. C. Dal Canto, G. H. Pezeshkpour, M. Yungbluth, F. Janotta, A. Aksamit, M. A. Martin, and A. S. Fauci.** 1986. Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. Science **233**:1089–1093.
21. **Kordel, M., R. Benz, and H. G. Sahl.** 1988. Mode of action of the staphylococcinlike peptide Pep 5: voltage-dependent depolarization of bacterial and artificial membranes. J. Bacteriol. **170**: 84–88.
22. **Letvin, N. L., M. D. Daniel, P. K. Sehgal, R. C. Desrosiers, R. D. Hunt, L. M. Waldron, J. J. MacKey, D. K. Schmidt, L. V. Chalifoux, and N. W. King.** 1985. Induction of AIDS-like disease in macaque monkeys with T-cell tropic retrovirus STLV-III. Science **230**:71–73.
23. **Lichtenstein, A., T. Ganzt, M. E. Selsted, and R. I. Lehrer.** 1986. In vitro tumor cell cytolysis mediated by peptide defensins of human and rabbit granulocytes. Blood **68**:1407–1410.
24. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
25. **Merrifield, R. B.** 1963. Solid phase synthesis. The synthesis of a tetrapeptide. J. Am. Chem. Soc. **85**:2149–2154.
26. **Narahashi, T.** 1974. Chemicals as tools in the study of excitable membranes. Physiol. Rev. **54**:813–889.
27. **Ohe, Y., H. Hayashi, and K. Iwai.** 1986. Human spleen histone H1. Isolation and amino acid sequence of main variant, H1b. J. Biochem. **100**:359–368.
28. **Price, R., B. Drew, J. Sidtis, M. Rosenblum, A. Scheck, and P. Cleary.** 1988. The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. Science **239**:586–592.
29. **Sabatier, J. M., G. Fontan, E. Loret, K. Mabrouk, H. Rochat, J. C. Gluckman, L. Montagnier, C. Granier, E. Bahraoui, and J. Van Rietschoten.** 1990. Large fragments of *nef*-protein and gp110 envelope glycoprotein from HIV-1. Int. J. Peptide Protein Res. **35**:63–72.
30. **Sahl, H. G., M. Kordel, and R. Benz.** 1987. Voltage dependent depolarization of bacterial membranes and artificial lipid bilayers by the peptide antibiotic nisin. Arch. Microbiol. **149**:120–124.
31. **Siomi, H., H. Shida, S. H. Nam, T. Nosaka, M. Maki, and M. Hatanaka.** 1988. Sequence requirements for nucleolar localization of human T-cell leukemia virus type 1 pX protein, which regulates viral RNA processing. Cell **55**:197–209.
32. **Snider, W. D., D. M. Simpson, S. Nielsen, J. W. M. Gold, C. E. Metroka, and J. B. Posner.** 1983. Neurological complications of acquired immune deficiency syndrome: analysis of 50 patients. Ann. Neurol. **14**:403–418.
33. **Sodroski, J., C. Rosen, F. Wong-Staal, S. Z. Salahuddin, M. Popovic, S. Arya, R. C. Gallo, and W. A. Haseltine.** 1985. *trans*-acting transcriptional regulation of human T-cell leukemia virus type III long terminal repeat. Science **227**:171–173.
34. **Tosteson, M. T., and D. C. Tosteson.** 1981. Mellitin forms channels in lipid bilayers. Biophys. J. **36**:109–116.
35. **Vazeux, R., N. Brousse, A. Jarry, D. Henin, C. Marche, C. Vedrenne, J. Mikol, M. Wolff, C. Michon, W. Rozenbaum, J. F. Bureau, L. Montagnier, and M. Brahic.** 1987. AIDS subacute encephalitis. Identification of HIV-infected cells. Am. J. Pathol. **126**:403–410.
36. **Viscidi, R. P., K. Mayur, H. M. Lederman, and A. D. Frankel.** 1989. Inhibition of antigen-induced lymphocyte proliferation by *tat* protein from HIV-1. Science **246**:1606–1608.
37. **Vogel, J., S. H. Hinrichs, S. K. Reynolds, P. A. Luciw, and G. Jay.** 1988. The HIV *tat* gene induces dermal lesions resembling Kaposi's sarcoma in transgenic mice. Nature (London) **335**:606–611.
38. **Young, J. D. E., C. G. B. Peterson, P. Venge, and Z. A. Cohn.** 1986. Mechanism of membrane damage mediated by human eosinophil cationic protein. Nature (London) **321**:613–616.
39. **Zasloff, M.** 1987. Magainins, a class of antimicrobial peptides from Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of precursor. Proc. Natl. Acad. Sci. USA **84**:5449–5453.